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ORIGINAL RESEARCH

Biotin Supplementation Ameliorates Murine Colitis by Preventing NF-κB Activation

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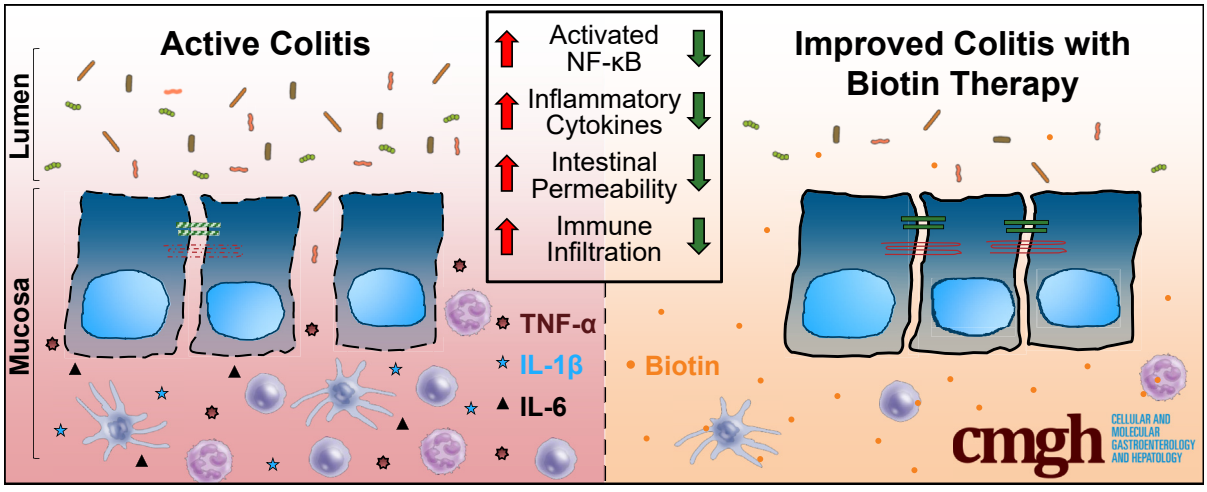

SUMMARY

Biotin deficiency causes an inflammatory bowel disease–like state, but biotin supplementation has never been tested as a therapeutic in established models for inflammatory bowel disease. We found that biotin therapy leads to delayed onset and severity of dextran sodium sulfate colitis mediated by decreased activation of nuclear factor-κB.

BACKGROUND & AIMS: Biotin is a water-soluble vitamin that is indispensable for human health. Biotin deficiency can cause failure-to-thrive, immunodeficiency, alopecia, dermatitis, and conjunctivitis. We previously reported that biotin deficiency also can lead to severe colitis in mice, which is completely reversed with supplementation. Our aim in this study was to determine if high-dose biotin supplementation can provide a therapeutic benefit in a preclinical model for inflammatory bowel disease (IBD) and to identify the molecular mechanism by which this occurs.

METHODS: Mice were challenged with dextran sodium sulfate to induce colitis and were treated with 1 mmol/L biotin to induce or maintain remission. Clinical response was monitored by the Disease Activity Index and fecal calprotectin levels. The colon tissue was investigated for histology, length, as well as expression of inflammatory cytokines (interleukin 6, tumor necrosis factor-α, interleukin 1β), intestinal permeability, tight junctions (zonula occludens-1 and claudin-2), and the transcription factor nuclear factor-κB (NF-κB).

RESULTS: Biotin therapy led to delayed onset and severity of colitis as well as accelerated healing. There was improvement in the Disease Activity Index, fecal calprotectin levels, colon length, and histology. In addition, biotin-treated mice had reduced expression of inflammatory cytokines, reduced intestinal permeability, and reduced activation of NF-κB.

CONCLUSIONS: Oral supplementation with biotin provides benefit for maintenance and induction of remission in the dextran sodium sulfate preclinical model for IBD. Biotin does this by reducing the activation of NF-κB, which prevents the production of inflammatory cytokines and helps maintain the integrity of the intestinal barrier. Clinically, the NF-κB pathway is important in the development of IBD and this finding suggests that biotin may have therapeutic potential for patients with IBD. *(Cell Mol Gastroenterol Hepatol 2020;9:557–567; https://doi.org/10.1016/j.jcmgh.2019.11.011)*

Keywords: Biotin; Inflammatory Bowel Disease; Colitis; Therapeutics.

Biotin is a water-soluble vitamin and an essential micronutrient that must be obtained from exogenous sources such as dairy, liver, egg yolk, and vegetables, or from commensal bacteria.[1] Its classic role is as a covalently bound coenzyme for cytoplasmic carboxylases used in fatty acid homeostasis, gluconeogenesis, and other metabolic pathways,[2] but emerging data also have identified a role in cellular stress response,[3] gene regulation,[4,5] and immune responses.[6–10] Clinically, biotin deficiency, whether induced by dietary means or by mutation of the intestinal biotin uptake system (ie, sodium-dependent multivitamin transporter [SMVT], *Slc5a6*), is associated with failure to thrive, microcephaly, osteoporosis, immunodeficiency, alopecia, dermatitis, and conjunctivitis.[11,12] In our previous studies to determine the relative contribution of SMVT toward total carrier-mediated biotin absorption, we made the serendipitous finding that SMVT knockout mice also develop colonic crypt abscesses, neutrophil infiltration, submucosal edema, and dysplastic changes.[13] In the current report, we extend our findings on the effect of biotin deficiency and show that dietary-induced biotin deficiency leads to an increase in the inflammatory marker fecal calprotectin, which is used routinely to monitor the progression of patients with inflammatory bowel disease (IBD).[14] Importantly, we show that these mice fully recover from their IBD-like state with biotin supplementation.

Our goal in this study was to determine if biotin supplementation also could be used as treatment in a preclinical model for IBD. For this, we examined the most widely used experimental model for IBD, the dextran sodium sulfate (DSS) colitis model.[15,16] Mice were challenged with DSS in drinking water to induce colitis; after 7 days they had increased weight loss, Disease Activity Index (DAI) scores, calprotectin, proinflammatory cytokines, intestinal permeability, and histologic scores, as expected. In addition, they also had decreased levels of the biotin transporter SMVT. The clinical relevance of this finding was confirmed when we identified that there also was a decrease of SMVT in biopsy specimens from patients with active ulcerative colitis (UC). Next, we used the DSS model to examine the ability of biotin supplementation to ameliorate signs of colitis and found that all parameters measuring the development of colitis trended toward baseline with biotin therapy. Finally, we identified the mechanism by which biotin exerts its therapeutic effect by showing decreased levels of active phosphorylated nuclear factor-κB (NF-κB) in biotin-treated mice. NF-κB is a pleiotropic transcription factor that drives expression of prosurvival genes in intestinal epithelial cells, and it also coordinates expression of proinflammatory genes from cells of the innate and adaptive immune system.[17,18] Increased inflammation promotes epithelial permeability, which exacerbates colitis. Indeed, NF-κB is a pivotal regulator in the development of IBD.[19,20]

In recent years, several new medications have been approved, and many more are under development, for the treatment of UC and Crohn's disease.[21–25] For each medication, there is a risk-to-benefit evaluation to consider and, depending on the side-effect profile, patients will need to be monitored for adverse events.[26] We propose that biotin would be an ideal medication for IBD therapy because it can be delivered orally and has minimal risk for complications. Notably, biotin deficiency often is overlooked in the setting of IBD and there have been several reports of biotin deficiency in patients with IBD.[27–31] The data from mice treated with biotin to prevent or treat colitis suggest that biotin therapy may provide benefit to patients with IBD.

Results

The Dietary Model for Biotin Deficiency Induces an IBD-Like State, Which Is Prevented With Biotin Supplementation

In the first series of experiments, we examined dietary biotin deficiency to further characterize the phenotype that develops. Mice received a biotin-deficient diet and showed the first signs of disease after 7 weeks when they began to develop alopecia and weight loss. As time progressed, they had decreased activity levels, hunched posture, poor skin turgor, and their stools became soft and bloody. This was prevented with 1 mmol/L biotin supplementation added to their drinking water (Figure 1*A* and *B*). We further tested stool samples at week 14 for fecal calprotectin, a noninvasive biomarker for inflammation in the gastrointestinal (GI) tract, and found that it was increased in mice with biotin deficiency (Figure 1*C*). Inflammation was most pronounced in the cecum of mice with biotin deficiency and histology showed loss of goblet cells, altered crypt architecture, infiltration of lymphocytes and neutrophils, with expansion of the lamina propria. Mice treated with biotin supplementation had normal histology without colitis (Figure 1*D*). Although no mouse model entirely recapitulates patients with IBD, this model reproduces many of the findings including weight loss, bloody diarrhea, increased fecal calprotectin, altered crypt architecture, and infiltration of neutrophils and lymphocytes to the mucosa and submucosa.

The Biotin Transport Pathway Is a Clinically Relevant Target for IBD Treatment

Because biotin therapy was able to reverse the IBD-like features described earlier, the next series of experiments were designed to validate if the biotin transport pathway would be a relevant drug target in IBD. To address a role for biotin in the treatment of colitis, we induced severe disease in mice using 3% DSS in drinking water. The mice in this

Abbreviations used in this paper: **DAI, Disease Activity Index; DSS, dextran sodium sulfate; ELISA, enzyme-linked immunosorbent assay; FITC, fluorescein isothiocyanate; GI, gastrointestinal; IBD, inflammatory bowel disease; IL, interleukin; NF-κB, nuclear factor-κB; PCR, polymerase chain reaction; SMVT, sodium-dependent multivitamin transporter; TNF, tumor necrosis factor; UC, ulcerative colitis.**

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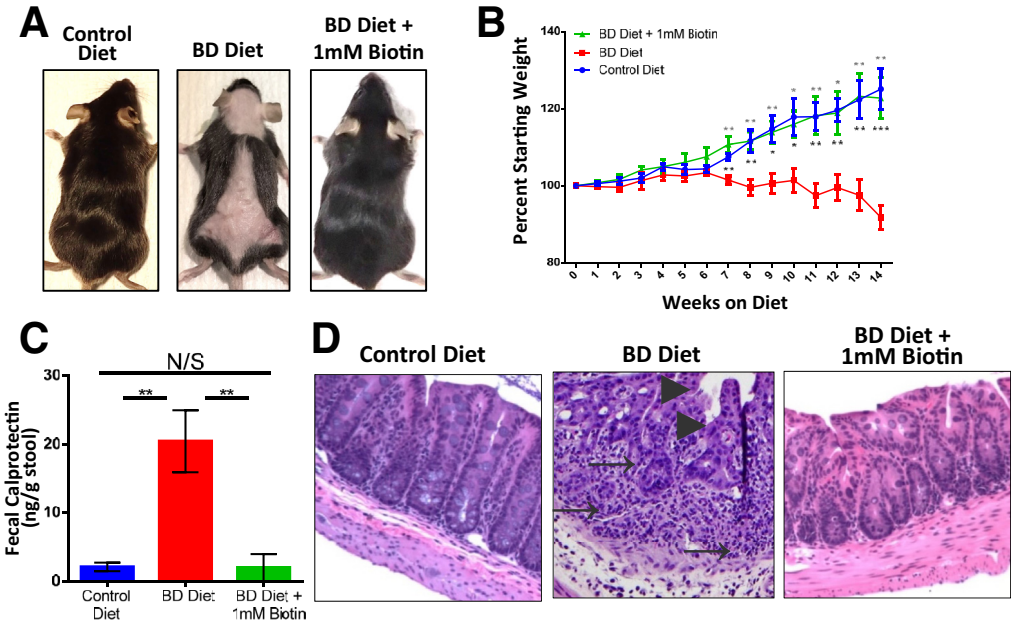

Figure 1. Chronic biotin deficiency induces an IBD-like state that completely resolves with biotin supplementation. C57BL/6J mice received a control diet or a biotin-deficient diet with and without 1 mmol/L biotin supplementation for 14 weeks. (*A*) Representative images comparing a control mouse, a mouse on the deficient diet ,and a mouse that received the deficient diet and treatment with 1 mmol/L biotin. (*B*) Body weight was recorded weekly for mice in each group, significance was calculated relative to the BD diet group. (*C*) A stool pellet was collected from mice in each group the day before death and used to quantify the fecal calprotectin level. (*D*) Representative histology from the cecum of a mouse in each group. Mice with biotin deficiency have erosions (*arrowheads*), loss of goblet cells, and altered crypt architecture with increased infiltration of lymphocytes and neutrophils into the lamina propria (*arrows*), and expansion of the submucosa, while mice that were supplemented with biotin did not have cecal inflammation. $^{*}P < .05$, $^{**}P < .01$, and $^{***}P < .001$. BD, biotin deficiency.

model typically develop a robust response within 7 days that can be measured by standard parameters including DAI, colon length, and histologic scoring (Figure 2*A*–*E*). We wanted to study the biotin transport pathway in this acute model for colitis and found that the localized biotin transport pathway in the colon was down-regulated, as judged from protein isolated from the distal colon to quantify expression of SMVT (Figure 2*F*). These results suggested that physiologic biotin uptake may be limited in the setting of colitis.

To determine if there is clinical relevance of these findings, we quantified SMVT expression by real-time polymerase chain reaction (PCR) using tissue from patients with colitis and controls. We found that SMVT was decreased significantly in patients with moderate/severe UC compared with controls (Figure 2*G*). To extend this finding, we reviewed a larger, publicly available gene expression database[32] (data accessible at NCBI GEO database, accession GDS3268) with 202 biopsy specimens taken from patients with controlled UC, active UC, and healthy controls.[33] We searched for expression levels of the SMVT gene, *Slc5a6*, in the sigmoid colon and the data have been normalized to samples from patients without disease. Interestingly, SMVT is reduced significantly in patients with inflamed UC, but there is no difference between healthy controls and patients with uninflamed UC (Figure 2*H*). Overall, these findings show that in mice with DSS colitis and in human beings with active UC, the biotin transport pathway is altered in the colon and this raises the possibility that it could be a target for therapy.

Biotin Therapy Helps Maintain Remission in DSS Colitis

To determine if biotin can be used as a therapeutic for the maintenance of remission to colitis, we used a 1.5% DSS model to induce mild/moderate colitis. Starting with healthy mice, 1 group received 1 mmol/L biotin in their drinking water for 1 week before DSS challenge. On day 0, the experimental and treatment groups had DSS added to drinking water and the treatment group also was continued on biotin (Figure 3*A*). An additional control group did not receive biotin or DSS. We found that disease in the biotin treatment group was attenuated in comparison with the mice without supplementation. In addition, the onset of disease was delayed, and the peak of disease was decreased with biotin therapy, as measured by DAI (Figure 3*B*). The colons from DSS challenged mice contained blood and were shortened significantly compared with controls and those that received DSS with biotin therapy. There was no difference between mice that received biotin therapy and controls lacking DSS exposure (Figure 3*C* and *D*). Fecal calprotectin also was quantified by enzyme-linked immunosorbent assay (ELISA) and found to be increased in the DSS group compared with controls and mice that received DSS with biotin therapy. Again, there was no significant

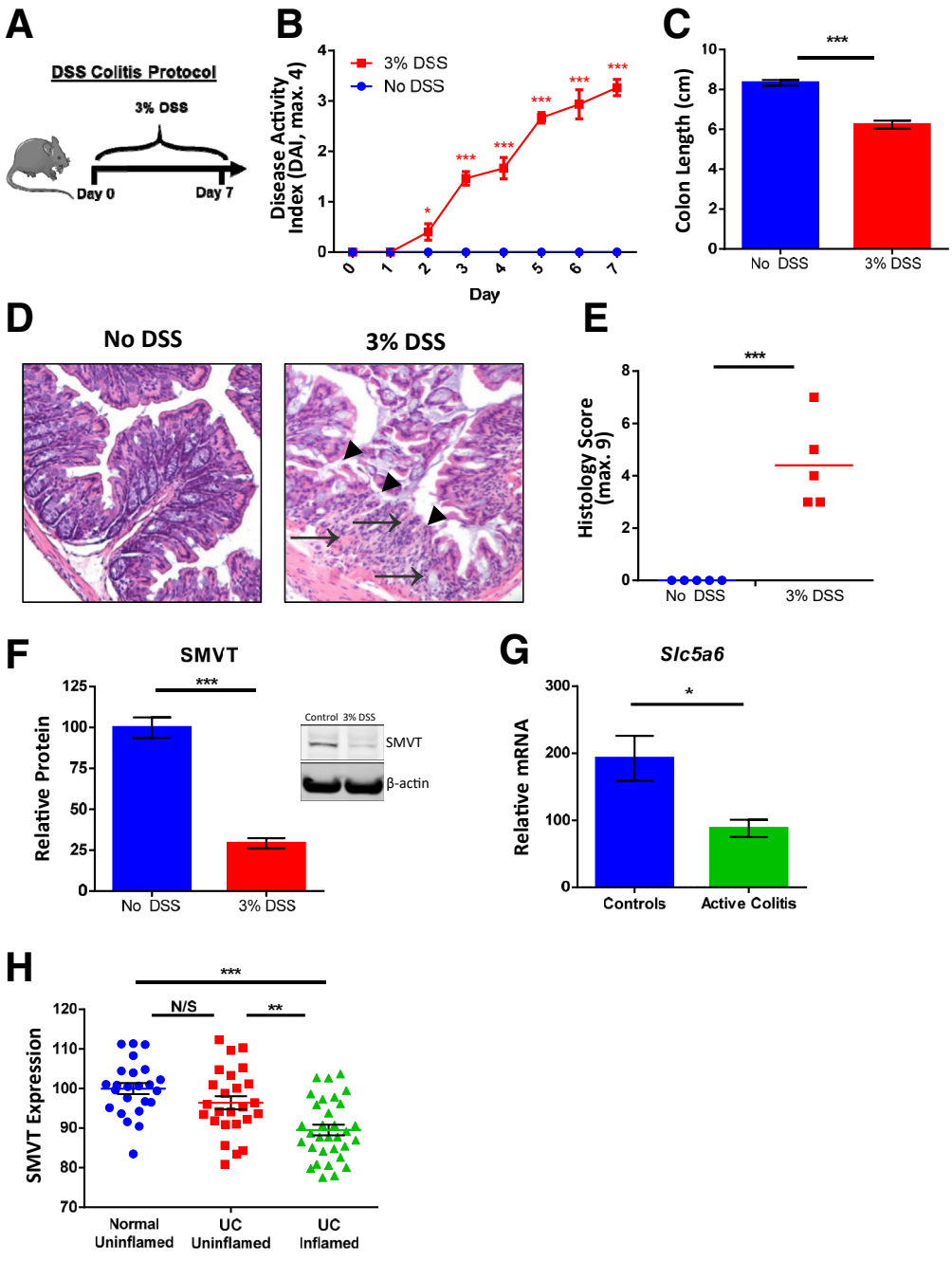

Figure 2. SMVT expression was reduced in human beings and mice with colitis. (*A*) Experimental protocol. (*B*) DAI was calculated daily. (*C*) On day 7, the mice were killed and colon length was determined. (*D*) Representative histology from the distal colon of mice treated with DSS and controls. DSS-treated mice developed mucosal erosion (*arrowheads*), with loss of goblet cells, altered crypt architecture, and infiltration of neutrophils and lymphocytes (*arrows*). (*E*) Total histologic score. (*F*) Protein was isolated from a segment of the distal colon and SMVT expression was quantified by Western blot and normalized to β-actin and the healthy control. (*G*) *Slc5a6* levels were quantified by quantitative PCR from patients with moderate/severe left-sided colitis and normalized to β-actin and healthy controls. (*H*) *Slc5a6* levels from the sigmoid colon in patients with UC was normalized to healthy controls using data available from GEO accession GDS3268. $^{*}P < .05$, $^{**}P < .01$, and $^{***}P < .001$. max, maximum; mRNA, messenger RNA.

difference between the group that received biotin therapy and the no-DSS control (Figure 3*E*). Finally, histology showed mucosal erosion, distorted crypts, loss of goblet cells, and infiltration of neutrophils and lymphocytes in the mucosal and submucosal layers of the colon wall of mice that received DSS. In contrast, mice that received biotin treatment maintained much of the mucosal lining with well-preserved crypts and goblet cells, and they had only mild infiltration into the lamina propria (Figure 3*F*). Histologic scoring confirmed significant improvement in colitis with biotin therapy (Figure 3*G*). Overall, these data show that biotin therapy helps to attenuate the development of colitis.

Biotin Therapy Enhances Induction of Remission to DSS Colitis

The next set of experiments were designed to determine if there is a role for biotin therapy in the induction of remission to colitis. Mice received 1.5% DSS in their drinking water for 7 days to induce moderate colitis. On day 0, the DSS was removed and half the mice received water, while the other half received therapy with 1 mmol/L biotin. Again, there was a control group that did not receive biotin or DSS. The groups were monitored until day 4 when the experiment was ended and all mice were killed (Figure 4*A*). Mice that received biotin therapy recovered more quickly with complete resolution of

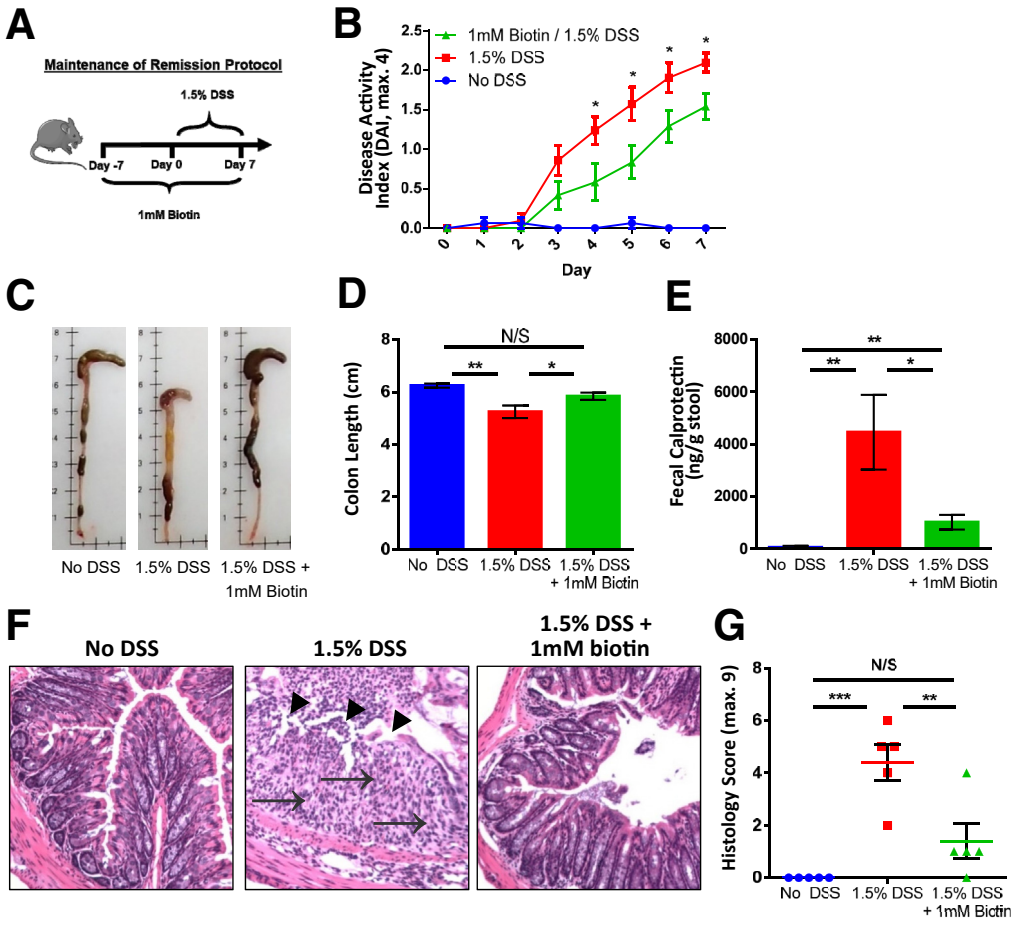

Figure 3. Biotin therapy helps maintain remission and ameliorate the development of colitis. (*A*) Experimental protocol. (*B*) DAI was calculated daily and significance is shown for biotin treatment. (*C*) On day 7, the mice were killed and a representative image of a colon from each group is shown. (*D*) Average colon length for each group. (*E*) A stool pellet was collected from mice in each group before killing and used to quantify the fecal calprotectin level. (*F*) Representative histology from the distal colon of a mouse in each group. DSS-treated mice had mucosal erosion (*arrowheads*) and substantial infiltration of neutrophils and lymphocytes in the lamina propria with loss of crypts and goblet cells (*arrows*). (*G*) Total histologic score. $^{*}P < .05$, $^{**}P < .01$, and $^{***}P < .001$. max, maximum.

disease, as measured by DAI (Figure 4*B*). The colons from mice that had received only water after DSS appeared shortened and atrophic compared with mice that had received biotin therapy and the no-DSS control. Nearly all the mice in all groups had solid stools by that point, although the average colon length still was shortened in the group that had received only water after DSS. There was no difference between mice that received biotin induction therapy and controls (Figure 4*C* and *D*). The fecal calprotectin level was increased in the group that received only water after DSS, compared with mice that received biotin therapy and controls. Again, there was no significant difference between the group that received biotin therapy and the control group (Figure 4*E*). Histology in mice from both groups showed marked improvement from the peak of disease, but the group that received only water still had mild crypt loss and neutrophil and lymphocyte infiltration (Figure 4*F* and *G*). Overall, these data indicate that biotin therapy is able to accelerate healing during induction of remission.

Biotin Therapy Leads to a Reduction in Inflammatory Cytokines and Intestinal Permeability

We next examined localized cytokine production to investigate the mechanism by which biotin was able to ameliorate colitis in the models for both maintenance and induction of remission. We previously have seen that inflammatory cytokines are up-regulated in the setting of biotin deficiency[34] and that those cytokines can be normalized with biotin supplementation.[35] To determine if there were similarities between the biotin deficiency and the DSS colitis models, we examined 3 proinflammatory cytokines that have a pivotal role in the pathogenesis of IBD: interleukin (IL)6, tumor necrosis factor (TNF)α, and IL1β.[36–38] Samples from the distal colons of mice in the maintenance of remission experiment were assayed by quantitative real-time-PCR. IL6 levels were increased significantly in mice that received DSS, although they were near baseline with biotin supplementation. TNFα and IL1β levels also were increased with DSS colitis, but cytokine levels did not completely return to baseline levels with biotin therapy (Figure 5*A*). There are similar trends for mice during the induction of remission (Figure 5*B*). These data complement earlier studies showing that biotin levels can affect innate and adaptive immune responses.[6,8–10,13,35,39]

To address whether or not biotin therapy alters mucosal permeability we performed a fluorescein isothiocyanate (FITC)-dextran permeability assay after the induction of remission protocol. We noted increased levels of FITC-dextran in the plasma of mice that had received only water

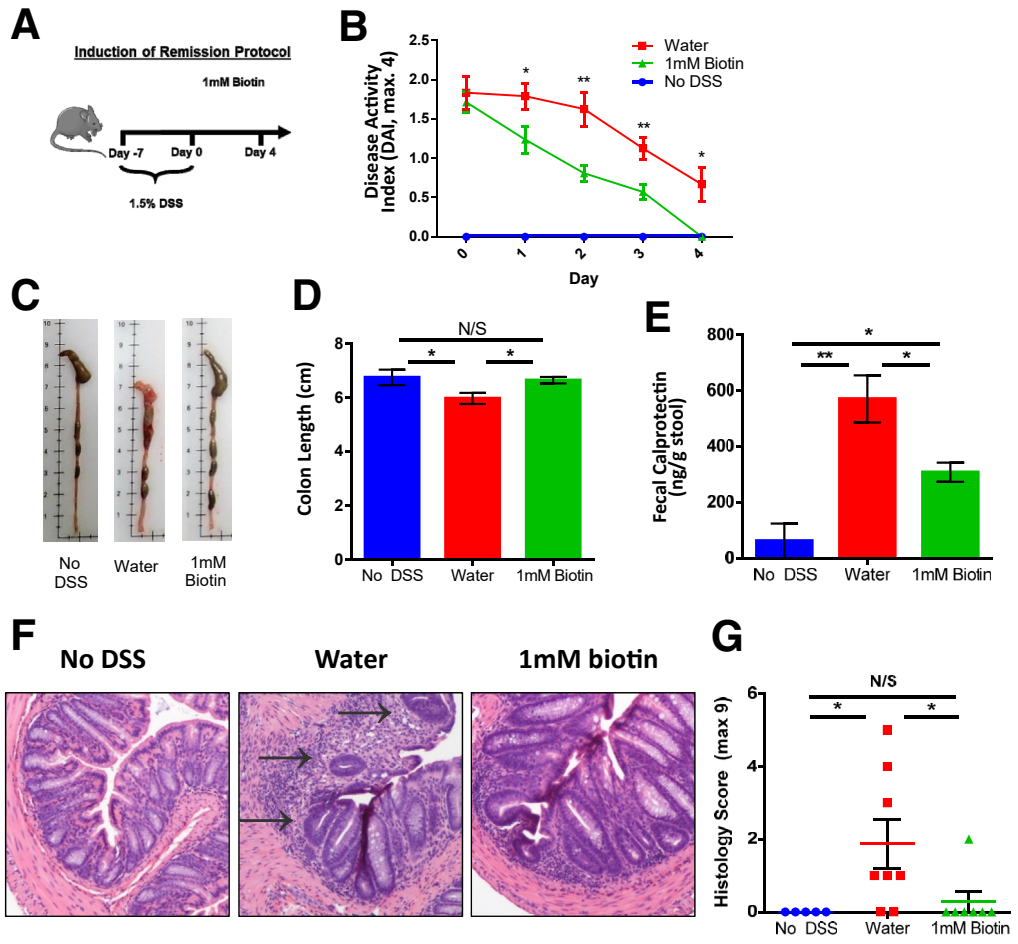

Figure 4. Biotin therapy accelerates induction of remission to DSS colitis. (*A*) Experimental protocol. (*B*) DAI was calculated daily and significance is shown for biotin treatment. (*C*) On day 4, the mice were killed and a representative image of a colon from each group is shown. (*D*) Average colon length. (*E*) A stool pellet was collected from mice in each group before killing and used to quantify the fecal calprotectin level. (*F*) Representative histology from the distal colon of a mouse in each group. Mice that received only water continued to have moderate infiltration along with loss of crypts and goblet cells (*arrows*). (*G*) Total histologic score. *$P < .05$ and **$P < .01$. max, maximum.

after DSS exposure, whereas mice that had received biotin therapy showed similar values to the controls that received no DSS (Figure 6*A*). These data show that biotin therapy helped reduce intestinal permeability and the results were confirmed by examining the expression of tight junction proteins. In the intestine, up-regulation of zonula occludens-1 is important to maintain the mucosal integrity,[40] while up-regulation of claudin-2 is associated with susceptibility to colitis and increased intestinal permeability.[41,42] Zonula occludens-1 levels in the distal colon were decreased in mice that had received only water after DSS, while they were close to baseline with biotin therapy (Figure 6*B*). Conversely, claudin-2 expression was increased in the distal colons of mice that had received only water after DSS while they were again close to baseline with biotin therapy (Figure 6*C*). Overall, these data support a model in which biotin supplementation helps to maintain mucosal integrity.

Biotin Therapy Reduces NF-κB Activation

Finally, we wanted to explore these changes at the molecular level to determine if NF-κB is affected by biotin therapy. NF-κB is considered one of the primary regulators in the development of IBD.[20] The 5 proteins in the NF-κB family dimerize and localize to the cytoplasm in unstimulated cells. Activation signals allow for nuclear translocation of the dimers and subsequent transactivation of target genes.[20,43] The p65 protein is the most abundant and best studied in the NF-κB family, and phosphorylation is the starting point in its activation.[43,44] We found that total NF-κB p65 levels were unchanged with biotin therapy (Figure 7*A*), while the phosphorylated active form (NF-κB p65 [phospho S529]) was increased in mice that did not receive biotin supplementation (Figure 7*B*). These results show that biotin therapy attenuates activation of NF-κB p65 and the inflammatory cascade that follows.

Discussion

In this report, we showed that the colitis that develops from biotin deficiency has many similarities to mouse models for IBD including weight loss, bloody stool, increased fecal calprotectin, and infiltration of lymphocytes and neutrophils to the intestinal wall. Importantly, disease and this IBD-like state can be prevented with biotin supplementation. Biotin is one of the water-soluble vitamins with transporters in both the small and large intestines.[45] The current understanding is that dietary biotin is absorbed primarily in the small intestine and biotin produced by commensal bacteria is absorbed in the large intestine. Because the colon has constant exposure

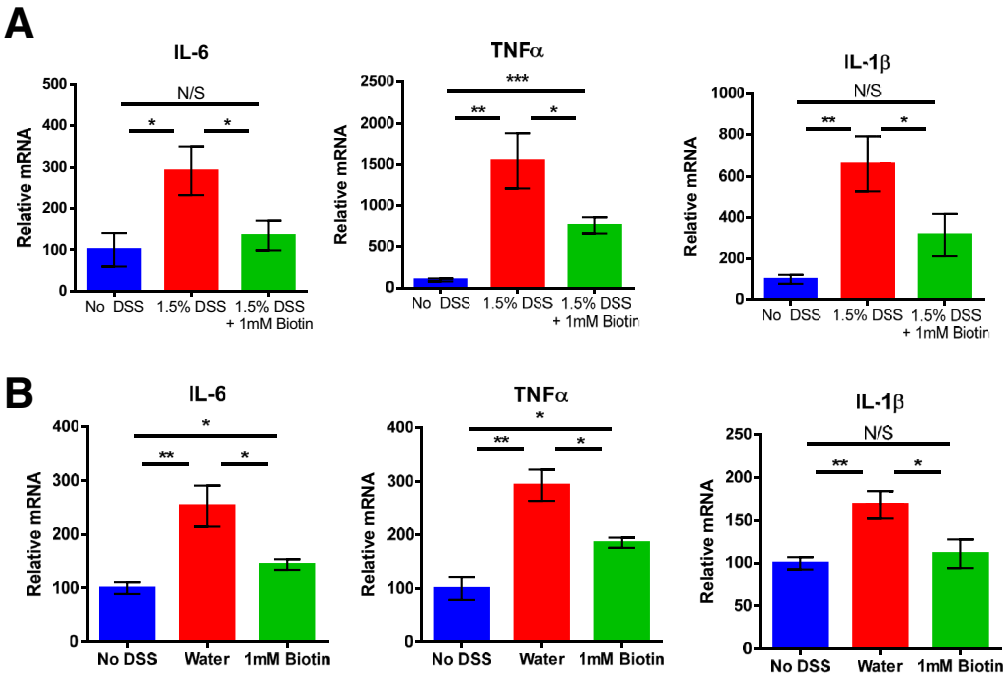

Figure 5. Biotin therapy leads to reduced proinflammatory cytokines in the colon. (*A*) Quantitative real-time-PCR was performed on tissue from the distal colon to quantify the cytokines IL6, TNFα, and IL1β from mice in the maintenance of remission protocol, and (*B*) the induction of remission protocol. Data have been normalized to glyceraldehyde-3-phosphate dehydrogenase and healthy controls. *$P < .05$ and **$P < .01$. mRNA, messenger RNA.

to biotin, it absorbs a substantial amount of total biotin.[46] These observations led us to evaluate if the biotin transport pathway is affected in an established mouse model for IBD. We used DSS to induce severe colitis, and examined mice at the peak of disease. We found that SMVT levels in the distal colon, where inflammation is most pronounced in the DSS model, were reduced significantly in mice with colitis when compared with healthy controls. The clinical relevance of this finding was supported by comparing gene expression in patients with UC and healthy controls. We found decreased expression of the SMVT biotin transporter in the distal colon of patients with active UC when compared with the distal colon of healthy controls. The same finding of decreased SMVT expression during active disease can be found in the proximal and descending colon as well.[32] Although this association cannot determine if reduced SMVT is a cause or result of a colitis flare, it does suggest that biotin supplementation should be explored for IBD treatment.

We then tested biotin supplementation in the DSS preclinical model for IBD to determine if it has a therapeutic effect. There is no expected direct interaction between biotin and DSS.[2] In the maintenance of remission model, mice began without disease and received biotin supplementation. They then were challenged with DSS to induce moderate colitis and we monitored the changes between the treatment and control groups for 7 days. The mice receiving biotin therapy still developed some signs of disease, but they were substantially less severe in comparison with mice that did not receive biotin. In biotin-treated mice, DAI showed a delayed onset and a reduced peak of disease, fecal calprotectin level was reduced, and histology showed only mild lymphocyte infiltration compared with more pronounced erosions and crypt

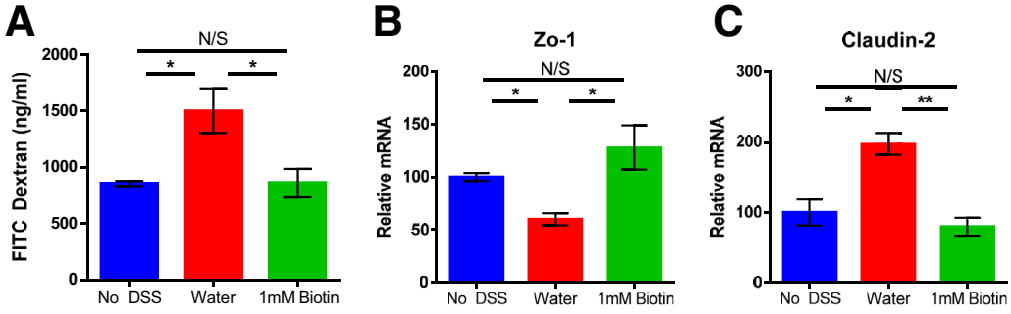

Figure 6. Biotin therapy helps maintain the integrity of the intestinal mucosa. (*A*) Mice from the induction of remission protocol received FITC-dextran by oral gavage 4 hours before killing. Plasma levels of FITC-dextran were measured as a marker of intestinal permeability. (*B*) Quantitative real-time-PCR was performed on tissue from the distal colon to quantify tight junction protein zonula occludens-1 (ZO-1) and (*C*) claudin-2. Data have been normalized to glyceraldehyde-3-phosphate dehydrogenase and healthy controls. *$P < .05$ and **$P < .01$. mRNA, messenger RNA.

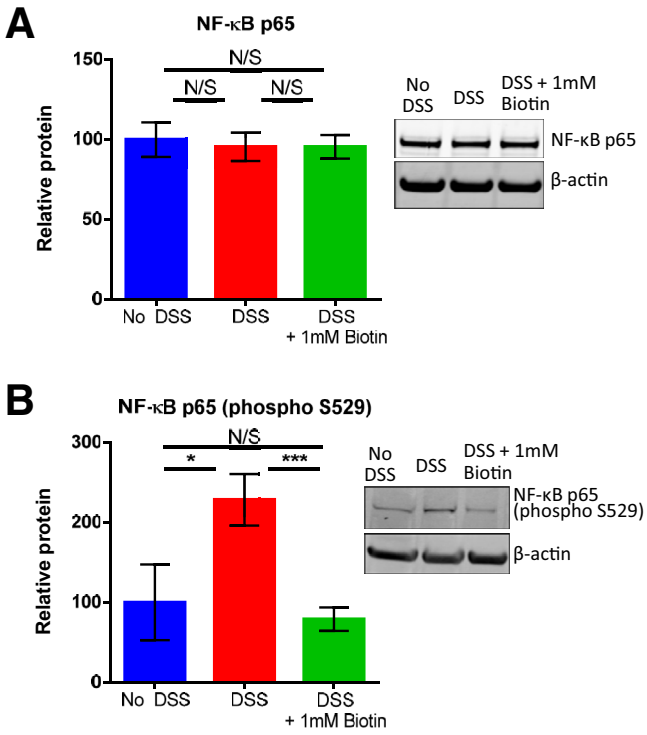

Figure 7. Biotin therapy leads to decreased levels of active phosphorylated NF-κB p65. Protein was isolated from a segment of the distal colon. (*A*) NF-κB p65 and (*B*) phosphorylated NF-κB p65 (phospho S529) expression was quantified by Western blot and normalized to β-actin and the healthy control. Data shown are the means ± SE. Statistical analyses were performed with a Student *t* test. $^{*}P < .05$ and $^{***}P < .001$.

loss in the untreated mice. Proinflammatory cytokines IL6, TNFα, and IL1β also were decreased in the treatment group. We repeated a similar set of experiments using an induction of remission model. In this model, mice began biotin therapy at the peak of disease and we compared remission rates with untreated mice after the DSS was removed. After 4 days on biotin, the DAI score of treated mice had returned to baseline and was improved significantly in comparison with untreated mice. Similar results were seen for colon length, calprotectin, and histology. Again, proinflammatory cytokines were decreased. In addition, in this model, we evaluated gut permeability and found that it was decreased with biotin treatment and that there were increased levels of zonula occludens-1 and decreased levels of the leaky tight-junction protein claudin-2. These data support our previous finding that the biotin transport pathway plays an important role in the maintenance of mucosal integrity.[34]

The mechanisms underlying the pathogenesis of biotin deficiency and biotin's therapeutic potential remains an active area of research and several advances have been made recently.[6,8,13,34,35,39,47–49] We previously reported on an SMVT knockout mouse that provided evidence that the SMVT system is exclusively responsible for intestinal biotin uptake and deletion of SMVT is sufficient to induce colitis.[2] SMVT levels also can decrease physiologically in response to cytokine activation of the NF-κB pathway.[39] Interestingly, NF-κB has a dual role in the GI tract by driving proinflammatory responses in immune cells and by up-regulating prosurvival genes in epithelial cells.[17] However, in the setting of IBD the primary role is orchestrating mucosal inflammation.[20] In the current study, we showed that biotin therapy is sufficient to attenuate activation of NF-κB p65 and propose this as the underlying mechanism of its therapeutic effect. If infiltrating cells have reduced activation of NF-κB, then they will produce decreased amounts of inflammatory cytokines that subsequently will lead to reduced intestinal permeability and maintenance of mucosal integrity. The specific mechanism(s) linking biotin and NF-κB is unclear but could be mediated via the different cellular pathways that are affected by biotin availability. Further studies are required to define this relationship.

IBD develops from a combination of genetic predisposition, environmental triggers, dysbiosis in the microbiome, and complex interactions in the host immune system, making it challenging to model IBD accurately in preclinical models.[50,51] As mentioned, biotin deficiency has been identified in patients with IBD and it may have profound implications in the GI tract that have not been fully explored. We are optimistic that the data presented here will serve as the foundation for future clinical studies to determine if biotin supplementation should be used as adjunct therapy in IBD. Biotin is available over the counter, is affordable, and it has minimal side effects, making it an ideal therapeutic if clinical trials can show similar efficacy to what we have seen in this preclinical model.

Materials and Methods

All authors had access to the study data and reviewed and approved the final manuscript.

Mice

C57BL/6J mice were purchased from Jackson Laboratories (Ellsworth, ME) and housed at University of California Irvine in compliance will all University Laboratory Animal Resources guidelines. Mice aged 8–10 weeks were used at the start of all experiments and representative experiments were confirmed with male and female mice to ensure there were no sex differences. Experiments were performed in compliance with all federal and local guidelines and approved by the Institutional Animal Care and Use Committee and the University of California Irvine.

Biotin-Deficient and Control Diet

The biotin-deficient diet (Envigo, Madison, WI) has no added biotin and the protein source is 300 g/kg spray-dried, egg-white solids. Egg white is high in avidin, which bind the luminal biotin produced by the host microbiome.[7,52] The biotin-control diet (Envigo) has the same make-up and protein source as the deficiency diet, but is supplemented with 0.004 g/kg biotin. Mice received the diet ad libitum and pair-feeding was performed.

Fecal Calprotectin ELISA

Fecal calprotectin was quantified by ELISA using the DuoSet Mouse S100A8/S100A9 Heterodimer kit (R&D

Table 1. List of Primer Sequences Used for Quantitative Real-Time-PCR

Gene name	Forward and reverse primer sequences, 5′-3′
Mouse claudin-2	TTAGCCCTGACCGAGAAAGA; AAAGGACCTCTCTGGTGCTG
Mouse ZO-1	TTCAAAGTCTGCAGAGACAATAGC; TCACATTGCTTAGTCCAGTTCC
Mouse IL6	GAGGATACCACTCCCAACAGACC; AAGTGCATCATCGTTGTTCATACA
Mouse TNF-α	CATCTTCTCAAAATTCGAGTGACAA; TCGGAGTAGACAAGGTACAACCC
Mouse IL1β	CTCTCCAGCCAAGCTTCCTTGTGC; GCTCTCATCAGGACAGCCCAGGT
Mouse GAPDH	CTACAGCAACAGGGTGGTGG; TATGGGGGTCTGGGATGG
Mouse SMVT	CGTAGGAACTTTGGTAGCCCTGG; CTTAGGTGTGATGGGTCTCTCC
Human SMVT	TGTCTACCTTCTCCATCATGGA; TAGAGCCCAATGGCAAGAGA
Human β-actin	CATCCTGCGTCTGGACCT; TAATGTCACGCACGATTTCC

GAPDH, glyceraldehyde-3-phosphate dehydrogenase; ZO-1, zonula occludens-1.

Systems, Minneapolis, MN) according to the manufacturer's recommendations. Briefly, a stool pellet was collected, weighed, and immediately frozen. The night before the assay, pellets were incubated at 4°C with 1 mL fecal extraction buffer (0.1 mol/L Tris, 0.15 mol/L MaCl, 1.0 mol/L urea, 10 mmol/L CaCl2, 0.1 mol/L citric acid, 5 g/L bovine serum albumin, 0.25 mmol/L thimerosal, pH 8; Hycult Biotech, Wayne, PA). The supernatant was collected after the pellet was broken up with vortexing and ceramic beads. The dilution with extraction buffer was calculated assuming a density of 1 mg/mL and results were normalized to the stool weight.

Histologic Analysis

De-identified H&E-stained sections from DSS-treated mice were scored by a pathologist according to published methods.[53] Briefly, the score is the sum of neutrophil infiltration (0–2), lymphocyte infiltration (0–2), erosion (0–3), and crypt loss (0–2), with a maximum score of 9.

DSS Colitis

DSS salt (molecular weight, 36,000–50,000; MP Biomedicals, Irvine, CA) was added to drinking water for 1 week at 3% to induce severe colitis or 1.5% for mild/moderate colitis. The solution was changed every 2–3 days. Mice were observed daily and the DAI was calculated according to published protocols.[54] Briefly, the score given is the average of a score for weight loss (0, none; 1, 2%–5%; 2, 5%–10%; 3, 10%–15%; 4, >15%), stool consistency (0, normal; 2, loose stool; 4, diarrhea), and blood in stool (0, negative; 2, guaiac positive, 4; gross bleeding).

Biotin Therapy

Biotin (Sigma-Aldrich) was dissolved in drinking water to a concentration of 1 mmol/L. The solution was changed every 2–3 days and mice received the treatment ad libitum.

FITC Dextran Permeability Assay

Intestinal permeability was determined in vivo using methods previously described.[55] Briefly, FITC-dextran (FD4; Sigma-Aldrich) was dissolved in phosphate-buffered saline to 100 mg/mL and administered to mice at 44 mg/100 g body weight. Mice were fasted overnight and gavaged with FITC-dextran on the last day of the experiment. After 4 hours, mice were bled, plasma was separated, and measurements were taken by fluorimeter at 488 nm (Synergy BioTek Plate Reader, Winooski, VT). The concentration was calculated based on a stander curve generated by serial dilutions of the FITC-dextran stock in phosphate-buffered saline.

Quantitative Real-Time PCR Analysis

Total messenger RNA was isolated from the mouse distal colon with Qiazol and the RNeasy Kit (Qiagen, Hilden Germany) using 1.2-mm silica beads (Fisherbrand, Hampton, NH). To remove DSS from purified messenger RNA we performed further messenger RNA purification by lithium chloride as described.[56] Complementary DNA was synthesized using the Verso complementary DNA synthesis kit (Invitrogen, Carlsbad, CA). Expression levels of human SMVT was determined using formalin-fixed paraffin-embedded biopsy samples collected from the sigmoid colon of patients at University of California Irvine with moderate/severe UC. RNA was purified from the samples using the Quick-RNA Formalin-Fixed Paraffin-Embedded Kit (Zymo Research, Irvine, CA). Samples were quantified by real-time quantitative PCR using gene-specific primers (Table 1) with a CFX96 real-time iCycler (Bio-Rad, Hercules, CA). SYBR green was used for mouse complementary DNA samples, and the iTaq Universal One-Step Kit (Bio-Rad) was used for human RNA samples.

Western Blot

For Western blot analysis, tissues/cells were homogenized in RIPA buffer (Sigma) containing complete protease inhibitor cocktail (Roche, Basel, Switzerland) using 1.2-mm silica beads (Fisher, Hampton, NH) and the Fisherbrand Bead Mill 24 Homogenizer. Total protein homogenates were cleared by centrifugation at 12,000*g* for 20 minutes, and an equal amount (65 μg) of the total proteins was loaded on a 4%–12% mini gel (Invitrogen). The proteins then were transferred to a polyvinylidene difluoride membrane and probed simultaneously with anti-mouse SMVT, NF-κB, phospho NF-κB p65 (ser536) (raised in rabbit), and monoclonal β-actin antibody (raised in mouse). The blots then

were incubated with anti-rabbit/anti-mouse IR 800 dye and anti-mouse IR 680 dye (LI-COR, Lincoln, NE) secondary antibodies (1:25,000) for 1 hour at room temperature. Relative expression was quantified by comparing the fluorescence intensities in an Odyssey Infrared imaging system (LI-COR) using Odyssey application software (version 3.0) with respect to corresponding β-actin.

Statistical Analysis

The data are expressed as means ± SEM. Differences were analyzed by a Student t test and was considered significant when the P value was less than .05. Experiments were performed at least 3 times to confirm consistent results and data from the most recent experiment are shown.

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Author contributions
Jonathan Skupsky, Subrata Sabui, and Hamid M. Said were responsible for the study concept and design; Jonathan Skupsky, Subrata Sabui, and Michael Hwang acquired data; Jonathan Skupsky, Subrata Sabui, and Manando Nakasaki analyzed and interpreted the data; Jonathan Skupsky drafted the manuscript; Michael D. Cahalan and Hamid M. Said critically revised the manuscript; Jonathan Skupsky and Subrata Sabui performed the statistical analysis; Jonathan Skupsky, Michael D. Cahalan, and Hamid M. Said obtained funding; Jonathan Skupsky, Subrata Sabui, and Michael Hwang provided technical support; and Michael D. Cahalan and Hamid M. Said supervised the study.

Conflicts of interest
The authors disclose no conflicts.

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