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Combined Supplementation with Vitamin B-6 and Curcumin is Superior to Either Agent Alone in Suppressing Obesity-Promoted Colorectal Tumorigenesis in Mice

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ABSTRACT

Background: Obesity increases the colorectal cancer risk, in part by elevating colonic proinflammatory cytokines. Curcumin (CUR) and supplemental vitamin B-6 each suppress colonic inflammation.

Objectives: We examined whether the combination of CUR and vitamin B-6 amplifies each supplement's effects and thereby suppress obesity-promoted tumorigenesis.

Methods: Male Friend Virus B (FVB) mice (4-week-old; $n = 110$) received 6 weekly injections of azoxymethane beginning 1 week after arrival. Thereafter, they were randomized to receive a low-fat diet (10% energy from fat), a high-fat diet (HFD; 60% energy from fat), a HFD containing 0.2% CUR, a HFD containing supplemental vitamin B-6 (24 mg pyridoxine HCl/kg), or a HFD containing both CUR and supplemental vitamin B-6 (C + B) for 15 weeks. Colonic inflammation, assessed by fecal calprotectin, and tumor metrics were the primary endpoints. The anti-inflammatory efficacy of the combination was also determined in human colonic organoids.

Results: HFD-induced obesity produced a 2.6-fold increase in plasma IL-6 ($P < 0.02$), a 1.9-fold increase in fecal calprotectin ($P < 0.05$), and a 2.2-fold increase in tumor multiplicity ($P < 0.05$). Compared to the HFD group, the C + B combination, but not the individual agents, decreased fecal calprotectin (66%; $P < 0.01$) and reduced tumor multiplicity and the total tumor burden by 60%–80% ($P < 0.03$) in an additive fashion. The combination of C + B also significantly downregulated colonic phosphatidylinositol-4,5-bisphosphate 3-kinase, *Wnt*, and NF-κB signaling by 31%–47% ($P < 0.05$), effects largely absent with the single agents. Observations that may explain how the 2 agents work additively include a 2.8-fold increased colonic concentration of 3-hydroxyanthranillic acid ($P < 0.05$) and a 1.3-fold higher colonic concentration of the active coenzymatic form of vitamin B-6 ($P < 0.05$). In human colonic organoids, micromolar concentrations of CUR, vitamin B-6, and their combination suppressed secreted proinflammatory cytokines by 41%–93% ($P < 0.03$), demonstrating relevance to humans.

Conclusions: In this mouse model, C + B is superior to either agent alone in preventing obesity-promoted colorectal carcinogenesis. Augmented suppression of procancerous signaling pathways may be the means by which this augmentation occurs. *J Nutr* 2021;151:3678–3688.

Keywords: vitamin B-6, curcumin, obesity, inflammation, colorectal cancer

Introduction

Obesity is a prominent risk factor for colorectal cancer (CRC), effecting a 1.5- to 2-fold increase in men and a 1.2- to 1.5-fold increase in women (1), and mechanistic studies have demonstrated it to be genuinely causal in nature (2). Both animal and human studies suggest that low-grade, chronic inflammation of the colonic mucosa is an important factor in mediating the procarcinogenic effects of obesity in colorectal tumorigenesis (3, 2, 4). We and others have shown that elevations in proinflammatory cytokines and activation of

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procarcinogenic signaling pathways are present in the colonic mucosa of obese laboratory rodents (3, 2) and humans (4) as compared with lean controls. In obese mice, genetic ablation of either TNF-α or IL-1β signaling results in the attenuation of various biomarkers of colonic carcinogenesis (5, 6), underscoring the causal role played by these inflammatory cytokines. The phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K)/protein kinase B (Akt) pathway plays a central role in the regulation of cell growth, proliferation, survival, and metabolism. Activation of this pathway is recognized as an instrumental step in tumorigenesis of many cancers, including those of the colon (7). Excess adiposity is observed to activate this pathway (8), which is unsurprising since a variety of systemic mediators commonly elevated in the setting of obesity activate the PI3K/Akt pathway, including insulin, insulin-like growth factor, TNF-α, and IL-6. Among the downstream targets of PI3K and Akt is NF-κB, a pivotal proinflammatory pathway that enhances the survival of premalignant cells and possesses other procarcinogenic actions (9). In addition, activated NF-κB upregulates the expression of proinflammatory cytokines such as TNF-α and IL-6, thereby creating an auto-multiplying loop (10).

Curcumin (CUR), a dietary polyphenol, possesses anti-inflammatory and antineoplastic activity in both cell culture and animal models, including suppression of colonic tumorigenesis in laboratory rodents (11, 12). In humans, daily supplementation with CUR at well-tolerated doses diminished the numbers of colonic aberrant crypt foci (13), an intermediary biomarker of CRC, and, when used as an adjunct to conventional therapy, suppressed the activity of ulcerative colitis (14). In addition, robust inverse relationships exist between vitamin B-6 exposure and inflammation in both animals and humans (15). Further, the observation that the provision of modest supplementation of dietary vitamin B-6 in replete animals can suppress experimental colitis (16) not only indicates the effect is present in the colon but that the relationship is a genuinely causal one. Moreover, a meta-analysis of prospective clinical studies also demonstrated inverse relationships between the risk of CRC and vitamin B-6 status, measured by plasma pyridoxal phosphate (pyridoxal 5'-phosphate; PLP) (17).

Combinations of chemopreventive agents designed to suppress colonic inflammation and the accompanying carcinogenesis possess several advantages over single agents (2, 18, 19, 20). Combining agents can produce additive or synergistic efficacy by maximizing suppression of a single procancerous cell signaling pathway and/or target multiple pathways (2, 20). Also, the required dose of each agent in the combination regimen can often be reduced due to enhanced efficacy, minimizing side effects (18, 19). In this study, we sought to: *1*) determine whether the combination of CUR and vitamin B-6 would be more efficacious than CUR or vitamin B-6 alone in preventing obesity-induced inflammation and tumorigenesis; *2*) examine the underlying cellular pathways mediating the effects; and *3*) determine whether vitamin B-6 and CUR possess similar anti-inflammatory effects in a human colonic context.

Methods

Chemicals, reagents, and antibodies

Azoxymethane (AOM) was purchased from Sigma-Aldrich. Curcumin (as Curcumin C3 Complex, a mixture of curcuminoids containing 79.4% curcumin, 17.8% demethoxycurcumin, and 2.8% bisdemethoxycurcumin) was provided by Sabinsa Corporation. The V-PLEX Proinflammatory Panel 1 (mouse) Kit and Tris Lysis Buffer (pH 7.5), 1% Triton X-100, were obtained from Meso Scale Discovery. Protease and phosphatase inhibitors were obtained from Boston BioProducts. The Nuclear Extraction Kit was from Abcam. Protein concentrations were determined using the bicinchoninic acid assay (BCA assay) method (Pierce). SuperSignal West Femto Maximum Sensitivity Substrate and CL-XPosure Film were from Thermo Fisher Scientific. Antibodies for phospho-PI3K (#4228S), PI3K (#4257S), phospho-Akt (#9271S), Akt (#9272S), phospho-NF-κB p65 (#3033S), β-Catenin (#8480S), and Lamin B1 (#13435S) were from Cell Signaling Technology. The β-Actin antibody (sc-47778) was from Santa Cruz. All antibodies used in Western blots were at 1:1000 dilutions.

Animals, diet, and dosage

The protocol was approved by the Institutional Animal Care and Use Committee of Tufts University (H2015-155). Diets were purchased from Research Diets. The high-fat diet (HFD; #D12492) and low-fat diet (LFD; #D12450B) contained 60% and 10% of their calories as lard, respectively (Research Diets). FVB mice were chosen because they are susceptible to both AOM-induced colorectal tumorigenesis (21, 22) and to diet-induced obesity (23). Male FVB mice (4 weeks old) were obtained from the Jackson Laboratory, and 2 mice were housed in each cage to minimize the confounding effects of social isolation and to minimize any possible cage effect. Up until the week following the final AOM injection, all mice were given free access to food (AIN-93G; #D10012G) and water. The compositions of D10012G, D12450B, and D12492 rodent diets are provided in **Supplemental Table 1**. Starting from the second week after arrival, mice were given 6 weekly injections of AOM (*5* mg/kg intraperitoneally at the first injection and 10 mg/kg intraperitoneally thereafter; see **Figure 1**A). One week after the sixth AOM injection, mice were randomly assigned to receive 1 of 5 experimental diets: *1*) LFD control (vitamin B-6, 6 mg/kg as pyridoxine HCl); *2*) HFD control (vitamin B-6, 6 mg/kg); *3*) HFD containing 0.2% CUR (wt%); *4*) HFD containing additional vitamin B-6 (18 mg/kg, for a total of 24 mg/kg); and *5*) HFD containing CUR and the supplemental level of vitamin B-6 (C + B). The sample size (21 mice in each group) was extrapolated from an earlier rodent study using curcumin alone (11). Body composition was determined by Echo-900 MRI at 21 weeks after arrival. Mice were killed by isoflurane asphyxiation at 22 weeks after arrival. Colonic tumors were inspected and measured and colonic mucosa were collected as described previously (2).

Custom CUR, vitamin B-6, and C + B diets were produced by Research Diets by blending the designated amounts of CUR and/or vitamin B-6 with HFD, with necessary modifications in content. The dose of CUR (0.2 wt%) used in this study is equivalent to approximately 1 g/day for a 60-kg adult human, based on the equivalent surface area dosage conversion method (24). The supplemental level of vitamin B-6 used is 4 times the recommended basal requirement of mice (25). Equivalent doses of CUR and vitamin B-6 are readily achievable in humans and are well tolerated.

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Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the authors and do not necessarily reflect the view of the USDA.

Supplemental Figure 1 and Supplemental Tables 1 and 2 are available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/jn/.

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Abbreviations used: Akt, protein kinase B; AOM, azoxymethane; C + B, curcumin + vitamin B-6; CRC, colorectal cancer; CUR, curcumin; HAA, 3-hydroxyanthranilic acid; HFD, high-fat diet; LFD, low-fat diet; PI3K, phosphatidylinositol-4,5-bisphosphate 3-kinase; PicA, picolinic acid; PL, pyridoxal; PLP, pyridoxal 5'-phosphate; p-p65, phospho-NF-κB p65; S1P, sphingosine-1-phosphate; SGK1, serum and glucocorticoid-regulated kinase 1.

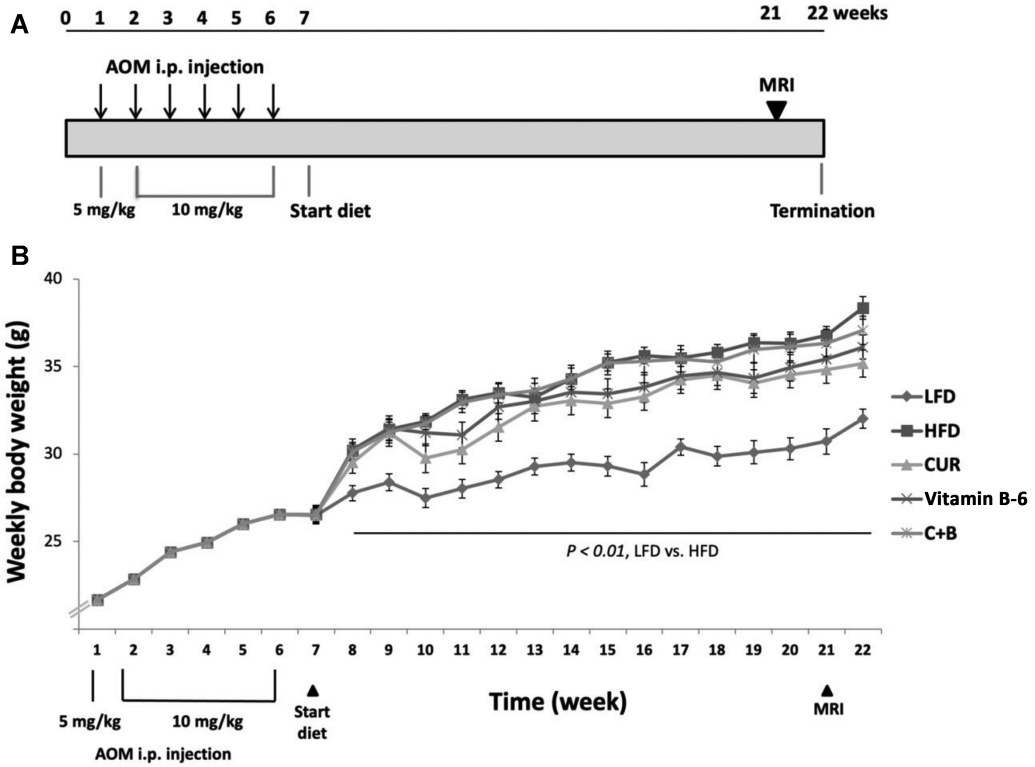

FIGURE 1 (A) Experimental design and (B) weekly body weights of AOM-treated FVB mice fed an LFD or HFD alone or supplemented with CUR, vitamin B-6, or both for 15 weeks. Values are means ± SEs; $n = 21$. Beginning at week 8, the LFD group differed from all HFD-fed groups at a P value < 0.01 (1-way ANOVA and post hoc Tukey's test). The 4 HFD groups did not differ from each another. Abbreviations: AOM, azoxymethane; C + B, curcumin + vitamin B-6; CUR, curcumin; HFD, high-fat diet; LFD, low-fat diet.

Determination of proinflammatory cytokines in plasma and calprotectin in feces

Plasma IL-1β, IL-6, and TNF-α concentrations were determined by the V-PLEX Proinflammatory Panel 1 (mouse) kit on a chemiluminescence platform (MesoScale Discovery) according to the manufacturer's instructions. For fecal calprotectin measures, frozen fecal samples obtained from the colon upon killing were lysed in ice-cold PBS containing 1% BSA, 0.05% Tween-20, and protease inhibitor. Protein concentrations were determined using the BCA method. Fecal calprotectin levels were measured by Mouse S100A8/S100A9 Heterodimer DuoSet ELISA according to the manufacturer's instructions (R&D Systems) and were normalized to protein concentrations.

Western blot analyses

The nuclear and cytoplasmic fractions of colonic mucosa were prepared using the Nuclear Extraction Kit (Abcam) according to the manufacturer's instructions. Protein concentrations were determined using the BCA method. Proteins of interest were probed and visualized as reported previously (2, 20). Band intensity was normalized to Lamin B1 (nuclear fraction) or β-actin (cytoplasmic fraction) and measured by Quantity One 1-D Analysis Software (Bio-Rad). Twelve colonic mucosa samples per group were used in Western blot analyses, and mucosa collected from 2 individual mice were pooled (i.e., a total of 6 lanes per group with at least 2 independent experiments). Statistical analyses were performed based on all 6 lanes.

Measurement of sphingosine-1-phosphate in plasma and colonic mucosa

Colonic sphingosine-1-phosphate (S1P) was measured by ELISA as previously described (16). Briefly, colonic mucosa was transferred to 300 μL of homogenization buffer [20 mM of Tris–HCl; 20% glycerol; 1 mM of B-mercaptoethanol; 1 mM of EDTA; 1 mM of Na orthovanadate; 15 mM of NaF; 1 mM of PMSF; a protease inhibitor cocktail; 0.5 mM of deoxypyridoxine; and 40 mM of B-glycerophosphate (Sigma)] and homogenized using a vortex for 4 cycles of 30 seconds, returning samples to ice between cycles. The samples were spun at 10,000 × g for 10 minutes (4°C), and S1P was measured in the supernatant according to manufacturer's instructions (Echelon Biosciences). Colonic S1P concentrations were normalized to the protein concentration.

Measurement of B-6 vitamers and kynurenines in plasma and colonic mucosa

Colonic mucosa was weighed and homogenized in a 3% ice-cold trichloroacetic acid solution at a 1:5 dilution. After pestle homogenization, use of a vortex for 1 minute, and sonication for 10 minutes, homogenates were snap frozen in liquid nitrogen and stored at −20°C. B-6 vitamers and kynurenines in the plasma and colonic supernatants were analyzed by BEVITAL by LC-MS/MS (26, 27). These analytes included PLP, pyridoxal (PL), 4-pyridoxic acid, pyridoxine, tryptophan, kynurenine, kynurenic acid, anthranilic acid, 3-hydroxykynurenine, xanthurenic acid, 3-hydroxyanthranilic acid, picolinic acid, and quinolinic acid.

Culture of human colonic organoids

Colonic organoids are 3-dimensional colon-like structures composed of functional, live epithelial crypt cells that self renew and spatially organize (28). Colonoscopic biopsy samples were obtained from 4 obese patients (BMI ≥30 kg/m^2) undergoing a screening colonoscopy at Tufts Medical Center, none of whom were regular users of nonsteroidal anti-inflammatory drugs (NSAIDs), had inflammatory bowel disease, or had CRC. The protocol was approved by the Tufts Medical Center Institutional Review Board (no. 11652), and informed consent was obtained from all subjects. Colonic tissue (5–8 biopsies) was placed in cold PBS and transported to the Koch Institute at MIT. Isolated colonic crypts were embedded in growth factor–reduced Matrigel (Corning) and diluted 3:4 in culture medium (advanced DMEM containing

TABLE 1 Final body weight, final body composition, and colonic neoplasms in AOM-treated FVB mice fed LFD or HFD alone or supplemented with CUR, vitamin B-6, or both for 15 weeks[1]

Group	Final body weight, g	Percentage fat mass	Tumor multiplicity (tumors per mouse)	Tumor burden (total volume of tumors per mouse, mm^3)	Tumor incidence[2]
LFD	32.0 ± 0.55^{b}	27.0 ± 0.79^{b}	0.64 ± 0.18^{b}	18.4 ± 7.14^{ab}	45%
HFD	38.4 ± 0.65^{a}	34.0 ± 0.55^{a}	1.38 ± 0.21^{a}	36.8 ± 9.26^{a}	81%
CUR	35.2 ± 0.77^{b}	31.0 ± 0.71^{ab}	0.81 ± 0.19^{ab}	25.4 ± 14.3^{ab}	52%
Vitamin B-6	36.1 ± 0.69^{b}	32.0 ± 1.37^{ab}	0.95 ± 0.22^{ab}	17.2 ± 7.38^{ab}	59%
C + B	37.1 ± 0.79^{ab}	33.0 ± 0.72^{ab}	0.50 ± 0.13^{b}	8.18 ± 3.60^{b}	45%

[1] Values are means ± SEs; $n = 21$. Labeled means in a column without a common letter differ at a P value < 0.05 (1-way ANOVA and post hoc Tukey's test). The tumor multiplicity was square root transformed to satisfy distributional assumptions for the ANOVA model. The tumor incidence was compared between groups using the Chi square test. Abbreviations: AOM, azoxymethane; C + B, curcumin + vitamin B-6; CUR, curcumin; HFD, high-fat diet; LFD, low-fat diet.
[2] The tumor incidence did not differ significantly between the groups.

Wnt3a, Rspondin-1, Noggin, and other growth factors) into 24-well plates (Olympus) at a density of ~500 crypts in 50 μL total volume per well, as previously described (29). The culture medium was changed every 2 days and organoids were passaged 1:3 to 1:5 every week. For long-term storage, the organoids were frozen and stored in liquid N2.

Determination of proinflammatory cytokines in colonic organoids

Human colonic organoids (between 5–10 passages) were seeded in 24-well plates for 48 hours at a density of ~200 cells in 25 μL total volume per well. The organoids were challenged with 10 μg/mL of LPS (variant O11:B4, Sigma-Aldrich) or vehicle for 48 hours. The culture medium was then collected and assayed for IL-1β, IL-6, IL-8, and TNF-α by a V-PLEX Proinflammatory Panel 1 (human) Kit on the chemiluminescence platform (MesoScale Discovery), according to the manufacturer's instructions. To determine the anti-inflammatory effects of CUR and pyridoxal, organoids were seeded in 48-well plates for 24 hours and then challenged with 10 μg/mL of LPS and CUR, vitamin B-6, or C + B for 48 hours. The culture medium was then collected and subjected to cytokine analyses as described above.

Statistical analyses

Data are presented as means ± SEs for the indicated number of independently performed experiments. All statistical analyses were performed using SAS Version 9.3 (SAS Institute). A 1-way ANOVA with Tukey's test was used for the comparison of differences among groups. The tumor multiplicity was square root transformed in order to satisfy distributional assumptions for the ANOVA model. The tumor incidence was compared using the Chi square test. Due to the multiplicity of signaling pathways, comparisons with the Benjamini–Hochberg method were employed to control the false discovery rate (30). The nature of the interactions between CUR and vitamin B-6 (i.e., synergistic, additive, or neither) in vivo and on cytokine production in colonic organoid experiments were analyzed based on the "model-free tests for synergy" by Laska et al. (31). A P value < 0.05 was considered statistically significant.

Results

Development of HFD-induced obesity in FVB mice

Food intake and body weight were assessed weekly (Figure 1B). No differences in energy intake, behavior, or appearance were observed among the 5 groups over the course of the experiment (data not shown). One mouse died after the second AOM injection. Also, 1 week after randomization 1 mouse in the HFD group died prematurely; the causes of death in each instance were not evident. Mice consuming the HFD without additives promptly achieved a significantly greater body weight 1 week after switching to the HFD, compared to the LFD mice ($P < 0.01$), and significant differences remained throughout the experiment. The final body weights of mice consuming the HFD and LFD were 38.4 ± 0.65 g and 32.5 ± 0.55 g, respectively (**Table 1**), an 18% difference ($P < 0.01$). Further, the proportion of the body comprised of fat mass in the HFD mice compared with the LFD mice was 26% greater (34% compared with 27%, respectively; $P < 0.01$). Altogether, the 60% HFD successfully induced adiposity in the FVB mice. Supplementation of CUR or vitamin B-6 resulted in a reduced final body weight compared to the HFD control group ($P < 0.03$). Interestingly, the combination of C + B did not cause a significantly lower final body weight compared to the HFD control, an effect likely due to the smaller tumor multiplicity and burden possessed by this group (Table 1).

C + B, but not each individual agent, diminishes colon tumorigenesis

HFD-induced obesity, in conjunction with 6 injections of AOM, resulted in a 2.2-fold increase in tumor multiplicity compared to the lean mice (Table 1; $P < 0.05$). The HFD-fed mice harbored 1.38 ± 0.21 tumors each, whereas LFD-fed mice had 0.64 ± 0.18 tumors each. Supplementation with C + B significantly suppressed tumor development in mice, as evidenced by 64% and 78% reductions in tumor multiplicity and the burden, respectively, compared to the HFD control ($P < 0.03$), and a test for synergy (31) demonstrated it did so for both of these endpoints in an additive fashion. In contrast, neither CUR nor vitamin B-6 alone significantly suppressed colorectal tumorigenesis. The tumor incidence was not significantly different between the 5 groups, although the combination regimen resulted in a level as low as that seen in the LFD control. Thus, the combination regimen of C + B substantially attenuated 2 of the 3 metrics of CRC, in contrast to the singular interventions, whose effects were much smaller and statistically nonsignificant. The box-plot figure of tumor multiplicity and burden is available in **Supplemental Figure 1**.

Suppression of obesity-induced inflammation by the combination regimen

The consumption of the HFD resulted in a 2.81-fold elevation in the plasma IL-6 concentration compared to the level observed in the LFD group (**Figure 2**A; $P < 0.02$). Supplementation with CUR, vitamin B-6, or their combination did not result in statistically significant reductions in IL-6, although the numerical reduction in the C + B compared with the HFD group approached significance ($P = 0.07$). There were no significant differences in the mean plasma concentrations of IL-1β and TNF-α between the 5 groups (data not shown).

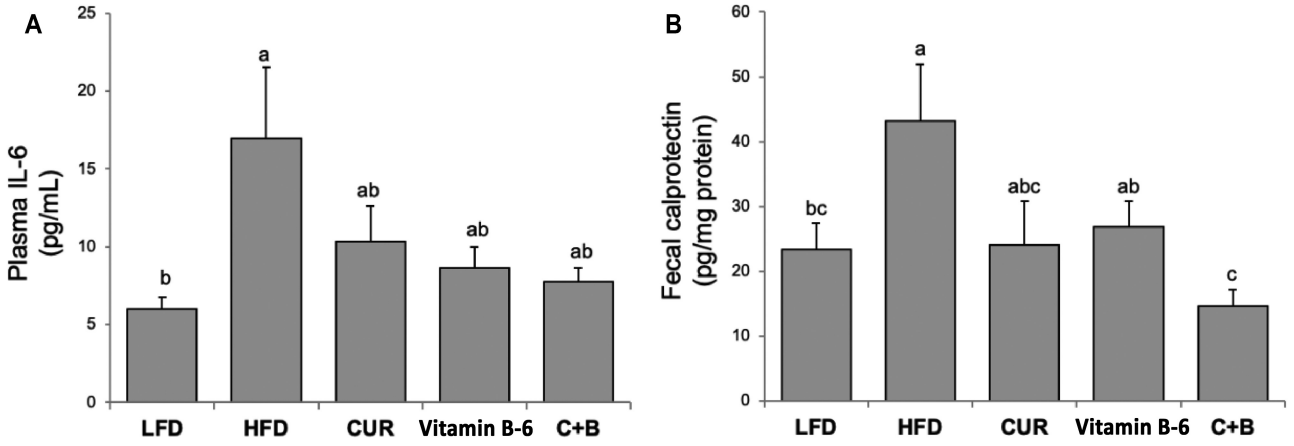

FIGURE 2 Effects of CUR, vitamin B-6, and C + B on (A) plasma IL-6 and (B) fecal calprotectin in AOM-treated FVB mice fed an LFD or HFD alone or supplemented with CUR, vitamin B-6, or both for 15 weeks. Frozen fecal samples were obtained in the colon upon euthanasia and then measured by ELISA. The fecal level of calprotectin was normalized by protein concentrations. Values are means ± SEs; $n = 10$ for plasma IL-6; $n = 20$ for fecal calprotectin. Labeled means without a common letter differ at a P value < 0.05 (1-way ANOVA and post hoc Tukey's test). Abbreviations: AOM, azoxymethane; C + B, curcumin + vitamin B-6; CUR, curcumin; HFD, high-fat diet; LFD, low-fat diet.

Calprotectin is a neutrophil-derived protein, and the concentration of fecal calprotectin is a widely accepted marker of intestinal inflammation in clinical and preclinical settings (32). As shown in Figure 2B, the HFD resulted in a 1.85-fold increase in calprotectin compared to the LFD ($P < 0.05$). The combination of C + B greatly reduced the fecal calprotectin level by 66% compared to HFD group ($P < 0.01$). In contrast, single supplementation did not cause significant suppression compared to the HFD group. The statistical method established by Laska et al. (31) indicates that the observed effects on tumorigenesis and intestinal inflammation produced by the C + B combination are additive, but not synergistic. Altogether, the combination treatment demonstrates a sizeable efficacy in suppressing obesity-induced tumorigenesis and intestinal inflammation that is not observed with the single-agent supplements.

The C + B combination modulates multiple procarcinogenic signaling pathways in the colon

Wnt.

Excessive activation of *Wnt* signaling in the colonic epithelium is an instrumental early step in more than 85% of sporadic CRC cases (33). Compared to LFD-fed lean mice, HFD-fed obese mice expressed a significantly higher concentration of intranuclear β-catenin (**Figure 3**A and B; $P < 0.01$), the proximate effector of canonical *Wnt* signaling and an accepted metric of *Wnt* activation (34). Vitamin B-6 supplementation alone, as well as the combination of C + B, led to significant reductions in nuclear β-catenin compared to the HFD group ($P < 0.05$).

PI3K, Akt, and serum and glucocorticoid-regulated kinase 1.

Increased activation of the PI3K signaling pathway (by phosphorylation) is a common event for several different pathways by which obesity is thought to increase the risk of CRC (35, 36). As shown in Figure 3A and C, the combination of C + B—but neither agent alone—significantly diminished the activation of PI3K signaling induced by HFD, by 46% ($P < 0.03$). However, Akt phosphorylation at Ser473, a frequent means by which PI3K activates Akt and mediates upregulation of downstream procarcinogenic pathways, was not altered in the total cellular lysate by any of the interventions compared to the HFD control ($P > 0.1$; data not shown).

Various other signal molecules can substitute for Akt's intermediary role in mediating the procarcinogenic downstream events stimulated by PI3K activation, such as serum and glucocorticoid-regulated kinase 1 (SGK1) (37). However, no difference was observed between LFD, HFD, or different intervention groups regarding the level of phospho-SGK1, which reflects the activation of SGK signaling ($P > 0.1$; data not shown).

NF-κB.

A prominent downstream target of PI3K signaling is NF-κB, a potent proinflammatory protein (35). When activated, NF-κB translocates into the cell nucleus and exerts its transcriptional activities. Phosphorylation of p65, the most important functional subunit of NF-κB, at Ser536 is a critical modification that further enhances the transcriptional activities of NF-κB (38). We therefore determined the concentration of phospho-p65 (p-p65) in the nuclear fraction of the colonic mucosa, an accepted metric of NF-κB activation (38). The pattern of p-p65 protein expression recapitulates what was observed with PI3K activation: only the combination of C + B significantly suppressed p-p65 in comparison to the HFD control, while neither agent alone produced significant suppression (Figure 3A and D). This observation indicates that CUR and vitamin B-6 work cooperatively to attenuate obesity-promoted activation of NF-κB signaling.

Plasma and colonic concentrations of B-6 vitamers

We also sought to investigate the mechanism(s) by which vitamin B-6 might exert its effects, especially in combination with curcumin. Of the various vitameric forms of B-6, PLP is the only active coenzyme form of the vitamin (39). In plasma, vitamin B-6 supplementation alone or in combination with CUR resulted in significantly higher concentrations of PLP compared to the groups receiving the basal requirement of the vitamin (**Figure 4**A). In the colonic mucosa, it is of particular interest that only the C + B combination group, and not the vitamin B-6 supplementation group, achieved a significantly

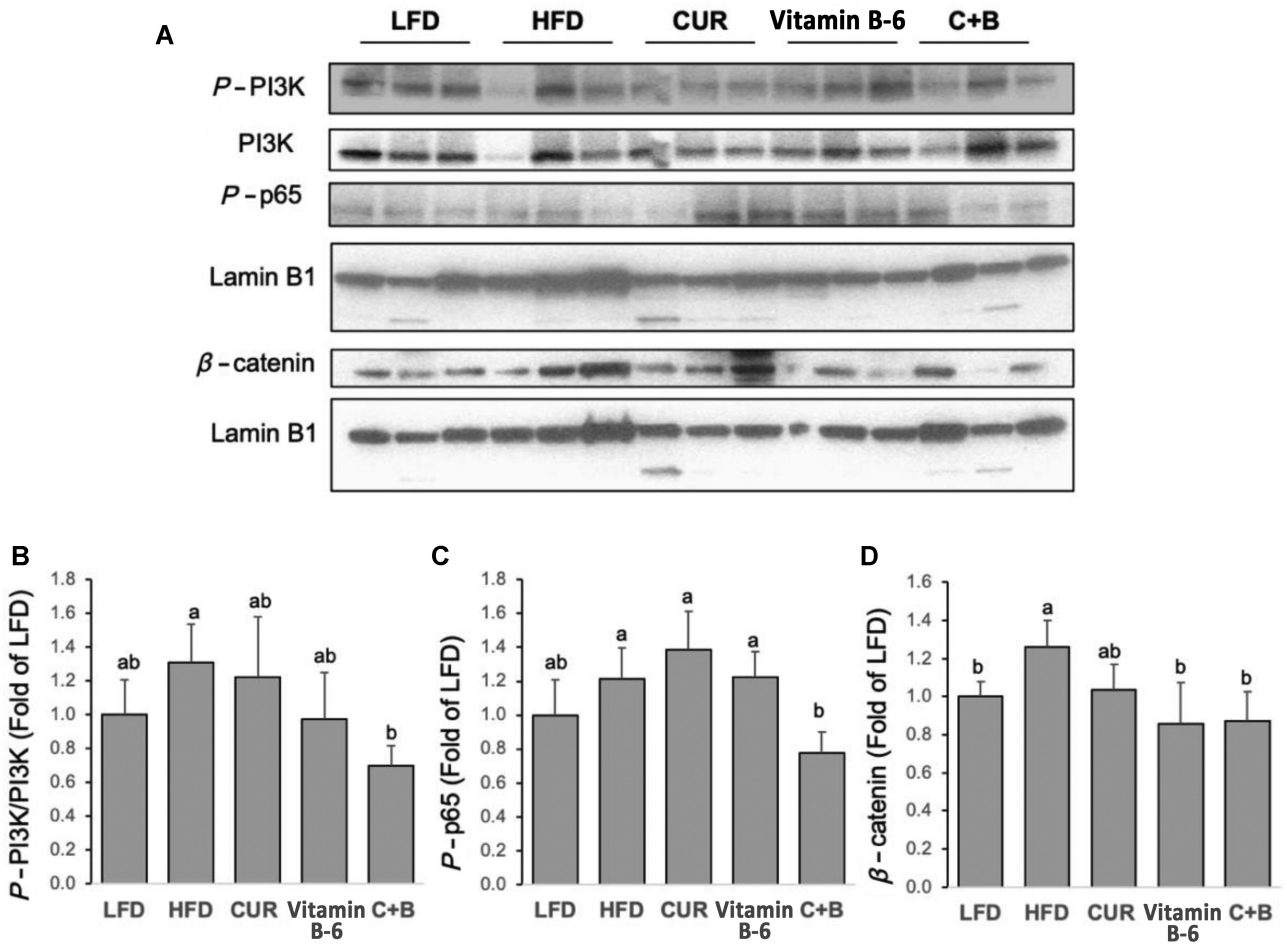

FIGURE 3 Effects of CUR, vitamin B-6, and C + B on expression levels of multiple procarcinogenic signaling pathways in the colonic mucosa of AOM-treated FVB mice fed an LFD or HFD alone or supplemented with CUR, vitamin B-6, or both for 15 weeks: (A) representative Western blot bands, (B) β-catenin (nuclear fractions), (C) phospho-PI3K (p-PI3K) and PI3K (total cellular lysates), and (D) p-p65 (nuclear fractions). Each lane contained colonic mucosa collected from 2 individual mice that were pooled for immunoblot analyses. A total of 6 lanes per group were tested, which necessitated the use of 2 gels. The figure shows a picture of 1 of the 2 gels, and statistical analyses were performed based on all 6 lanes. Band intensity was normalized to Lamin B1 (nuclear fraction) or β-actin (whole-tissue lysate). Values are means ± SEs; $n = 6$. Labeled means without a common letter differ at a P value < 0.05 (1-way ANOVA and post hoc Tukey's test). Multiple comparisons with the Hochberg method were employed to control the false discovery rate (30). Abbreviations: AOM, azoxymethane; C + B, curcumin + vitamin B-6; CUR, curcumin; HFD, high-fat diet; LFD, low-fat diet; PI3K, phosphatidylinositol-4,5-bisphosphate 3-kinase; p-p65, phospho-p65.

greater concentration of the active co-enzyme, PLP ($P < 0.04$), compared to the HFD control (Figure 4B).

Mucosal S1P is not diminished to a significant extent by single agents or C + B

S1P is a sphingolipid with potent chemotactic activity for immune cells and is an activator of NF-κB and signal transducer and activator of transcription 3 (STAT3) (40, 41). The catabolism of S1P is catalyzed by a PLP-dependent enzyme, S1P lyase (15). HFD-induced obesity produced a 2.5-fold elevation in the concentration of colonic S1P compared to the LFD-fed, lean mice (**Figure 5**A; $P < 0.01$). Supplementation with CUR, vitamin B-6, or the C + B combination did not produce a significant attenuation of S1P concentrations in the colonic mucosa.

Kynurenine metabolism was modified by the combination regimen

Another metabolic pathway that utilizes PLP as a cofactor and that modifies the inflammatory response is the catabolism of tryptophan through the kynurenine pathway (15). Specifically, concentrations of 3-hydroxyanthranilic acid (HAA) are altered in the afflicted intestinal tissue of patients with inflammatory bowel disease (42) and in several animal models, where either exogenous administration of HAA or genetically enhancing its tissue concentration suppresses inflammation and its downstream effects (43, 44). The majority of kynurenine metabolites in the colonic mucosa did not differ in the 5 diet groups (**Supplemental Table 2**). However, colonic levels of HAA and picolinic acid (PicA) were significantly elevated by the C + B combination regimen (Figure 5B and C; $P < 0.01$). Also, CUR alone significantly increased the level of HAA, and the change was comparable to that of the C + B combination.

Human colonic organoids

In order to explore whether the anti-inflammatory effects of vitamin B-6 and CUR have relevance to the human colon, we utilized the model of human colonic organoids (28). No significant differences in the numbers of organoids or their morphology were observed among control, LPS, or different treatment groups. CUR at 10–30 μM and vitamin B-6 at 100–300 μM resulted in significant reductions of IL-1β, IL-6, IL-8, and TNF-α compared to the levels observed in LPS-treated

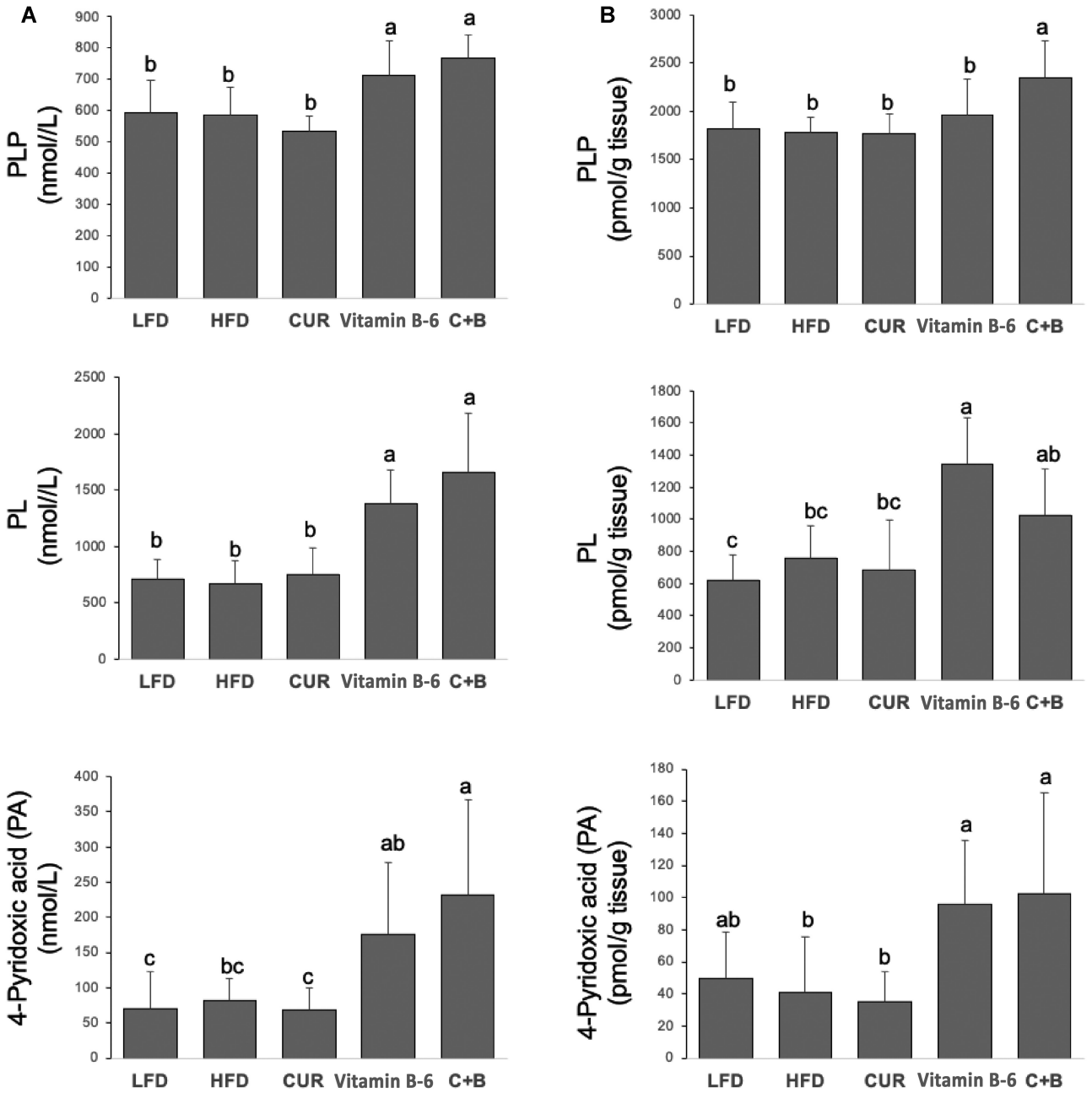

FIGURE 4 Effects of CUR, vitamin B-6, and C + B on the concentrations of B-6 vitamers and metabolites in (A) plasma and (B) colonic mucosa of AOM-treated FVB mice fed an LFD or HFD alone or supplemented with CUR, vitamin B-6, or both for 15 weeks. Values are means ± SEs; $n = 10$. Labeled means without a common letter differ at a P value < 0.05 (1-way ANOVA and post hoc Tukey's test). Abbreviations: AOM, azoxymethane; C + B, curcumin + vitamin B-6; CUR, curcumin; HFD, high-fat diet; LFD, low-fat diet.

organoids (**Figure 6**B). Halving the concentrations of CUR and vitamin B-6 (i.e., CUR at 5–15 μM plus vitamin B-6 at 50–150 μM) also led to significant reductions of all cytokines compared to the LPS control, except the lowest dose combination did not significantly diminish TNF-α. In contrast to the in vivo study, according to Laska et al.'s method (31), a significant augmentation of the anti-inflammatory effect was not observed in the organoids when vitamin B-6 was combined with CUR.

Discussion

At the very low dietary concentrations that were studied, we demonstrated that the combination of supplemental CUR and vitamin B-6 effectively suppresses, in an additive fashion, the formation of obesity-promoted colonic neoplasms by 60%–80% in male FVB mice as compared to the HFD control, attenuates biochemical evidence of colonic inflammation, and downregulates the activity of several relevant procarcinogenic signaling pathways, including *Wnt*, PI3K, and NF-κB. There do exist prior observations in rodent models of CRC that each of these agents can alone suppress tumorigenesis (12, 45). However, the 2 avenues by which vitamin B-6 is hypothesized to produce an anti-inflammatory effect are distinct from those avenues by which curcumin is thought to exert anti-inflammatory effects, and thus our rationale for combining these agents was the idea that the 2 might act in a complementary—and therefore an additive—fashion. Moreover, our prior

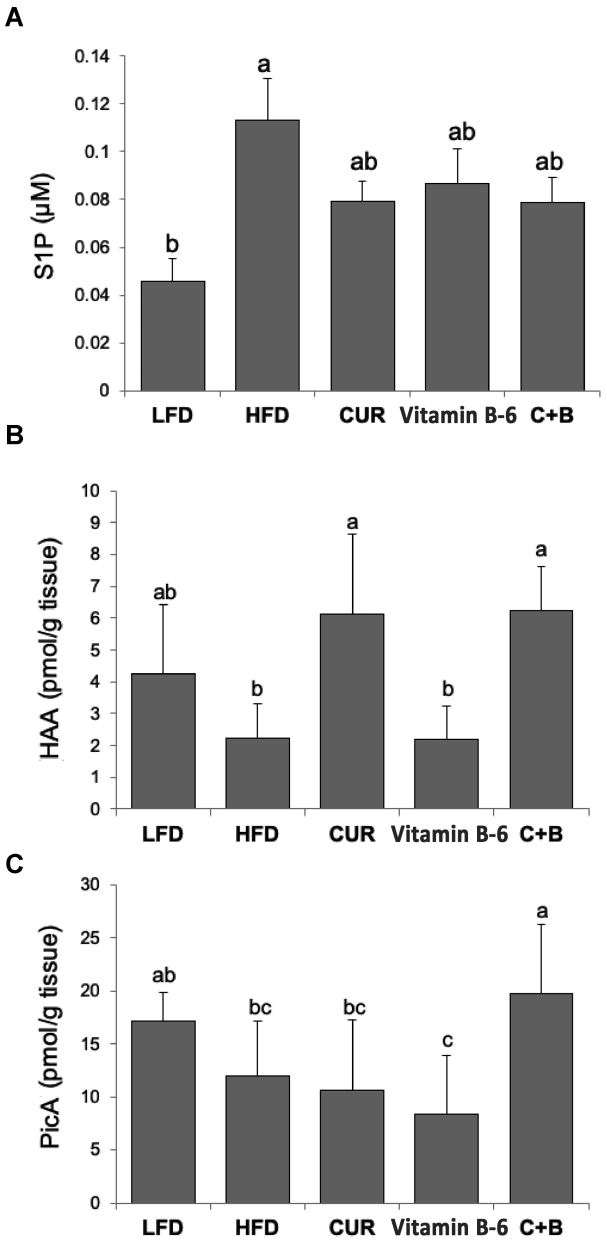

FIGURE 5 (A) Effects of CUR, vitamin B-6, and C + B on the colonic concentrations of S1P in AOM-treated FVB mice fed an LFD or HFD alone or supplemented with CUR, vitamin B-6, or both for 15 weeks. Values are means ± SEs; $n = 20$. Labeled means in a column without a common letter differ at a P value < 0.05 (1-way ANOVA and post hoc Tukey's test). Effects of CUR, vitamin B-6, and C + B on concentrations of (B) kynurenine metabolites HAA and (C) PicA in the colonic mucosa. Values are means ± SEs; $n = 10$. Labeled means without a common letter differ at a P value < 0.05 (1-way ANOVA and post hoc Tukey's test). Abbreviations: AOM, azoxymethane; C + B, curcumin + vitamin B-6; CUR, curcumin; HAA, 3-hydroxyanthranilic acid; HFD, high-fat diet; LFD, low-fat diet; PicA, picolinic acid; p65, NF-κB p65; S1P, sphingosine-1-phosphate.

publication, demonstrating the potent suppressive effect of supplemental vitamin B-6 in a mouse model of colitis (16), was a compelling reason why this vitamin was chosen to be a component of the combination regimen. Our observations confirm this idea, demonstrating that the combination of C + B is more effective in producing these effects than the individual agents, and it does so even in the setting of the heightened risk that accompanies diet-induced obesity, thereby demonstrating an additive effect when the 2 agents are used together and attesting to the superiority of the combination regimen. We confined our study to male mice, since a high-fat diet does not reliably induce obesity in female mice (46); moreover, obesity is a substantially more potent promoter of CRC risk among men than women (1), and thus focusing on males made our study more relevant to the gender most affected by obesity-promoted CRC.

In agreement with our prior studies of HFD-induced obesity (3, 2, 4), excess adiposity generated a substantial degree of biochemical inflammation, both systemically and in the colon, compared to the LFD-fed, lean mice (Figure 2). Supplementation with the C + B combination induced a 66% reduction in fecal calprotectin, compared to the HFD control, whereas no significant reduction was observed by either agent alone. Although the design of this study does not prove that it was this inflammation that activated the signaling pathways, prior experiments by us and others in colonocyte cell cultures and in intact animals have consistently shown the genuine causal roles that proinflammatory cytokines play in activating *Wnt*, NF-κB, and epithelial hyperproliferation in the colonic mucosa (5, 6).

We examined 2 pathways through which vitamin B-6 has been reported to exert anti-inflammatory effects: kynurenine and the sphingolipid metabolism. The kynurenine pathway is a cellular route by which tryptophan is catabolized by multiple PLP-dependent enzymatic reactions into a number of metabolites collectively known as kynurenines (15). Some kynurenines have been reported to exert immunomodulatory effects, including HAA, quinolinic acid, and PicA (15). We measured a broad array of kynurenines in the colonic mucosa and found that only HAA and PicA were altered by the interventions (Figure 5B and C). CUR alone and the C + B combination each increased the concentration of HAA several-fold, whereas only the C + B combination also resulted in higher concentrations of PicA. HAA is a potent redox active metabolite with antioxidant, anti-inflammatory, immunosuppressive, and pro-apoptotic effects (15, 44). Its anti-inflammatory activity has been shown to act, in part, by suppressing PI3K and NF-κB activation (47). PicA is thought to be neuroprotective (48), but a clear anti-inflammatory role has not yet been defined. PLP is also a cofactor in the catabolism of the highly proinflammatory lipid S1P by S1P lyase (15): in a murine colitis model, those mice lacking sphingosine kinase 1, the enzyme that synthesizes S1P, were resistant to the induction of colitis (37). In the present study, the trend towards a reduction in colonic S1P produced by each agent alone and in combination suggests that this pathway might also play an anti-inflammatory role, but further studies are required to determine whether this is a genuine effect.

PLP is the only biologically active coenzyme form of vitamin B-6. Supplemental vitamin B-6 at 4 times the basal requirement raised plasma PLP, but did not produce a significant rise in colonic PLP (Figure 4), consistent with a phenomenon of tissue saturation observed in prior studies (16). In contrast, the addition of CUR to vitamin B-6 supplementation produced a significant increase in colonic PLP content compared to the other groups, apparently by diminishing the proportion of the vitamin that was in its nonphosphorylated form. Whether the lower concentration of PL present in the colons of the C + B mice indicates that CUR retards the hydrolysis of PLP by PLP-phosphatases to the inactive PL form is a hypothesis that has yet to be tested. Thus, in addition to modulating the PLP-dependent catabolic pathway of tryptophan towards an anti-inflammatory profile, CUR may also enable the colon to achieve

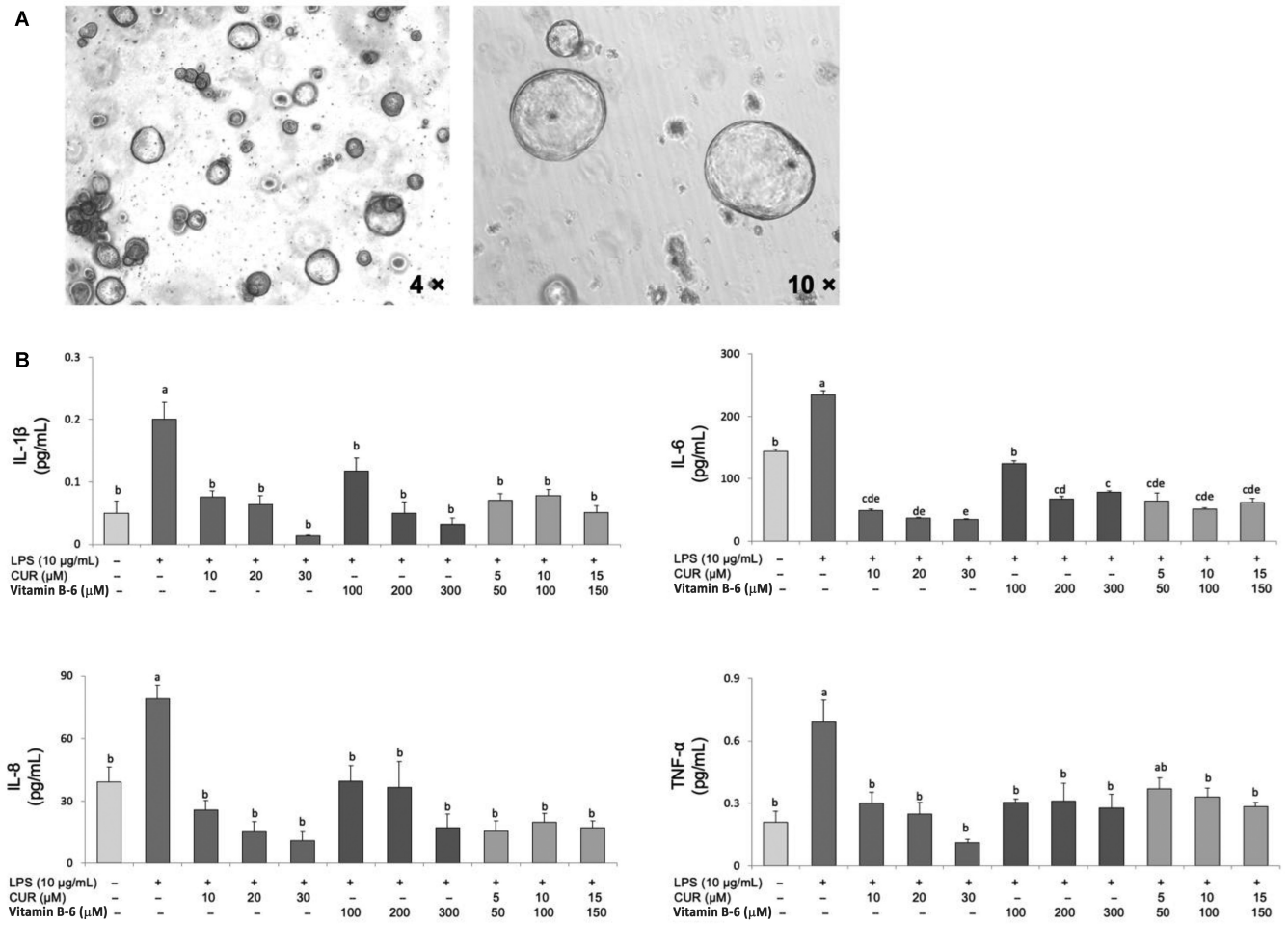

FIGURE 6 (A) Representative images of human colonic organoids. (B) Effects of CUR, vitamin B-6 (pyridoxal), and C + B on the concentrations of IL-1β, IL-6, IL-8, and TNF-α in LPS-treated human colonic organoids. Values are means ± SEs; $n = 4$. Labeled means without a common letter differ at a P value < 0.05 (1-way ANOVA and post hoc Tukey's test). Abbreviations: C + B, curcumin + vitamin B-6; CUR, curcumin.

higher concentrations of PLP. However, we cannot exclude the possibility that vitamin B-6 might conversely impact the CUR metabolism in a manner that enables the combination regimen to exert enhanced suppressive effects on tumor and inflammation metrics. Exploring this possibility will also require future studies.

These data also demonstrate that the C + B combination efficaciously downregulates *Wnt*, PI3K, and NF-κB signaling in the colonic mucosa (Figure 3). Notably, Akt activation was unchanged as assessed by phosphorylation at Ser473, although we cannot exclude the possibility that it was activated at another amino acid residue, as has been previously reported (49). Typically, phosphorylation of Akt by PI3K is considered a principal intermediary effector of downstream signaling, including *Wnt* and NF-κB. We therefore speculated that an Akt-independent pathway might be mediating the downstream effects of PI3K activation. However, we observed that SGK1, which plays an intermediary role in mediating PI3K signaling and upregulating *Wnt* and NF-κB in human colon cancer cell lines (41), was unchanged by our dietary interventions. Other PI3K-dependent Akt-independent pathways (50) might therefore be involved in C + B's chemopreventive function, which warrants further investigation.

In accordance with the observations in mice, micromolar concentrations of the C + B combination exerted a substantial inhibitory effect against LPS-stimulated overexpression of proinflammatory cytokines in human organoids. However, 1 distinct difference from our in vivo observations is that the suppression of the cytokine release produced by the combination was neither synergistic nor additive. This might simply reflect a diminished sensitivity to detecting treatment differences in the organoid model or, alternatively, it might indicate that the added efficacy in vivo requires interactions between the epithelial cells of the crypt and the underlying myofibroblasts, immune cells, and other elements of the lamina propria, or other elements present in the intact animal.

This study has limitations. Although our data are all consistent with the concept that the C + B regimen suppressed tumorigenesis by suppressing biochemical inflammation and downregulating the PI3K/NF-κB/*Wnt* pathways, we rely on prior observations that prove the causal roles of inflammation in obesity-promoted tumorigenesis (3, 2, 4). Also, in this study, we did not distinguish between procarcinogenic effects of adiposity per se compared with those directly due to the HFD. Prior studies, however, have shown that when the conditions are segregated, high-fat diets and obesity can each, independently, enhance colonic inflammation and tumorigenesis (39). Lastly, although prior research examining the utility of curcumin has been criticized because ill-defined mixtures of curcumoid compounds have been used, making it difficult to compare the

results of 1 study to another (51): we circumvented this issue by using a curcuminoid preparation that has been precisely chemically defined.

In summary, we have demonstrated that the combination regimen of C + B suppresses obesity-promoted biochemical inflammation in the colonic mucosa, downregulates several important procancerous and proinflammatory signaling pathways, and suppresses the formation of colonic neoplasms in AOM-injected FVB male mice. Notably, the combination regimen was superior to either agent alone in most respects, including the suppression of tumorigenesis. The doses of CUR and vitamin B-6 used in this study were well tolerated and caused no adverse effects. Comparable human doses have been consistently well tolerated in clinical studies (14). Future clinical trials are needed to determine whether the antitumor effects conveyed by this combination regimen will also possess utility for CRC chemoprevention in overweight and obese human populations.

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References

1. Jochem C, Leitzmann M. Obesity and colorectal cancer. Recent Results Cancer Res 2016;208:17–41.
2. Wu X, Koh GY, Huang Y, Crott JW, Bronson RT, Mason JB. The combination of curcumin and salsalate is superior to either agent alone in suppressing pro-cancerous molecular pathways and colorectal tumorigenesis in obese mice. Mol Nutr Food Res 2019;63(8):1801097.
3. Jain SS, Bird RP. Elevated expression of tumor necrosis factor-alpha signaling molecules in colonic tumors of Zucker obese (fa/fa) rats. Int J Cancer 2010;127(9):2042–50.
4. Pfalzer AC, Leung K, Crott JW, Kim SJ, Tai AK, Parnell LD, Kamanu FK, Liu Z, Rogers G, Shea MK, et al. Incremental elevations in TNFalpha and IL6 in the human colon and procancerous changes in the mucosal transcriptome accompany adiposity. Cancer Epidemiol Biomarkers Prev 2018;27(12):1416–23.
5. Guo C, Kim SJ, Frederick A-LM, Li J, Jin Y, Zeng H, Mason JB, Liu Z. Genetic ablation of tumor necrosis factor-alpha attenuates the promoted colonic *W nt* signaling in high fat diet-induced obese mice. J Nutr Biochem 2020;77:108302.
6. Pfalzer AC, Crott JW, Koh GY, Smith DE, Garcia PE, Mason JB. Interleukin-1 signaling mediates obesity-promoted elevations in inflammatory cytokines, *Wnt* activation, and epithelial proliferation in the mouse colon. J Interferon Cytokine Res 2018;38(10):445–51.
7. Danielsen SA, Eide PW, Nesbakken A, Guren T, Leithe E, Lothe RA. Portrait of the PI3K/AKT pathway in colorectal cancer. Biochim Biophys Acta 2015;1855(1):104–21.
8. Park S, Kim J, Seo Y, Sung M. Effects of diet-induced obesity on colitis-associated colon tumor formation in A/J mice. Int J Obes 2012;36(2):273–80.
9. Hoesel B, Schmid JA. The complexity of NF-κB signaling in inflammation and cancer. Mol Cancer 2013;12(1):1–15.
10. Wang S, Liu Z, Wang L, Zhang X. NF-kappaB signaling pathway, inflammation and colorectal cancer. Cell Mol Immunol 2009;6(5):327–34.
11. Kubota M, Shimizu M, Sakai H, Yasuda Y, Terakura D, Baba A, Ohno T, Tsurumi H, Tanaka T, Moriwaki H. Preventive effects of curcumin on the development of azoxymethane-induced colonic preneoplastic lesions in male C57BL/KsJ-db/db obese mice. Nutr Cancer 2012;64(1):72–9.
12. Huang M-T, Lou Y-R, Ma W, Newmark HL, Reuhl KR, Conney AH. Inhibitory effects of dietary curcumin on forestomach, duodenal, and colon carcinogenesis in mice. Cancer Res 1994;54(22):5841–7.
13. Carroll RE, Benya RV, Turgeon DK, Vareed S, Neuman M, Rodriguez L, Kakarala M, Carpenter PM, McLaren C, Meyskens FL, Jr., et al. Phase IIa clinical trial of curcumin for the prevention of colorectal neoplasia. Cancer Prev Res 2011;4(3):354–64.
14. Hanai H, Iida T, Takeuchi K, Watanabe F, Maruyama Y, Andoh A, Tsujikawa T, Fujiyama Y, Mitsuyama K, Sata M, et al. Curcumin maintenance therapy for ulcerative colitis: randomized, multicenter, double-blind, placebo-controlled trial. Clin Gastroenterol Hepatol 2006;4(12):1502–6.
15. Ueland PM, McCann A, Midttun O, Ulvik A. Inflammation, vitamin B6 and related pathways. Mol Aspects Med 2017;53:10–27.
16. Selhub J, Byun A, Liu Z, Mason JB, Bronson RT, Crott JW. Dietary vitamin B6 intake modulates colonic inflammation in the IL10-/- model of inflammatory bowel disease. J Nutr Biochem 2013;24(12):2138–43.
17. Larsson SC, Orsini N, Wolk A. Vitamin B6 and risk of colorectal cancer: a meta-analysis of prospective studies. JAMA 2010;303(11):1077–83.
18. DiMarco-Crook C, Xiao H. Diet-based strategies for cancer chemoprevention: the role of combination regimens using dietary bioactive components. Annu Rev Food Sci Technol 2015;6:505–26.
19. Xiao H, Yang CS. Combination regimen with statins and NSAIDs: a promising strategy for cancer chemoprevention. Int J Cancer 2008;123(5):983–90.
20. Wu X, Pfalzer AC, Koh GY, Tang S, Crott JW, Thomas MJ, Meydani M, Mason JB. Curcumin and salsalate suppresses colonic inflammation and procarcinogenic signaling in high-fat-fed, azoxymethane-treated mice. J Agric Food Chem 2017;65(33):7200–9.
21. Olivo-Marston SE, Hursting SD, Perkins SN, Schetter A, Khan M, Croce C, Harris CC, Lavigne J. Effects of calorie restriction and diet-induced obesity on murine colon carcinogenesis, growth and inflammatory factors, and microRNA expression. PLoS One 2014;9(4):e94765.
22. Nambiar PR, Girnun G, Lillo NA, Guda K, Whiteley HE, Rosenberg DW. Preliminary analysis of azoxymethane induced colon tumors in inbred mice commonly used as transgenic/knockout progenitors. Int J Oncol 2003;22(1):145–50.
23. Cranford TL, Enos RT, Velázquez KT, McClellan JL, Davis JM, Singh UP, Nagarkatti M, Nagarkatti PS, Robinson CM, Murphy EA. Role of MCP-1 on inflammatory processes and metabolic dysfunction following high-fat feedings in the FVB/N strain. Int J Obes 2016;40(5):844–51.
24. Freireich EJ, Gehan EA, Rall DP, Schmidt LH, Skipper HE. Quantitative comparison of toxicity of anticancer agents in mouse, rat, hamster, dog, monkey, and man. Cancer Chemother Rep 1966;50(4):219–44.
25. Reeves PG. Components of the AIN-93 diets as improvements in the AIN-76A diet. J Nutr 1997;127(5):838S–41S.
26. Ueland PM, Midttun O, Windelberg A, Svardal A, Skalevik R, Hustad S. Quantitative profiling of folate and one-carbon metabolism in large-scale epidemiological studies by mass spectrometry. Clin Chem Lab Med 2007;45(12):1737–45.
27. Midttun O, Hustad S, Ueland PM. Quantitative profiling of biomarkers related to B-vitamin status, tryptophan metabolism and inflammation in human plasma by liquid chromatography/tandem mass spectrometry. Rapid Commun Mass Spectrom 2009;23(9):1371–9.
28. Almeqdadi M, Mana MD, Roper J, Yilmaz ÖH. Gut organoids: mini-tissues in culture to study intestinal physiology and disease. Am J Physiol Cell Physiol 2019;317(3):C405–19.
29. Pleguezuelos-Manzano C, Puschhof J, van den Brink S, Geurts V, Beumer J, Clevers H. Establishment and culture of human intestinal organoids derived from adult stem cells. Curr Protoc Immunol 2020;130(1):e106.

30. Benjamini Y, Hochberg Y. Controlling the false discovery rate–a practical and powerful approach to multiple testing. J R Stat Soc Series B Stat Methodol 1995;57(1):289–300.
31. Laska EM, Meisner M, Siegel C. Simple designs and model-free tests for synergy. Biometrics 1994;50:834–41.
32. Poullis A, Foster R, Shetty A, Fagerhol MK, Mendall MA. Bowel inflammation as measured by fecal calprotectin: a link between lifestyle factors and colorectal cancer risk. Cancer Epidemiol Biomarkers Prev 2004;13(2):279–84.
33. Taketo MM. Shutting down *Wnt* signal-activated cancer. Nat Genet 2004;36(4):320–2.
34. Wu X, Tu X, Joeng KS, Hilton MJ, Williams DA, Long F. Rac1 activation controls nuclear localization of β-catenin during canonical *Wnt* signaling. Cell 2008;133(2):340–53.
35. Taniguchi K, Karin M, NF-κB, inflammation, immunity and cancer: coming of age. Nat Rev Immunol 2018;18(5):309–24.
36. Kulp SK, Yang Y-T, Hung C-C, Chen K-F, Lai J-P, Tseng P-H, Fowble JW, Ward PJ, Chen C-S. 3-Phosphoinositide-dependent protein kinase-1/Akt signaling represents a major cyclooxygenase-2-independent target for celecoxib in prostate cancer cells. Cancer Res 2004;64(4):1444–51.
37. Snider AJ, Kawamori T, Bradshaw SG, Orr KA, Gilkeson GS, Hannun YA, Obeid LM. A role for sphingosine kinase 1 in dextran sulfate sodium-induced colitis. FASEB J 2009;23(1):143–52.
38. Oeckinghaus A, Hayden MS, Ghosh S. Crosstalk in NF-kappaB signaling pathways. Nat Immunol 2011;12(8):695–708.
39. Pfalzer AC, Nesbeth P-DC, Parnell LD, Iyer LK, Liu Z, Kane AV, Chen CO, Tai AK, Bowman TA, Obin MS. Diet- and genetically-induced obesity differentially affect the fecal microbiome and metabolome in apc 1638N mice. PLoS One 2015;10(8):e0135758.
40. Liang J, Nagahashi M, Kim EY, Harikumar KB, Yamada A, Huang W-C, Hait NC, Allegood JC, Price MM, Avni D. Sphingosine-1-phosphate links persistent STAT3 activation, chronic intestinal inflammation, and development of colitis-associated cancer. Cancer Cell 2013;23(1):107–20.
41. Schwiebs A, San Juan MH, Schmidt KG, Wiercinska E, Anlauf M, Ottenlinger F, Thomas D, Elwakeel E, Weigert A, Farin HF. Cancer-induced inflammation and inflammation-induced cancer in colon: a role for S1P lyase. Oncogene 2019;38(24):4788–803.
42. Huhn M, Juan MHS, Melcher B, Dreis C, Schmidt KG, Schwiebs A, Collins J, Pfeilschifter JM, Vieth M, Stein J. Inflammation-induced mucosal KYNU expression identifies human ileal Crohn's disease. J Clin Med 2020;9(5):1360.
43. Polyzos KA, Ovchinnikova O, Berg M, Baumgartner R, Agardh H, Pirault J, Gisterå A, Assinger A, Laguna-Fernandez A, Bäck M. Inhibition of indoleamine 2, 3-dioxygenase promotes vascular inflammation and increases atherosclerosis in apoe−/− mice. Cardiovasc Res 2015;106(2):295–302.
44. Parrott J, Redus L, Santana-Coelho D, Morales J, Gao X, O'Connor J. Neurotoxic kynurenine metabolism is increased in the dorsal hippocampus and drives distinct depressive behaviors during inflammation. Transl Psychiatry 2016;6(10):e918.
45. Komatsu S-i, Watanabe H, Oka T, Tsuge H, Nii H, Kato N. Vitamin B-6–supplemented diets compared with a low vitamin B-6 diet suppress azoxymethane-induced colon tumorigenesis in mice by reducing cell proliferation. J Nutr 2001;131(8):2204–7.
46. Yakar S, Nunez NP, Pennisi P, Brodt P, Sun H, Fallavollita L, Zhao H, Scavo L, Novosyadlyy R, Kurshan N. Increased tumor growth in mice with diet-induced obesity: impact of ovarian hormones. Endocrinology 2006;147(12):5826–34.
47. Lee K, Kwak J-H, Pyo S. Inhibition of LPS-induced inflammatory mediators by 3-hydroxyanthranilic acid in macrophages through suppression of PI3K/NF-κB signaling pathways. Food Funct 2016;7(7):3073–82.
48. Grant RS, Coggan SE, Smythe GA. The physiological action of picolinic acid in the human brain. Int J Tryptophan Res 2009;2:71–9.
49. Lučić I, Rathinaswamy MK, Truebestein L, Hamelin DJ, Burke JE, Leonard TA. Conformational sampling of membranes by Akt controls its activation and inactivation. Proc Natl Acad Sci 2018;115(17):E3940–9.
50. Lien EC, Dibble CC, Toker A. PI3K signaling in cancer: beyond AKT. Curr Opin Cell Biol 2017;45:62–71.
51. Nelson KM, Dahlin JL, Bisson J, Graham J, Pauli GF, Walters MA. The essential medicinal chemistry of curcumin: miniperspective. J Med Chem 2017;60(5):1620–37.