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Hydrostatic intestinal edema induced signaling pathways: potential role of mechanical forces

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Abstract

Background—Hydrostatic intestinal edema initiates a signal transduction cascade that results in smooth muscle contractile dysfunction. Given the rapid and concurrent alterations in the mechanical properties of edematous intestine observed with the development of edema, we hypothesize that mechanical forces may serves as a stimulus for activation of certain signaling cascades. We sought to examine if isolated similar magnitude mechanical forces induced the same signal transduction cascades associated with edema.

Methods—The distal intestine from adult male Sprague Dawley rats was longitudinally stretched for two hours to 122.5% its original length, correlating with the interstitial stress seen with edema. Wet to dry ratios, myeloperoxidase activity, nuclear STAT-3 and NF-kappa B DNA binding, STAT-3 phosphorylation, myosin light chain phosphorylation, baseline and maximally stimulated intestinal contractile strength, and iNOS and sodium hydrogen exchanger 1–3 mRNA in stretched and adjacent control segments of intestine were compared.

Results—Mechanical stretch did not induce intestinal edema or an increase in myeloperoxidase activity. Nuclear STAT-3 DNA binding, STAT-3 phosphorylation and nuclear NF-kappa B DNA binding were significantly increased in stretched seromuscular samples. There was increased expression of sodium hydrogen exchanger 1 but no increase in iNOS expression. Myosin light chain phosphorylation was significantly decreased in stretched intestine as was baseline and maximally stimulated intestinal contractile strength.

Conclusions—Intestinal stretch, in the absence of edema/inflammatory/ischemic changes, leads to activation of signaling pathways known to be altered in intestinal edema. Edema may initiate a mechanotransductive cascade that is responsible for subsequent activation of various signaling cascades known to induce subsequent contractile dysfunction.

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MeSH Keywords

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Introduction

The global hypothesis of our laboratory is that intestinal edema serves as a potential initiator and/or amplifier of dysfunctional signaling cascades and downstream effects. To that end, our group has developed an animal model of intestinal edema in which edema develops in the absence of inflammation, ischemia, and/or ischemia reperfusion induced injury.[1] In previously published work, we have demonstrated that this model of resuscitation-induced (hydrostatic) intestinal edema created by high volume crystalloid resuscitation and mesenteric venous hypertension (as a surrogate to abdominal packing) induces smooth muscle contractile dysfunction and consequent ileus. [2] Interrogation of the myosin light chain$_{20}$ (MLC) phosphorylation/de-phosphorylation regulatory pathway has revealed multiple signal transduction cascades that are activated in response to edema development, including NF-kappa B and signal transduction and activator of transcription (STAT)-3. [3, 4][Uray, In Press, Critical Care Medicine] While all signal transduction cascades affecting contractility ultimately converge on the MLC phosphyorylation/de-phosphorylation apparatus, the impetus for activation of NF-kappa B and STAT-3 transcription factors is not entirely clear.

In our animal model, edema develops early (within 30 minutes) and is sustained for 12–24 hours. [1, 5] As edema is an early finding, the potential initiator(s) for activation of dysfunction signaling pathways should be rapidly induced by edema. Subsequently, we demonstrated that during the first 30 minutes, there are rapid and concurrent alterations in the mechanical properties of intestine (stress/strain) and increases in interstitial pressure. Early observations in our laboratory demonstrated the alterations in the mechanical properties of intestinal tissue following edema development are early and sustained [6, 7] noting that they may serve as a mechanotransductive signal initiator. [5] With the knowledge that cellular mechanotransducers are ion channels/ion exchangers and cytoskeletal filaments, and that ion channel/exchanger mediated effects are more rapid than canonical pathways for transcription factor activation and protein synthesis dependent signaling, we initially demonstrated that there is increased expression of the mechanosensitive sodium hydrogen exchanger (NHE) with edema development, and NHE inhibition decreased STAT-3 activation and partially restored contractile function without edema resolution. Additionally, administration of hypertonic saline (shown previously to decrease edema and reverse contractile dysfunction) prevented the early and late mechanical changes induced by edema and decreased NHE expression. [5, 8, 9]

A mechanical explanation for alteration in cellular properties, or mechanotransduction, is not a novel concept. Stretch in rat cardiomyocytes has been shown to activate the JAK (janus kinase)/STAT pathway, NF-kappa B, and increase NHE activity. [10–16] Mechanical forces additionally have been shown to be important in intestinal epithelial cell biology. [17] There is, however, little work published in the literature about signaling pathways altered/activated by mechanical forces in the intestine as a potential contributor to seromuscular dysfunction.

We sought to examine the effects of similar magnitude mechanical forces on intestinal smooth muscle in a setting free of edema and developed a novel apparatus that recreates the calculated engineering stress/strain relationship in an ex-vivo, perfused rodent intestinal model. After mimicking the stress/strain relationship of edema (without edema development or inflammation), we interrogated the MLC phosphorylation/de-phosphorylation apparatus, the NHE system (a putative mediator of mechanical signaling), as well as nuclear transcription factors activation we have associated with resuscitation-induced edema formation and

contractile dysfunction (STAT-3 and NF-kappa B).[11, 13–16] We hypothesized that we could replicate the signal transduction activation profile induced by edema by approximating the calculated mechanical forces, thereby demonstrating a potential role for mechanical forces in the initiation of various signaling pathways in intestinal edema.

Materials and Methods

Intestinal Stretch Model

All procedures were approved by the University of Texas Animal Welfare Committee and were consistent with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* (HSC-AWC-07-111).

Longitudinal Stretch Animal Model

Male Sprague Dawley rats (230–310g) were fasted for 12–16 hours with free access to water. After general anesthesia was induced with isoflurane, a midline laparotomy was performed. The distal portion of the small intestine (ileum) was exteriorized with minimal manipulation.

In order to stress the tissue longitudinally, an apparatus was created to grasp and stretch the small intestine. (Figure 1) The amount of stress induced was estimated using the equation for the longitudinal stress of a thin-walled cylindrical pressure vessel:[18]

$$\tau = (P r_i^2)/(r_i^2 - r_o^2) \qquad \text{Equation 1}$$

τ: longitudinal stress

P: pressure

r_i: inner radius

r_o: outer radius

Published values of the average inner and outer circumferential lengths in 230g rats were used to determine an estimate of the inner and outer radii of the ileum.[19] An interstitial pressure of 506.63Pa (as seen with intestinal edema) was used for the pressure variable.[5] This yields an estimate of 0.50kPa of stress on the small intestine with edematous pressure. In order to relate this stress to an amount of longitudinal stretch necessary to mimic an equivalent stress, published relationships between longitudinal Kirchhoff's stress, a stress value that resembles that of the thin-walled cylindrical pressure vessel equation, and longitudinal Green's strain in 200g–240g rats were consulted. A stress of 0.50kPa was estimated to correlate to a 25% Green's strain[19–21]. Longitudinal Green's strain is demonstrated by the following equation:

$$\varepsilon = (\lambda^2 - 1)/2 \qquad \text{Equation 2}$$

ε: strain

l: length of the intestine

λ: $l_{pressurized}/l_{zero\ load}$

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The pressurized length represents the length of the stretched intestine, where the zero load length represents the length of the intestine prior to manipulation. Using this equation, we estimated that the intestine must be stretched to 122.5% of its original length to mimic the edematous stress felt longitudinally. The stretch apparatus was connected to a computer (Dell, Round Rock, TX) and the amount of stretch was monitored using Lab View (National Instruments, Austin, TX)

Each group consisted of 8 rats. The ileum was stretched for two hours. In previous experiments, STAT-3 was initially activated in both treatment (i.e., edema) and control rats; the levels decline rapidly in control animals at 2 hours and remain relatively constant thereafter. [4] The stretched tissue was controlled to a segment of adjacent intestine that grasped in a similar manner, but was not stretched. At the conclusion of the experiment, intestinal tissue from both the control and stretched sections were harvested; the mucosa and seromuscular layers were separated by scraping and immediately frozen in liquid nitrogen.

Wet to Dry Ratio

Wet to dry ratios were measured in stretched and control sections of intestine to determine the amount of edema development. After collection, the tissue was blotted dry, immediately weighed, and allowed to dry in a 60°C oven for a period of two to three days until the dry weight did not change. The wet to dry ratio was then calculated based on the following equation.

$$\text{Wet to Dry Ratio} = (\text{wet weight} - \text{dry weight})/(\text{dry weight}) \quad \text{Equation 3}$$

Preparation of Nuclear and Cytoplasmic Extracts

Frozen tissue was ground with a mortar and pestle over liquid nitrogen. Cytoplasmic and nuclear extracts were prepared using a commercially available nuclear extract kit (Active Motif, Carlsbad, CA) following the manufacturer's suggested protocol. Briefly, samples (ground tissue) were homogenized with a polytron in cold hypotonic buffer containing protease inhibitors (Protease Inhibitor Cocktail, Sigma Aldrich, St. Louis, MO) and phosphatase inhibitors (2 mM orthovanadate and 2 mM sodium fluoride). The homogenized samples were incubated at 4°C for 15 minutes and then centrifuged at 850xg at 4°C for 10 minutes. The supernatant was saved. The residual pellet was re-suspended in cold hypotonic buffer containing protease and phosphatase inhibitors and incubated on ice for 15 minutes. After the addition of 0.05% detergent (Nonidet P-40), the samples were vortexed briefly and centrifuged at 14,000xg at 4°C for 10 minutes. The supernatant was saved and combined with the first supernatant and called the cytoplasmic extract. The pellet was resuspended in lysis buffer, vortexed for 30 seconds, and incubated on ice for 30 minutes. The samples were then centrifuged at 14,000xg at 4°C for 10 minutes. The resultant supernatant was called the nuclear extract. All samples were aliquotted and stored at −80°C.

Myeloperoxidase Activity

Myeloperoxidase activity was measured in cytoplasmic extracts from seromuscular samples as previously published. [22] Briefly, samples were diluted 1:5 and added to a 96 well plate and incubated with 100 microliters of SureBlue™ tetramethylbenzidine (TMB) microwell peroxidase substrate (KPL, Gaithersburg, MD) for 20 minutes at 37°C. The reaction was stopped by addition of 100 microliters of TMB Stop Solution (KPL, Gaithersburg, MD), and the optical density was measured at 450 nm. To quantitate myeloperoxidase activity, a standard curve using horseradish peroxidase was developed. Each sample was assayed in duplicate, and the values were normalized to protein concentration.

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Nuclear STAT-3 DNA Binding

Nuclear STAT-3 DNA binding was measured in nuclear extracts of the seromuscular layers using a commercially available STAT-3 transcription factor assay kit (TransAm STAT-3, Active Motif, Carlsbad, CA) following the manufacturer's suggested protocol. Briefly, nuclear extracts were added to a 96-well plate in which the oligonucleotide containing the STAT-3 consensus binding site was immobilized. After incubation with nuclear lysates for one hour, a STAT-3 antibody was added followed by incubation with a secondary anti-rabbit horseradish peroxidase (HRP) conjugated immunoglobulin G (IgG) antibody. Wells were washed after each incubation period. A colorimetric HRP substrate was then added. The colorimetric reaction was stopped with oxalic acid. The plate was read at 450 nm with a reference wavelength of 655nm. To quantitate STAT-3 binding, a standard curve using IL-6 activated HepG2 cells was developed. Each sample was assayed in duplicate, and STAT-3 nuclear activation was normalized to nuclear protein concentration.

Phosphorylated STAT-3

Phosphorylated STAT-3 was measured in cytoplasmic extracts from the seromuscular layers using a commercially available enzyme-linked immunosorbent assay (ELISA) kit following the manufacturer's suggested protocol and as previously described (PathScan Phospho-STAT-3 (Tyr705) Sandwich ELISA Kit; Cell Signaling Technology, Beverly, MA). [4] Briefly, cytoplasmic extracts were added to individual wells of a 96-well plate coated with a STAT-3 rabbit monoclonal antibody. After overnight incubation at 4°C, the wells were washed, and a primary Phospho-STAT-3 mouse monoclonal antibody was added. After 1 hour of incubation, the wells were washed again, and a secondary anti-mouse IgG HRP conjugated antibody was added. After 30 minutes of incubation, the wells were washed, and an HRP substrate was added. After approximately 30 minutes of color development at 25°C, a stop solution was added, and the plate was read at 405 nm. To quantitate phosphorylated STAT-3, a standard curve using IL-6 activated HepG2 cells was developed. Each sample was assayed in duplicated and normalized to cytoplasmic protein concentration.

Nuclear NF-kappa B DNA Binding

Nuclear NF-kappa B DNA binding was measured in the nuclear extracts of the seromuscular samples utilizing a commercially available transcription factor assay kit (TransAM NF-kappa B p65, Active Motif, Carlsbad, CA) following the manufacturer's suggested protocol. Briefly, nuclear extracts were added to each well of a 96 well plate in which an oligonucleotide containing the NF-kappa B consensus sequence was immobilized. After incubation with lysates, a NF-kappaB p65 antibody was added followed by incubation with a HRP conjugated secondary antibody. Wells were washed after each incubation period. A colorimetric HRP substrate was then added. The reaction was stopped with oxalic acid. The plate was read at 450 nm with a reference wavelength of 655 nm. A recombinant p65 standard was used to generate a standard curve. Each sample was assayed in duplicate and normalized to total nuclear extract protein.

MLC Phosphorylation

Cytoplasmic extracts were separated using sodium dodecyl sulfate-polyacrylamide gel (12.5%) electrophoresis. After transfer to polyvinyldifluoride membranes, western blotting was performed by incubating the membranes with a rabbit polyclonal IgG myosin light chain antibody (MLC2 (A-20), 1:400 dilution, Santa Cruz Biotechnology, Santa Cruz, CA) overnight at 4°C. A secondary anti-rabbit IgG-HRP conjugated antibody was obtained from Sigma-Aldrich (St. Louis, MO). Enhanced chemical luminescence was used to visualize the proteins on the membranes (ECL Plus, Amersham Biosciences, Piscataway, NJ). Quantification of bands was performed with ImageJ software (National Institutes of Health, Bethesda, MD).

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Intestinal Contractility

Contractile activity was measured in the stretched and un-stretched strips of small intestine according to methods which we have previously published. [2] Briefly, whole thickness strips were mounted in 25 mL organ baths filled with Krebs-Ringer solution (103mM NaCl, 4.7mM KCl, 2.5mM $CaCl_2$, 25mM $NaHCO_3$, 1.1mM NaH_2PO_4, and 15nM glucose). The solution was buffered with albumin to avoid edema formation during incubation in the tissue chamber and gassed with 5% CO_2-95% O_2. Isometric force was monitored by an external force displacement transducer (Quantametrics, Newtown, PA) connected to a PowerLab data acquisition system (AD Instruments, Colorado Springs, CO). Each strip was stretched to optimal length then allowed to equilibrate for at least 30 min. After the equilibration period, 30 min of basal contractile activity data (contractile strength and frequency) was recorded. The intestinal strips were then treated with 10^{-5} M carbachol and contractile activity data recorded for 5 minutes.

After recording contractile activity, the strip of intestine was removed, blotted lightly, and weighed. The length of each strip was measured. The cross-sectional area of each strip was calculated from length and weight data by assuming that the density of smooth muscle was 1.05 g/cm^3. All force development was normalized to tissue cross-sectional area. Contractile activity was calculated as the area under the curve for 10 minutes. Maximally stimulated contractile activity was calculated as the area under the curve for 2 minutes immediately following carbachol treatment. Contractile frequency was measured over the same time periods.

RNA Extraction

RNA was isolated from frozen seromuscular tissue samples using RNA-Bee (Tel-Test, Inc., Friendswood, TX) following the manufacturer's suggested protocol. After isolation, RNA samples were treated immediately treated with DNase (to remove genomic DNA contamination) and an RNAse inhibitor.

Real-time quantitative PCR

1 μl of the RNA stock solution was reverse transcribed in triplicate at 50°C for 30 minutes using 1× RT buffer, 300nM specific reverse primer, 500 μM dNTPs and Superscript II (LTI, Bethesda, MD). After 10 minutes at 75°C the RT reaction (10μl) was added to 40 μl of PCR mix containing 1× PCR buffer, 400nM primers, 4 mM $MgCl_2$, Taq Polymerase, and 100 nM fluorogenic probe. Amplification and quantitation based on real-time monitoring of amplification was carried out using an ABI Prism 7700 (Applied Biosystems, Foster City, CA) performing 40 cycles of 95°C for 12 sec and 60°C for 1 min, following a 1 minute denaturation step at 95°C. Values of transcripts in unknown samples was obtained by interpolating their Ct (PCR cycles to threshold) values on a standard curve derived from known amounts of cognate, specific amplicons. Transcript levels were normalized to the level of 36B4 RNA. All determinations were performed in triplicate and with a no RT control. The sequences of all primers and TaqMan probes used for amplification reaction assays are summarized in Table 1.

Statistical Analysis

All values are represented as mean ± standard error of the mean (SEM). Values were compared using a paired 2-tailed t-test. A p value of ≤ 0.05 was considered significant.

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Results

Wet to Dry Ratios

There was no significant difference in wet to dry ratio in control and stretched specimens (2.92 ± 0.10 versus 2.65 ± 0.33). This indicates that stretch does not induce development of tissue edema.

Myeloperoxidase Activity

There was no significant difference in myeloperoxidase activity in control and stretched specimens (1e-2 ± 0.008 versus 0.4e-2 ± 0.006). Values are expressed as myeloperoxidase activity per microgram protein.

Nuclear STAT-3 DNA Binding

Nuclear STAT-3 DNA binding was significantly increased in the seromuscular layer between control and stretched segments (1.92 ± 0.11 versus 2.78 ± 0.26). Values are expressed as nuclear STAT-3 DNA binding per microgram protein.

STAT-3 Phosphorylation

STAT-3 phosphorylation was measured in the seromuscular layer in stretched and unstretched controls. There was a significant increase in STAT-3 phosphorylation in the seromuscular (2.03 ± 0.43 versus 0.95 ± 0.08) layers in stretched intestine as compared to unstretched controls. Values are expressed as phosphorylated STAT-3 per microgram protein.

Nuclear NF-kappa B DNA Binding

There was a significant increase in nuclear NF-kappa B DNA binding in seromuscular samples of stretched intestine as compared to unstretched controls (0.68 ± 0.11 versus 0.33 ± 0.07). Values are expressed as nuclear NF-kappa B DNA binding per microgram protein.

MLC Phosphyorylation

There was a significant decrease in MLC phosphorylation in stretched intestine as compared to unstretched controls (0.27 ± 0.11 versus 0.64 ± 0.16). Values are expressed as the ratio of phosphorylated to un-phosphorylated MLC.

Contractile Activity

There was a significant decrease in basal (1.49 ± 0.23 versus 3.51 ± 0.73; stretched versus unstretched) and maximally stimulated (2.17 ± 0.89 versus 6.82 ± 1.67; stretched versus unstretched) contractile strength induced by longitudinal stretch. There were no significant differences in contractile frequency with either basal or maximally stimulated contractile activity. Values are expressed as $g/cm^2/s$.

Expression of iNOS and NHE 1–3

There was no difference in expression of iNOS (1.3 fold, p=0.71). Longitudinal stretch significantly increased expression of NHE 1 (5.5 fold). There were significant trends towards increases in expression of NHE 2 (2.9 fold, p=0.1) and NHE 3 (4.5 fold, p=0.08). (Figure 2)

Discussion

In the results presented, we have demonstrated that reproduction of the early mechanical forces measured with edema development affects various mediators of the smooth muscle contractile apparatus and results in increased NHE expression, STAT-3 and NF-kappa B activation,

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decreased MLC phosphorylation, and decreased intestinal contractility. (Figure 3) We note that this pattern is similar to our previously published in-vivo data seen in intestinal edema induced by mesenteric venous hypertension and high volume crystalloid resuscitation, indicating that mechanical forces may be an important initiating stimuli for the activation of these signaling cascades in intestinal edema. [2–4, 8]

In our in-vivo animal model, edema develops early (i.e., 30 minutes) and is sustained for 12–24 hours. [1, 5] This suggests that potential initiators for activation of dysfunctional signaling cascades occur early in the course of edema. This led us to quantify mechanical changes in the early 30 minute window and we demonstrated there to be significant increases in interstitial pressure as well as changes in intestinal tissue stress/strain. Our hypothesis for the potential role of mechanical forces in edema bore out of the observation that mechanical changes are an early and concurrent finding in the early development of intestinal edema and that mechanical alterations are a sustained finding. [5–7] To that end we sought to recreate the measured mechanical forces in a setting separate from edema thereby excluding any other potential effect or components of edema that may serve as an initiating stimulus. By confirming that edema did not develop as a result of stretch, we approximated a system in which mechanical changes predominate.

Prior to undertaking this set of experiments, we initially evaluated the role of NHE, a known mechanoresponsive membrane channel. In preliminary data, NHE expression is increased with edema and NHE inhibition decreases STAT-3 activation and improves intestinal contractility and transit. [8] This suggests that mechanical stimuli/factors may be important for downstream alterations in cell signaling. Mechanical stretch has been shown to activate the STAT and NF-kappa B pathways as well as the NHE in rat cardiomyocytes. Traditionally, intestinal edema has not been evaluated as a mechanical stimulus, although it is widely appreciated as a physical alteration of tissue, with quantifiable force generation. [5–7]

While there are many known isoforms of NHE, several pieces of evidence point to NHE 1 as a major contributor to edema induced contractile dysfunction.[23, 24] Additonally, the K_i (inhibitory potency) of 5-(N-ethyl-N-isopropyl) amiloride (EIPA), which we and other investigators have used for NHE inhibition is significantly lower for NHE 1 than it is for other NHE isoforms.[25] We therefore suspect that the NHE 1 isoform plays the largest role in edema induced contractile dysfunction.

iNOS expression does not play a role in early mechanotransductive induced contractile dysfunction. We have shown presumably that iNOS may be involved in the cellular pathophysiological response to intestinal edema in our in vivo edema model. Administration of L-nil, an inhibitor of iNOS, has been shown to prevent edema induced ileus. 3 NF-kappa B is known to mediate downstream effects, in part, through transcription of iNOS, which modulates smooth muscle contractility via production of NO. The fact that expression of iNOS was not significantly increased with intestinal stretch does not rule out its role; it may be a late mediator in the global paradigm of edema induced dysfunction. Unlike STAT-3 and NF-kappa B, in which mRNA levels increase as early as 30 minutes following the induction of edema, iNOS mRNA levels do not increase for at least 6 hours after induction of edema, suggesting a transcription requiring sequence of events.[4] [Data not shown] Transcription of iNOS may serve as a second-hit, with NO potentially propagating old and/or initiating new dysfunctional signaling pathways.

Stretch in in-vivo intestinal edema likely occurs in a three dimensional direction, consisting of both longitudinal and circumferential components. A limitation of our study is that our model only tested the effects of stretch in the longitudinal direction. It is difficult to create a circumferential stretch model without other coexisting factors and without significant

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manipulation of the bowel that could confound the results. Nevertheless, we did perform another set of experiments to examine the effects of circumferential stretch. When the bowel is distended by injection of Kreb's solution to approximately 3 times its diameter (what one may clinically see with significant intestinal edema and abdominal compartment syndrome), there are significant increases in STAT-3 phosphorylation in the seromuscular layer (3.09 ± 0.52 versus 1.19 ± 0.27 in stretched versus control sections, respectively). There is also significant trends towards increased NF-kappa B activation (0.08 ± 0.07 versus 0.005 ± 0.3, p=0.19) and decreased MLC phosphorylation (0.32 ± 0.09 versus 0.63 ± 0.12, p=0.06). The lack of statistical significance is likely secondary to the difficulty in ensuring reproducibility of the amount of circumferential stretch, secondary to the limitations of the model.

Activation of certain signaling pathways, such as STAT-3 and NF-kappa B, may represent a host response to injury. However, in addition to any potentially protective effects, activation of these pathways does result in downstream deleterious effects resulting in intestinal contractile dysfunction. Therefore, we are interested in both the initiating stimuli as well as downstream effects of resultant activating signaling pathways.

Ischemic and or inflammatory injury frequently leads to neutrophil infiltration; a surrogate marker of this is myeloperoxidase activity. There is no significant difference between control and stretched segments of intestine, indicating no significant inflammatory/ischemic injury in our model.

In summary, we have shown that mechanical stretch to approximate measured forces induced early by edema, in the absence of edema, results in a similar signaling profile as that initiated by in-vivo intestinal edema. Based on these data, we suggest that mechanical forces may be an important initiating stimuli for activation of signaling pathways that culminate in end-organ dysfunction in intestinal dysfunction. Our findings are summarized in Figure 3. Elucidation of the intermediate components of this signal transduction cascade may allow targeted intervention either upstream to prevent mechanotransductive initiation of the pathway, or later interruption/amplification to mitigate against prolonged intestinal dysfunction.

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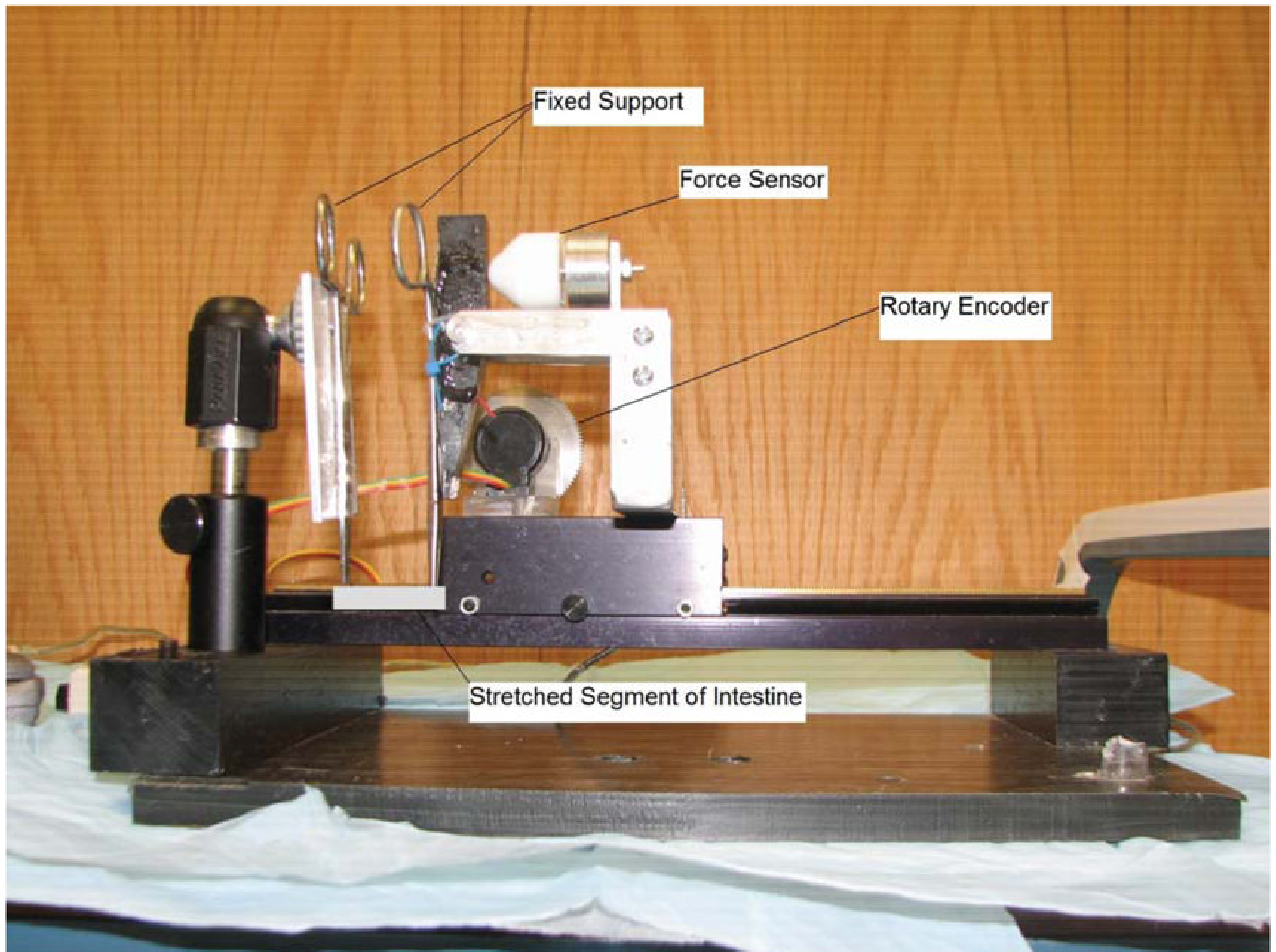

Figure 1.

Figure 1 demonstrates the longitudinal stretch apparatus. A section of intestine is grasped with hemostats and stretched to 122.5% of it's original length. Real time monitoring of the change in length is afforded by the rotary encoder connected to a computer. An adjacent section of intestine is also grasped with hemostats but is not stretched. The animal is kept alive and the intestine perfused throughout the duration of the experiment.

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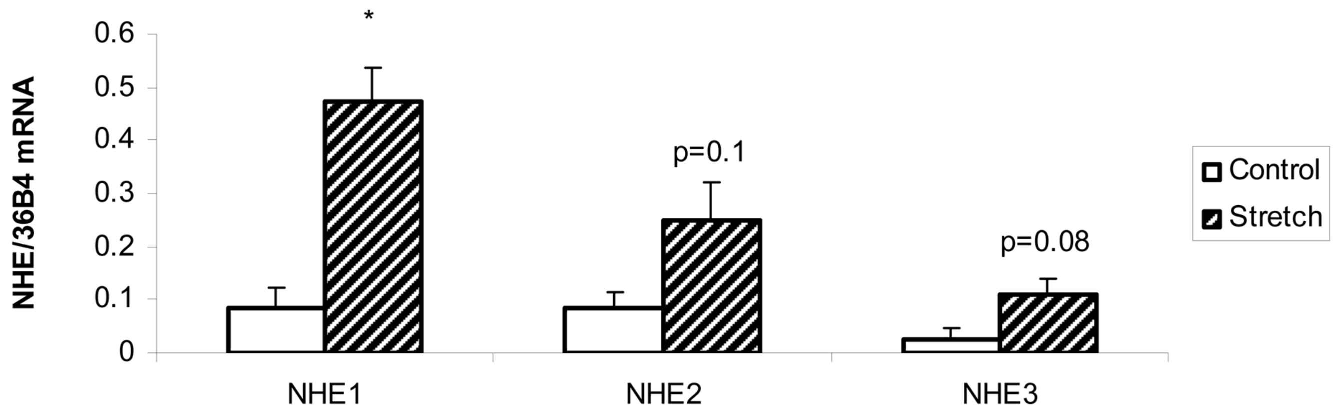

Figure 2.
There is significantly increased expression of NHE 1 in stretched seromuscular samples with significant trends towards increased NHE 2 and NHE 3 expression. * represents statistical significance versus control.

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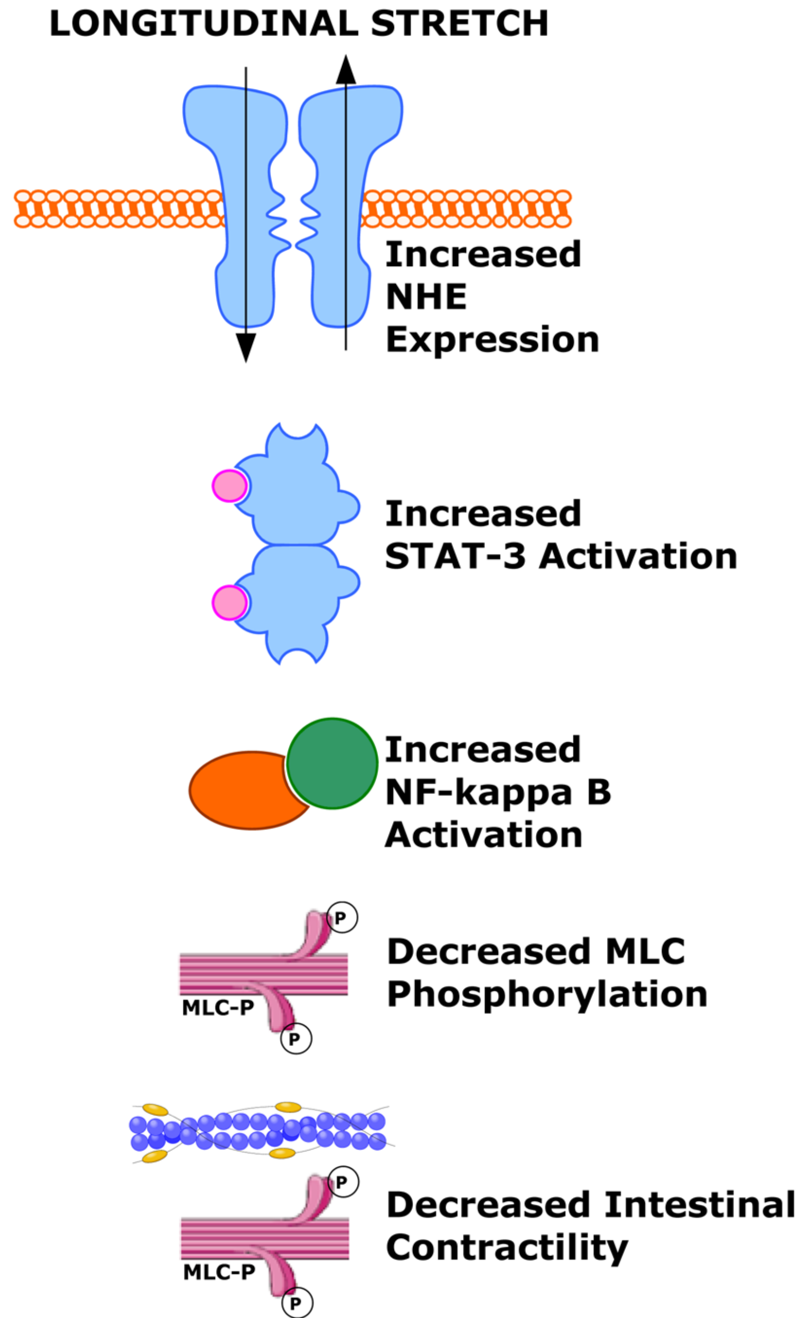

Figure 3.
Similarities between hydrostatic edema induced signaling profile and that induced by approximating measured interstitial pressure without edema. In both, there is increased expression of NHE, activation of NF-kappa B and STAT-3, decreased MLC phosphorylation, and decreased intestinal contractility. Given the concurrent early mechanical changes seen with edema development, mechanical forces may play a role in the initiation of certain signaling pathways.

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Table 1

Primers and probes utilized for qPCR analysis

Transcript	Accn. #	Forward Primer	Reverse Primer	Fluorogenic Probe
36B4	X15096	AGAGGTGCTGGACATCACAG	CATTGCGGACACCCTCTAG	CAGGCCCTGCACACTCGCTT
NHE1 (Slc9A1)	NM_012652	ACACAGTTCCTGGACCACCTT	TCCAGTGATGGTGGCCATA	TGACAGGCATCGAGGACATCTGTGG
NHE2 (Slc9A2)	NM_012653	TGGTATCCTGCTGGGATTCA	GCTCAATGACCCGGATGTT	AGCAGCGTTCACCACCCGTTTCA
NHE3 (Slc9A3)	NM_012654	ACGTGAAGGCCAACATCTCA	ACTTGCCAGCATCTTCATAGTGT	AGCAGTCGGCCACCACCGTG
iNOS	NM_012611	GAGAAGCTGAGGCCCAGG	ACCTTCCGCATTAGCACAGA	CAGTCTTGGTGAAAGCGGTGTTCTTTG