

Low Dietary Calcium Levels Modulate Mucosal Caspase Expression and Increase Disease Activity in Mice with Dextran Sulfate Sodium-Induced Colitis¹⁻³

Laetitia C. Pele,^{4,5} Vinay Thoree,⁵ Feras Mustafa,⁵ Shijun He,⁵ Loukia Tsaprouni,⁶ Neville A. Punchard,⁶ Richard P. H. Thompson,⁵ Stephen M. Evans,⁵ and Jonathan J. Powell^{4*}

⁴MRC Human Nutrition Research, Elsie Widdowson Laboratory, Cambridge CB1 9NL, UK; ⁵Gastrointestinal Laboratory, The Rayne Institute, St. Thomas' Hospital, London SE1 7EH, UK; and ⁶Division of Science, University of Luton, Luton LU1 3JU, UK

Abstract

Dietary calcium (Ca) positively modulates the susceptibility to colon cancer, but its effects on related or earlier colonic pathologies, such as inflammation and mucosal dysregulation, are poorly understood. We tested the effects of differing dietary Ca levels on acute dextran sulfate sodium (DSS)-induced colitis in mice. BALB/c mice received a normal Ca (NCa) diet (0.5% Ca), a high Ca (HCa) diet (1.5% Ca), a low Ca (LCa) diet (0.05% Ca), or a very low Ca (VLCa) diet (0.009% Ca) for 3 wk. Mucosal caspases 1, 3, and 9 were assessed by Western blotting, and the histological crypt score was assessed by microscopy. Half of the mice in each group received DSS (1.5%) for 20 d in their drinking water, and disease activity was assessed. Increasing or lowering dietary Ca increased mucosal caspases ($P < 0.0001$ vs. NCa). Crypt scores increased with decreasing dietary Ca levels ($P < 0.0001$, $r = -0.675$), indicating that elevated caspases in LCa groups reflected early subclinical inflammation. DSS-induced disease activity was higher in mice fed low dietary Ca levels [$P < 0.0001$, VLCa and DSS vs. NCa and DSS (NCaDSS) and $P < 0.005$, LCa and DSS vs. NCaDSS], and mice from the VLCa group were moribund within 11 d of DSS administration. Those in the HCa group did not differ greatly from controls. Together, these data indicate that Ca protects against DSS-induced colitis in mice. The mechanisms are unclear, but the calcium-sensing receptor and/or luminal precipitates of calcium phosphate microparticles may be involved. Whether these observations can be extended to patients with colitis or infectious diarrhea deserves consideration. J. Nutr. 137: 2475–2480, 2007.

Introduction

Disorders of the gastrointestinal tract are major contributors to ill health in any population. In underdeveloped countries, infectious disorders predominate; whereas in developed countries, idiopathic inflammation, cancers, and functional bowel disorders are more common. A series of rodent models and human epidemiological studies have demonstrated that changes to dietary calcium (Ca)⁷ intakes can influence susceptibility to colonic cancer or precancerous markers (1–7). High Ca (HCa)

intakes are protective, whereas low Ca (LCa) intakes increase susceptibility. Ca may also confer protection against intestinal infection and its clinical symptoms (8–10), although the nature of the relationship with dietary Ca is not well understood.

Increasing dietary Ca leads to increased levels of luminal Ca, chiefly due to absorption in the duodenum and/or proximal jejunum and resecretion in the mid- to distal intestine (11). Nonetheless, within the colonic lumen, Ca levels vary greatly, mainly as a result of absorption and resecretion processes for water, Ca, and phosphate ions. Ca and phosphate partially coprecipitate in the intestinal lumen, leading to the formation of calcium phosphate particles that typically are submicrometer or micrometer sized and that we have termed as “endogenous microparticles” (12). The luminal activities of both the soluble and insoluble fractions of Ca have been invoked in mucosal protection. Ca binding of cytotoxic luminal components (6,13–16) and/or the regulation of epithelial or mononuclear cell signaling (1–4,17,18) have all been suggested.

There has been increasing recognition that chronic inflammation often underlies tumorigenesis. In the colon, chronic inflammatory bowel disease is a significant risk factor for colonic cancer, and recent work has linked inflammation to tumorigenesis

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³ Supplemental Tables 1 and 2 and Supplemental Figures 1–3 are available with the online posting of this paper at jn.nutrition.org.

⁷ Abbreviations used: Ca, calcium; CMF-HBSS, calcium-free and magnesium-free HBSS; CaR, calcium-sensing receptors; DSS, dextran sulfate sodium; ECL, enhanced chemiluminescent; HCa, high calcium; LCa, low calcium; LCaDSS, low calcium and DSS; LPMNC, lamina propria mononuclear cells; NCa, normal calcium; NCaDSS, normal calcium and DSS; TCM, tissue culture medium; VLCa, very low calcium; VLCaDSS, very low calcium and DSS.

* To whom correspondence should be addressed. E-mail: jonathan.powell@mrc-hnr.cam.ac.uk.

at the molecular level. Inhibition of nuclear factor κ B (NF κ B) signaling, through deletion of IKK β , led to reduced colonic tumor formation in a mouse model of colitis-associated cancer (19). Specific inactivation of this pathway, in epithelial and/or myeloid cells, attenuated the formation of inflammation-associated tumors, indicating a conduit for myeloid cell signaling in epithelial cell responsiveness.

Despite the protective role of dietary Ca in colon cancer, there are few data on the influence of Ca on the severity of colitis. Recently, Zhu et al. (20) have shown that increased dietary Ca can protect against intestinal weight loss and peripheral TNF α production in IL-10 knockout mice. Neither the influence of low levels of dietary Ca on colitis nor the relationship between differing dietary levels and disease activity have been reported. Here we tested the effects of differing dietary Ca levels on acute dextran sulfate sodium (DSS)-induced colitis in mice.

Materials and Methods

Animals

Female BALB/c mice (age 5 wk), a strain known to be susceptible to oral DSS, were purchased from Harlan-Teklad (Shives Farm) and acclimatized for 2 wk. They were housed at 3 mice per cage, maintained under standard conditions (12 h light/dark cycle), and had free access to a nonpurified diet (Harlan-Teklad) and water prior to the beginning of the study. On d 1 of the study, mice were switched to one of the experimental diets. The study protocol was approved by the UK Home Office, project license PPL 70/433.

Calcium diets

Mice were randomly divided into 4 groups and were fed very low Ca (VLCa), low Ca (LCa), normal Ca (NCa), and high Ca (HCa) diets (Harlan-Teklad). Each group had 12 mice, housed at 3 mice per cage (i.e., 4 cages/group). Ca diets were from Harlan-Teklad. The baseline diet (i.e., VLCa) was composed of 16% protein and 4.4% fat (Table 1) and had a natural molar calcium:phosphate ratio of 0.3:1.

Thereafter, Ca was added in the form of dibasic calcium phosphate (CaHPO $_4$ ·2H $_2$ O) and calcium carbonate (CaCO $_3$) to maintain a calcium:phosphate ratio of 1.3:1, which is consistent with the normal ratios of these elements in a standard rodent nonpurified diet. Final Ca levels of the diets were 0.009% (very low), 0.05% (low), 0.5% (normal), and 1.5% (high). The unique identity codes for these diets are TD 96349, TD 01237, TD 01238, and TD 01239, respectively (Harlan-Teklad).

TABLE 1 Composition of the standard diet

Constituent	Diet, g/kg
Lactalbumin	180.00
Dextrose monohydrate	740.22
Coconut oil	30.00
Corn oil	10.00
Ethoxyquin (antioxidant)	0.01
Mineral mix ¹	13.37
Vitamin mix ²	10.00

¹ Supplying the following (mg/kg of diet): sodium chloride, 259.02; potassium citrate monohydrate, 770.06; potassium sulfate, 182.01; magnesium oxide, 84.00; manganese carbonate, 12.25; ferric citrate, 21.00; zinc carbonate, 5.60; cupric carbonate, 1.05; potassium iodate, 0.035; sodium selenite, 0.035; and chromium potassium sulfate, 1.92.

² Containing (mg/kg of diet): p-Aminobenzoic acid, 110.01; ascorbic acid, coated (97.5%), 1016.6; biotin, 0.44; vitamin B-12, 29.70; calcium pantothenate, 66.10; choline dihydrogen citrate, 3496.90; folic acid, 1.98; inositol, 110.10; menadione, 49.50; niacin, 99.10; pyridoxine HCl, 22.00; riboflavin, 22.00; thiamine HCl, 22.00; dry retinyl palmitate, 39.65; dry cholecalciferol, 4.40; dry vitamin E acetate, 242.30; and corn starch diluent, 4666.90.

Induction of colitis

Mice consumed their diets and ultra-high-purity water ad libitum for the duration of the study (3 wk, unless terminated; see below). On d 3, half of the mice in each group (i.e., 2 cages or $n = 6$ per group) received 1.5% DSS (wt:v) (ICN Pharmaceuticals) in their drinking water for the duration of the study. A prior pilot experiment indicated that this dose induced mild disease in mice fed normal dietary Ca levels (data not shown). Based on the pilot study, DSS-challenged mice were killed by cervical spine dislocation at d 20–21, as were corresponding controls from d 21, thus ensuring appropriate time to collect and process tissues. However, in the VLCa and DSS (VLCaDSS) group, the mice became moribund at d 11 and, under the animal licenses act, had to be killed; their respective control group had to be killed on d 12. For the same reasons, 2 mice from the LCa and DSS (LCaDSS) group had to be killed at d 18 with 2 respective controls killed at d 19.

Intestinal calcium levels

Immediately following death, intestinal contents were collected from the small intestine by gentle massage and then diluted to 10 mL with 1% HNO $_3$ (v:v, Fluka Puriss) at 40°C to ensure dissolution of Ca. The samples were analyzed for Ca content by inductively coupled plasma optical emission spectrometry with a v-groove nebulizer and a Scott-type double-pass spray chamber (21). Integration times were 3 s/increment. Ca was detected using a wavelength of 317.933 nm, and Ca concentrations were extrapolated from sample-based standard curves (21).

Assessment of inflammation

Inflammation was assessed in 4 ways, as previously reported for DSS-induced colitis (22,23): namely, clinical disease activity index, colon length, histological inflammation (crypt score), and IL-1 β secretion.

Clinical disease activity index. The clinical disease activity index was assessed using the method described by Cooper et al. (22; Supplemental Table 1) for each mouse, 2 times every 48 h. Because 2 mice from the LCaDSS group were terminated at d 18, their data were extended according to the extended last value principle (24).

Colon length. At necropsy, the colon length of each mouse was measured and recorded as a further indicator of disease severity (23).

Crypt score. Following cleansing with ice-cold PBS, colon segments (Supplemental Fig. 1) taken from each mouse were fixed in formalin and embedded in paraffin. All slides were stained with hematoxylin and eosin, and crypt scores were assessed according to Cooper et al. [(22); Supplemental Table 2].

Although crypt scores are commonly used to assess the severity of colitis, this measure was also used for mice not receiving DSS, as an early marker of histological inflammation (epithelial colonic architecture and/or integrity is progressively lost with inflammation); thus enabling us to address whether differing dietary Ca levels influence intestinal homeostasis. Crypt scores for each mouse were calculated as the grade for appearance \times the grade for percentage involvement.

IL-1 β secretion. Lamina propria mononuclear cells (LPMNC) were isolated from the cecum and rectum (Supplemental Fig. 1) using the modified protocol of Bull and Bookman (25). Briefly, fresh cecal and rectal samples (0.5 cm in length) were opened up and washed 3 times in 10 mL calcium-free and magnesium-free HBSS (CMF-HBSS; GibcoBRL, Life Technologies). The mucus layer was removed by incubation in CMF-HBSS containing 1 mmol/L dithiothreitol (Sigma) for 15 min at room temperature with gentle end-end mixing on a rotary mixer. After 3 more washes in CMF-HBSS, cecal and rectal samples were incubated in CMF-HBSS containing 1 mmol/L EDTA (Sigma) for 50 min at 37°C in 5% CO $_2$:95% air, and were mixed every 15 min to remove the epithelial cell layer. The samples were then washed 3 times in CMF-HBSS to remove traces of EDTA and teased apart using hypodermic needles. Subsequently, the specimens were incubated for 2–4 h at 37°C in 5% CO $_2$:95% air in tissue culture medium (TCM) [RPMI 1640 (GibcoBRL) supplemented with 10 kU/L penicillin, 100 mg/L streptomycin, 2 mmol/L

L-Glutamine, 40 mg/L gentamicin, and 10% (v:v) fetal calf serum containing 1 g/L collagenase type I (all from Sigma)]. Cells were thoroughly mixed at 20-min intervals for 1–2 min. When the tissues were totally broken down, the samples were washed and centrifuged 2 times in TCM at $400 \times g$ for 10 min at room temperature. Following washing, LPMNC were stained with 0.4% trypan blue (Sigma) and counted on a hemocytometer with light microscopy. Finally, cells were resuspended in TCM at a concentration of 1 million cells/mL.

Suspensions of 1 mL of cecal or rectal LPMNC were cultured in 5 mL polystyrene round bottom tubes (Marathon Laboratory Supplies) and incubated for 24 h at 37°C in 5% CO₂:95% air. Following incubation, cells were centrifuged at $400 \times g$ at 4°C, and the supernatants were collected and stored at –70°C until further analysis was performed.

ELISA, specific for mouse IL-1 β , as well as the necessary reagents were purchased from R&D systems and were conducted according to the manufacturer's protocol.

Mucosal caspase expression

At necropsy, a sample (0.5 cm) of the mid-distal colon (Supplemental Fig. 1) was immediately collected from each mouse in the control groups (i.e., without DSS) and was analyzed for caspase expression by Western blot. Collected tissues were dissected and placed in a petri dish with 100 μ L HBSS and cut into small pieces using forceps and scissors. After the addition of another 400 μ L HBSS, the tissue pieces were transferred into an Eppendorf tube along with 50 μ L of trypsin-EDTA. The samples were left to incubate for 10 min at 37°C and 50 μ L fetal calf serum was added per tube. Following centrifugation at $400 \times g$ for 5 min at 4°C, supernatants were discarded and pellets were homogenized with 400 μ L lysis buffer, using a manual homogenizer. Finally, tissues were allowed to stand for 10 min on ice and then centrifuged at $800 \times g$ for 5 min at 4°C. The supernatant containing the total tissue protein was collected and the protein measured by the Bradford assay.

To provide a sufficient sample for triplicate analysis, supernatants from each group were pooled in pairs (i.e., providing 3 samples, each pooled from 2 mice and analyzed in triplicate, per group). Samples, loaded as 30 μ g of protein per well, were separated by SDS-PAGE (with a 12% resolving gel) and then transferred onto an enhanced chemiluminescent (ECL) membrane by electroblotting as previously described (26). The membranes were stained with Ponceau-S solution until bands appeared and then washed with deionized water. The membranes were blocked using a blocking buffer for 20 min and then incubated overnight with 1:800 (v:v) dilutions in a blocking buffer of appropriate primary antibodies (Santa Cruz Biotechnology) to caspase 1 (goat polyclonal), caspase 3 (rabbit polyclonal), and caspase 9 (rabbit polyclonal). Finally, β -actin was measured to ensure equal protein loading, and the final

results were corrected for any minor variations in loading. The following day, membranes were washed for 5 min in PBS-Tween 20 for a total of 4 times and incubated for 2 h with the appropriate secondary antibody (Dako). For caspase 1, this was mouse anti-goat horseradish peroxidase conjugate, whereas for both caspase 3 and 9, goat anti-rabbit horseradish peroxidase conjugate was used (all at a dilution of 1:4000 (v:v) in a blocking buffer). Finally, following another 4 cycles of washing with PBS-Tween 20, the ECL membranes were incubated with ECL substrate for 1 min in darkness, followed immediately by exposure of the membranes to a blue light sensitive film (X-ograph). After development of the films using standard techniques, they were analyzed by densitometry (GelPro, Mediacybernetics).

Other histological analyses

DSS is a toxin that mainly affects the distal colon where it disrupts epithelial integrity, leading to increased permeability and thus bacterial access to the underlying mucosa. Indeed, DSS itself is taken up by subepithelial macrophages (23). To ensure that changes in dietary Ca did not simply affect DSS exposure to the colon (and hence the degree of colitis), we assessed the frequency of DSS-positive macrophages in the different groups. Paraffin-embedded colonic sections (3- μ m thick) were de-waxed and rinsed in distilled water. DSS staining was carried out with 0.5% toluidine blue (BDH) through graded alcohol solutions (95, 70, and 50%; 20 min each) followed by 2 min in 0.5% aqueous toluidine. After a final wash in 70% alcohol, slides were dehydrated in 95% alcohol and cleared in 100% xylene. DSS-positive macrophages were assessed and counted per 3 graticules under light microscopy, as previously reported (22).

Statistical analysis

All data are means \pm SEM. Statistical analyses were conducted with Stata statistical software (version 9, StataCorp LP) and Graphpad software (version 4).

Because caspase expression was only measured in healthy untreated mice (i.e., without DSS), differences between NCa and the other Ca groups were assessed using 1-way ANOVA. Kaplan-Maier survival curves were used to determine differences in survival. Differences were considered significant at $P < 0.05$. Repeated-measurements ANOVA was carried out to analyze the disease activity index and a resulting Dunnett's test performed to determine differences between NCa and the other Ca groups. Differences were considered significant at $P < 0.016$.

Luminal Ca content and IL-1 β data were transformed logarithmically (\log_e) to achieve normality. Interactions between Ca and DSS (as well as Ca or DSS main effects) for luminal Ca contents, colon length,

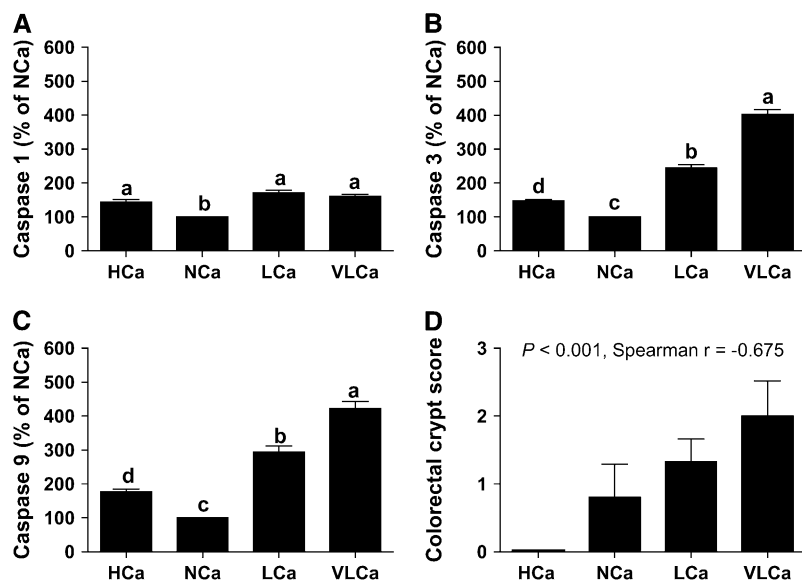


FIGURE 1 Colonic caspase expression (A–C) and crypt scores (D) in the distal colon of untreated mice fed VLCa, LCa, NCa, and HCa diets for up to 3 wk. Values are means \pm SEM ($n = 6$, except group NCa, $n = 5$). Means without a common letter differ, $P < 0.0001$.

crypt scores, and IL-1 β were assessed using 2-way ANOVA. Interactions and main effects were considered significant at $P < 0.05$. Additionally, associations between levels of dietary Ca and measured outcomes were evaluated using Spearman rank correlation coefficient.

Results

Effect of dietary Ca in healthy mice. An increase in dietary Ca has been associated with increased levels of apoptosis in colonic epithelial cells. Here, as a measure of cell death and in relation to dietary Ca content only, we measured colonic caspase expression. We showed that caspase 1, 3, and 9 were all increased in mice fed an HCa or L/VLCa diet compared with those from the NCa group (Fig. 1A–C; $P < 0.0001$ vs. NCa). Furthermore, an overall strong effect of Ca was demonstrated on distal colonic crypt score ($P = 0.0008$) with an inverse relationship (Fig. 1D; $r = -0.675$, $P < 0.0001$), suggesting a caspase-related protective effect in the HCa group and a caspase-detrimental effect, as a consequence of microscopic induction of inflammation, in the LCa and VLCa groups.

Effect of Ca in mice with DSS-induced colitis. Upon colitis induction, there was no evidence for interactions between treatment groups (i.e., Ca and Ca and DSS) in relation to colon length (Fig. 2C) and to luminal Ca content (Supplemental Figs. 2 and 3A). Similarly, dietary Ca did not inhibit tissue uptake of DSS (Supplemental Fig. 3B). However, as expected, a strong Ca effect alone was demonstrated ($P < 0.0001$), showing a positive association between dietary Ca levels and Ca luminal contents ($r = 0.699$, $P < 0.0001$; Supplemental Fig. 2).

Disease activity rapidly increased in mice fed low dietary Ca levels [Fig. 2A; $P < 0.0001$ VLCaDSS vs. NCa and DSS (NCaDSS) (d 0–11) and $P < 0.0001$ LCaDSS vs. NCaDSS (d 0–20), respectively], such that the VLCaDSS group became moribund by d 11 and was killed (Fig. 2B). Two mice from the LCaDSS group were killed on d 18, but the others survived to d 20, albeit with a high disease activity (Fig. 2A). Kaplan-Maier survival curves confirmed a significant effect of dietary Ca on mouse survival (Fig. 2B; $P < 0.001$).

There was no evidence for interactions between treatment groups in the mid- (Fig. 3A) and distal (Fig. 3B) colonic crypt scores, but a significant effect of DSS alone was observed ($P < 0.0001$). Additionally, there were no differences in IL-1 β secretion between treatment groups (Fig. 3D), although an interaction in cecal IL-1 β (Fig. 3C; $P = 0.046$) was observed between the Ca and Ca and DSS groups. Very low dietary Ca levels led to a significant decrease in cecal IL-1 β production (Fig. 3D; $P < 0.01$ VLCaDSS vs. NCaDSS).

Discussion

Protection against colonic cancer by increased dietary Ca has been shown in a number of animal models (2–6) and human epidemiological studies (7,14,15). Chronic colitis is a marked risk factor for the induction of precancerous lesions and the development of colonic cancer. It is increasingly recognized that inflammation commonly underlies tumorigenesis; as noted earlier; for example, nuclear factor- κ B activation has been shown to link inflammation and tumorigenesis in the colon (19). Here, we sought to investigate whether dietary Ca would protect against colonic inflammation as it does against colonic cancer.

Previous studies have indicated that dietary Ca promotes epithelial cell apoptosis in the distal colon (2,5,6), consistent with

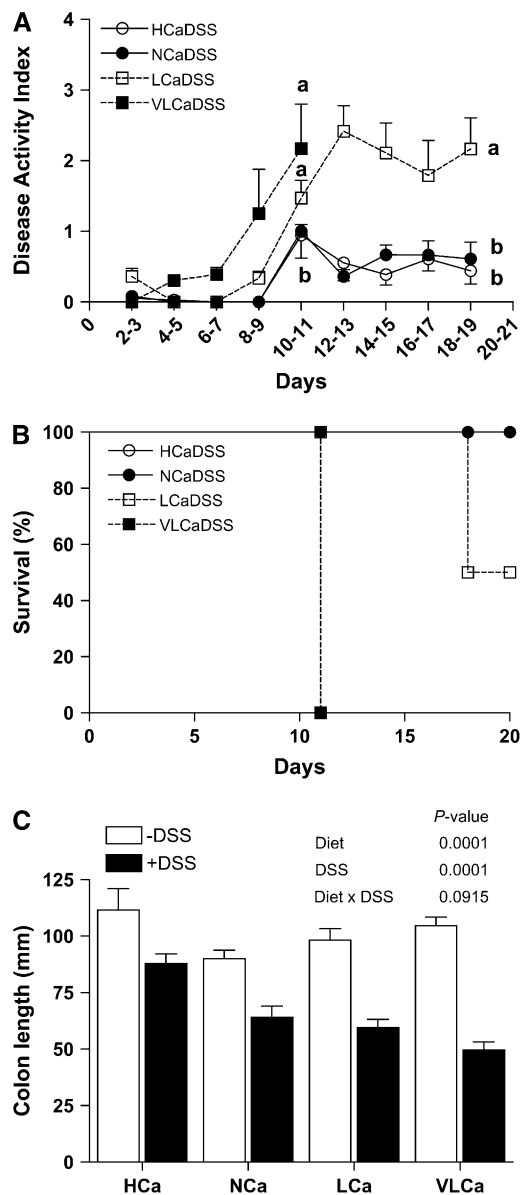


FIGURE 2 Disease activity index (A) and survival (B) in mice with DSS-induced colitis, and colon length (C) in mice with and without DSS-induced colitis fed VLCa, LCa, NCa, and HCa diets for up to 3 wk. Values are means \pm SEM ($n = 6$). In A, means without a common letter differ, $P < 0.005$.

our results from the colon that showed increases in apoptosis-inducing and executioner caspases, namely active caspase 9 and 3, respectively, in response to an HCa diet (Fig. 1A–C). Moreover, this seemed associated with a reduced crypt score even in the otherwise normal colon (Fig. 1D). The mechanisms are unclear, but there may be a general, nonspecific increase in caspase activity because the pro-IL1 β cleaving caspase, namely caspase 1, was also upregulated by increased dietary calcium (Fig. 1A–C).

Lowering the Ca levels of the diet to 1/10 or 1/50 of that of the control diet also led to marked increases in colonic caspase expression (Fig. 1A–C). However, on these occasions, caspase expression seemed to be associated with epithelial damage (Fig. 1D) and, presumably therefore, pathological increases in cell turnover (6,27). Taken together, these data extend previous

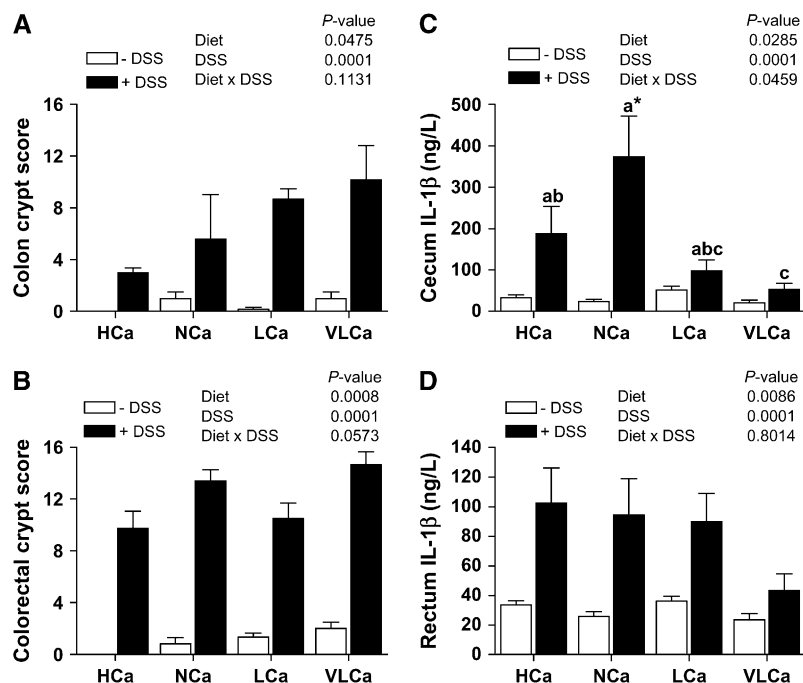


FIGURE 3 Crypt scores (A–B) and IL-1 β secretion (C–D) in mice with and without DSS-induced colitis fed VLCa, LCa, NCa, and HCa diets for up to 3 wk. Values are means \pm SEM ($n = 3$ –6). In C, means without a common letter differ, $P < 0.05$. *Different from the corresponding –DSS group, $P < 0.001$.

findings that dietary Ca may modulate mucosal apoptosis of the distal large intestine that, at high Ca levels, protects against constitutive epithelial cell damage.

To determine whether these observations could be extended to an induced-colitis model, we challenged half the mice in each group with a suboptimal, disease-inducing dose (1.5%) of DSS. DSS is a macrophage toxin and its lysosomal uptake can be observed in swollen macrophages of the colonic mucosa (23). Indeed the acute colitis model, as studied here, is a macrophage-dependent disease that even occurs in severe combined immunodeficiency mice, indicating that B and T cells are not required (28). Exact mechanisms are unclear, but Cooper et al. (22) demonstrated that the earliest lesion is the progressive loss and dropout of crypts that could be due to a direct toxic effect of DSS (28) or secondary to abnormal macrophage signaling, which can influence epithelial cell function (19). Thus, altered permeability may be coupled with exaggerated macrophage responses to the commensal flora (29). Following disruption of crypt architecture, acute DSS colitis is characterized by the production of proinflammatory cytokines [especially IL-1 β (30)], clinical disease (blood loss, diarrhea, and weight loss), and shortening of the colon. Distal disease is more prominent than proximal disease. In this study, mice from the NCa group responded as expected to low-dose DSS (Figs. 2, 3). However, mice fed LCa diets showed pronounced sensitivity to DSS (Fig. 2A–B). This was not a result of DSS perturbation of luminal Ca levels (Supplemental Fig. 3A) nor because changes to dietary Ca affected mucosal DSS uptake (Supplemental Fig. 3B). Moreover, in the most distal colon (colorectal region), DSS-induced disruption of crypt architecture and mononuclear cell production of IL-1 β was similar in all groups, with no obvious protection afforded by Ca. The benefit of dietary Ca thus appeared to be in limiting further DSS-induced disease, as shown by the clinical disease activity index (Fig. 2A). Surprisingly, in mice receiving DSS, IL-1 β production from mucosal mononuclear cells of the proximal colon was lower in the very low dietary Ca group (Fig. 3C). This may simply reflect negative feedback, such that IL-1 β secretion is minimized in very severe colitis, as was shown by Egger et al.

(28) when comparing differing doses of DSS. Alternatively, we have previously shown (31) that IL-1 β secretion may be induced when calcium phosphate is taken up into intestinal mononuclear cells. It is possible that early IL-1 β secretion is required in innate immune responses to limit further inflammation (29,32). Certainly, Rakoff-Nahoum et al. (27) have shown that intestinal epithelial cell Toll-like receptor signaling promotes constitutive secretion of proinflammatory cytokines and protects the mucosa from injury. Future work should address these possibilities and effects on other inflammatory cytokines, such as IL-6 and TNF α .

The mechanism of action of dietary Ca in influencing colonic health is unclear. However, 3 possibilities have been proposed and these are not mutually exclusive. First, the binding of Ca to biliary and fatty acids in the intestinal lumen limits their solubility and, therefore, their damaging effects on the colonic mucosa. Supplemental Ca has been shown to alter the ratio of fecal bile acids and to lower the concentration of water-soluble bile acids, thus reducing the cytotoxicity of fecal water (13,15,16). Binding of bile acids to luminal precipitates of calcium phosphate has been especially noted (16). Both Ca and phosphate are secreted into the mid-distal intestinal lumen and coprecipitate forming calcium phosphate particles (11). While calcium phosphate may bind and “detoxify” components of the luminal stream, we have proposed a second potential protective mechanism, namely, constitutive antigen transfection (12). In brief, calcium phosphate particles could bind luminal antigens and carry them into mucosal mononuclear cells as a mechanism of immunosurveillance and promotion of tolerance. Cross talk between intestinal mononuclear cells and epithelial cells is increasingly recognized (19), so low dietary Ca levels could exacerbate the break in tolerance and induction of inflammation that is precipitated by DSS.

Thirdly, a major recent focus has been on the role of calcium-sensing receptors (CaR) in modulating epithelial responses, including in the gastrointestinal tract (1,17,18). CaR are G protein-coupled receptors that respond to extracellular calcium levels, especially over the range of 0.05–5 mmol/L, and are expressed throughout the mammalian gastrointestinal tract,

including the apical and basolateral membranes of colonocytes (17,18). CaR activate diverse intracellular signaling pathways, including those involved in proliferation and apoptosis (1). Based on the contribution of CaR to cell differentiation and the finding that CaR expression correlates with the stage of differentiation of colonic tumors, Hebert et al. have proposed this as a primary mechanism that links dietary Ca and colonic cancer and perhaps also dietary Ca and colitis (17,33).

Several groups, including ours, continue to investigate the relationship between luminal Ca and intestinal health, and although mechanisms are currently not clear, the finding that dietary Ca can protect against colitis is important. Infectious colitis and ulcerative colitis are not uncommon in the Western world, whereas infectious colitis and diarrhea are especially prevalent in developing countries. Ca is a cheap and apparently safe dietary supplement; therefore, clinical trials are warranted to determine whether this has a role in the prevention or reduction of the incidence of colitis.

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