Intestinal edema decreases intestinal contractile activity via decreased myosin light chain phosphorylation

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Objective: **The purpose of this study was to investigate the effects of interstitial edema on intestinal contractile activity.**

Design: **Randomized animal study.**

Setting: **University laboratory.**

Subjects: **Male Sprague–Dawley rats.**

Intervention: **Intestinal edema was induced in rats by a combination of fluid infusion and mesenteric venous hypertension. Rats were divided into four groups: CONTROL, sham; RESUS, saline infusion only; RESUS+VH, saline infusion and venous hypertension; and VH, venous hypertension only. Edema development, basal contractile activity, maximum agonist-induced contractile response (measured as total force generation during the first 2 mins after carbachol treatment), and myosin light chain phosphorylation were measured in the distal small intestine.**

Measurements and Main Results: **The amount of interstitial fluid, indicated by the wet-to-dry ratio, increased significantly in both the RESUS and RESUS+VH groups as early as 30 mins after surgery compared with the CONTROL group. Whereas the tissue fluid remained significantly elevated in the RESUS+VH group up to 6 hrs after surgery, the RESUS group wet-to-dry ratios returned to CONTROL group levels by 2 hrs after surgery. Basal contractile activity was significantly less in the RESUS+VH group compared with either the RESUS group or the CONTROL group 6 hrs after surgery. Maximum contractile response decreased significantly in the RESUS+VH group compared with the RESUS group. Basal contractile activity and maximum contractile response did not change significantly in the VH group compared with the CONTROL group. The phosphorylated fraction of myosin light chain was significantly lower in the RESUS+VH group compared with the CONTROL group at 0.5, 2, and 6 hrs after surgery.**

Conclusion: **We conclude that edema decreases myosin light chain phosphorylation, leading to decreased intestinal contractile activity. (Crit Care Med 2006; 34:2630–2637)**

KEY WORDS: fluid balance; intestinal contractile activity; intestinal smooth muscle; ileus

Many different organ systems develop interstitial edema under a variety of pathologic and traumatic circumstances. Interstitial edema is typically thought to be deleterious and has been associated with dysfunction in several organ systems, including the pulmonary, cerebral, intestinal, and myocardial systems (1–5). In some cases, the mechanism by which edema affects function is known. For instance, in pulmonary edema, interstitial fluid eventually accumulates in the alveoli and interferes with gas exchange (1). In cerebral edema, fluid accumulation can result in increased intracranial pressure, consequently compromising cerebral blood flow (2). In other cases, such as intestinal edema, the mechanism by which edema causes dysfunction is unclear.

Intestinal interstitial edema often develops in trauma patients with abdominal injuries (6, 7). Damage-control laparotomy, abdominal packing, and supranormal resuscitation cause increased abdominal venous pressures and decreased plasma oncotic pressure (7–9). For instance, Balogh et al. (7, 8) showed that 42% of patients receiving supranormal resuscitation developed intra-abdominal hypertension that leads to increased intra-abdominal venous pressures. In its most severe manifestation, intestinal edema can cause abdominal compartment syndrome (the combination of increased intra-abdominal pressure and organ failure); however, more commonly, postresuscitation intestinal edema is associated with ileus, leading to increased morbidity, prevention of enteral feeding, and prolonged hospital stays (7, 10–12).

Postoperative ileus, defined as a decrease in bowel activity after surgery, can last for hours or for days. The mechanism responsible for the development of ileus is not well understood but is likely to be multifactorial and different for acute ileus (within hours after surgery) and prolonged postoperative ileus (>3 days) (13). The different mechanisms proposed for the development of ileus include neurogenic, inflammatory, and pharmacologic (14). As intestinal edema often develops during abdominal surgery, edema may also contribute to the development of postoperative ileus. Thus, the objective of this study was to examine the effects of edema on intestinal motility in the absence of the confounding effects of inflammation.

Experimental evidence from our laboratory shows that intestinal edema causes decreased intestinal transit and increased epithelial permeability (5). Decreased intestinal transit is most likely caused by decreased smooth muscle contractile activity, although alterations in contraction coordination and propagation can also contribute to decreased intestinal transit. Contractile activity of smooth muscle, including intestinal smooth muscle, is determined primarily by the phosphorylation status of the light chain of myosin (MLC) that allows

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interaction of myosin and actin (15). Thus, we investigated the effects of edema on small intestinal contractile activity and MLC phosphorylation. We hypothesized that edema decreases intestinal transit by decreasing intestinal smooth muscle contractile activity via a reduction in MLC phosphorylation.

MATERIALS AND METHODS

Animal Model. Male Sprague–Dawley rats weighing between 250 and 350 g were used for all experiments. All procedures were approved by the University of Texas Medical School Care and Use Committee and are consistent with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*. Animals were fasted overnight preceding surgery and randomly assigned to one of four experimental groups: sham (CONTROL), saline infusion only (RESUS), saline infusion and venous hypertension (RESUS+VH), or venous hypertension only (VH). After anesthetizing the animals with isoflurane, a fluid-filled catheter was inserted into the external jugular vein for saline infusion through a 0.5-cm incision in the neck. The abdominal cavity was opened with a 3-cm midline incision, and the small intestine was exteriorized. The superior mesenteric vein was constricted to the diameter of PE10 tubing (outside diameter, 0.61 mm) in one group of animals (RESUS+VH group). The intestine was then returned to the abdominal cavity, and the incision was closed. Saline was then infused into the jugular catheter (80 mL/kg 0.9% NaCl). Sham-operated animals underwent the same procedure with saline infusion but excluding the constriction of the mesenteric vein (RESUS group). In a third experimental group (VH), the superior mesenteric vein was constricted, but no resuscitation fluids were given. Control animals underwent sham surgery (abdominal incision and exteriorized intestine) without saline infusion or venous constriction (CONTROL group). The animals were allowed to recover. At 30 mins, 2 hrs, or 6 hrs after surgery, animals were killed, and the small intestine was collected and divided into three equal sections. The mid and proximal segments were used for wet-to-dry analysis. The proximal one third of the distal segment was divided into the mucosal and muscularis layers by scraping, and the muscularis layer was frozen for subsequent Western blot analysis. The remaining two thirds of the distal segment were used for contractile activity studies.

This acute rat model of interstitial hydrostatic edema mimics the clinical causes of gut edema in damage-control surgical procedures. No signs of ischemia or inflammation have been detected in this model, thus allowing us to examine the effects of edema without the confounding effects of ischemic injury (5, 16).

Wet-to-Dry Analysis. To determine the amount of edema development in the distal small intestine, wet-to-dry ratios were measured (17). Samples were weighed immediately after collection and then dried in a 60°C oven until the weight did not change (approximately 10 days). The wet-to-dry ratio was calculated as: (wet weight − dry weight)/dry weight.

Quantitation of Cytokines. Cytokine levels, including interleukin (IL)-1α, IL-1β, IL-2, IL-4, IL-6, IL-10, granulocyte–macrophage colony-stimulating factor, interferon-γ, and tumor necrosis factor-α, were measured in cytoplasmic extracts of muscularis samples using a Bio-Plex cytokine assay kit and the Bio-Plex Suspension Array System (Bio-Rad Laboratories, Hercules, CA) according to the manufacturer's instructions and Hulse et al (18). Each sample was assayed in duplicate and normalized to total protein.

Intestinal Contractile Activity. Intestinal contractile activity was measured 2 and 6 hrs after surgery. Strips (approximately 10 mm in length) from the distal third of the small intestine, two from each animal, were mounted in 15-mL organ baths filled with Krebs–Ringer solution (103 mM NaCl, 4.7 mM KCl, 2.5 mM $CaCl_2$, 25 mM $NaHCO_3$, 1.1 mM NaH_2PO_4, 15 mM glucose). The solution was buffered with albumin to avoid edema formation during incubation in the tissue chamber and gassed with 5% CO_2/95% oxygen. Isometric force was monitored by an external force displacement transducer (Grass FT.03; Grass Instrument, Quincy, MA) connected to a PowerLab (AD Instruments, Colorado Springs, CO). Each strip was stretched to optimal length (length at which maximum basal contractile activity was obtained) and then allowed to equilibrate for ≥30 mins. After equilibration, 30 mins of basal contractile activity data were recorded. The strips were then treated with varying doses of carbachol (a cholinergic agonist), from 10^{-3} M to 10^{-9} M, added to the bath, and 5 mins of data were recorded after addition of each dose. The strips were washed and allowed to re-equilibrate for 5 mins between each dose. After recording contractile activity, the length of each strip was measured, removed, blotted lightly, and weighed. Contractile activity was calculated as the area under the curve for 10 mins. Maximum contractile response was calculated as the area under the curve for the first 2 mins after addition of 10^{-5} M carbachol (the dose of carbachol that gives the maximum contractile response according to the dose-response curves). 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 and expressed as stress.

Western Blot Analysis. Frozen distal small intestine muscularis tissue was homogenized in 50 mM Tris (pH 7.4) containing protease inhibitors (Halt Kit Protease Inhibitor Cocktail, Pierce Chemicals, Rockford, IL) and phosphatase inhibitors (2 mM orthovanadate and 2 mM sodium fluoride). The samples were then sonicated (50 pulses at 50% at 4°C). After centrifugation to remove cellular debris, the supernatants were collected and subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis using standard methods.

MLC and MLC-P antibodies were obtained from Sigma-Aldrich (St. Louis, MO) and Cell Signaling Technology (Danvers, MA), respectively, and used at a 1:750 dilution. Secondary antimouse immunoglobulin G–horseradish peroxidase conjugate was obtained from Sigma-Aldrich Chemicals. A β-actin antibody (Sigma-Aldrich) was used for normalization. Enhanced chemical luminescence, followed by exposure to autoradiography film, was used to visualize the proteins on the membranes (ECL Plus, Amersham Biosciences, Piscataway, NJ). Bands on the film were quantitated using Image J software (19).

Statistics. All data are reported as mean ± SEM. Values were compared using an analysis of variance with a Newman–Keuls *post hoc* analysis. A p value of $<.05$ was considered significant. The correlation between wet-to-dry ratios and MLC phosphorylation was determined as follows. The best fit line was determined using the least-squares method. The F statistic derived from this correlation was used to determine the significance of the correlation.

RESULTS

Edema Development. As shown in Figure 1, there were no significant differences in CONTROL group wet-to-dry ratios over time. However, the amount of interstitial fluid, indicated by the wet-to-dry ratio, increased significantly as early as 30 mins after surgery in the RESUS+VH group compared with the CONTROL group (RESUS+VH, 4.53 ± 0.19, n = 6 vs. CONTROL, 3.49 ± 0.07, n = 6) and remained increased in the RESUS+VH group for ≥6 hrs after surgery (3.88 ± 0.09, n = 10) compared with the CONTROL group (3.40 ± 0.03, n = 10). In the RESUS group, a transient increase in wet-to-dry ratios occurred at 30 mins after surgery (RESUS, 4.44 ± 0.15, n = 6). However, the wet-to-dry ratios in the RESUS group (3.47 ± 0.04, n = 7) returned to CONTROL group levels (3.46 ± 0.04, n = 7) by 2 hrs after surgery. Wet-to-dry ratios in the VH group (3.60 ± 0.04, n = 6) and the RESUS group (3.55 ± 0.04, n = 11) were not significantly different from the CONTROL group 6 hrs after surgery.

Cytokine Levels. Cytokine levels, including IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-10, granulocyte–macrophage colony-stimulating factor, interferon-γ, and tumor necrosis factor-α, measured in intestinal muscularis cytoplasmic extracts in CONTROL and RESUS+VH groups at baseline (0 hrs), 30 mins, 2 hrs, and 6 hrs are shown in Figure 2. There was a slight

but significant decrease in granulocyte–macrophage colony-stimulating factor at 6 hrs in the RESUS+VH group compared with the CONTROL group. Otherwise, there were no significant differences in cytokine levels between CONTROL and RESUS+VH groups.

There were several significant changes in cytokines over time. IL-1α and IL-1β increased significantly at the 2- and 6-hr time points compared with 0 hrs (Fig. 2, *A* and *B*, respectively). IL-6 increased at the 30-min time point but returned to baseline by 2 hrs after surgery (Fig. 2*D*). Granulocyte–macrophage colony-stimulating factor decreased significantly at 2 and 6 hrs after surgery compared with 0 hrs (Fig. 2*F*). No significant changes occurred in IL-2 or IL-10 (Fig. 2, *C* and *E*, respectively) or interferon-γ (data not shown). IL-4 and tumor necrosis factor-α were below the detectable limits of the assay (data not shown).

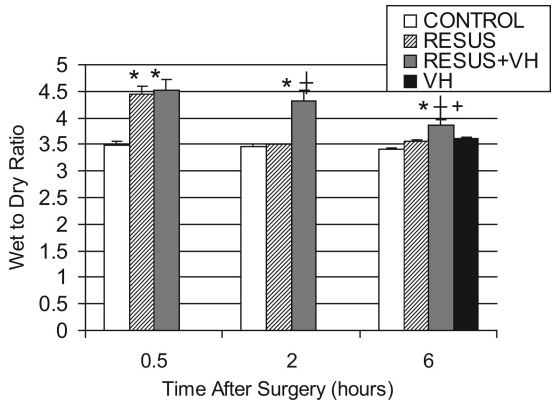

Figure 1. Wet-to-dry ratios expressed as mean ± SE, indicating the amount of edema development, in each group at 30 mins, 2 hrs, and 6 hrs after surgery. *CONTROL*, sham operation with no saline infusion or venous hypertension: n = 6, 7, and 10 for 30 mins, 2 hrs, and 6 hrs, respectively; *RESUS*, sham operation plus saline infusion: n = 6, 7, and 11 for 30 mins, 2 hrs, and 6 hrs, respectively; *RESUS+VH*, saline infusion plus venous hypertension: n = 6, 6, and 10 for 30 mins, 2 hrs, and 6 hrs, respectively; *VH*, venous hypertension only: n = 6; *$p < .05$ vs. CONTROL; †$p < .05$ vs. RESUS; +$p < .05$ vs. VH.

Basal Contractile Activity. Isolated strips from the distal third of the small intestine exhibited intrinsic rhythmic contractile activity. Basal contractile activity, calculated from unstimulated contractions in the distal small intestine per cross-sectional area, is shown in Figure 3. Two hours after surgery, basal contractile activity tended to decrease in the RESUS+VH group compared with the CONTROL group (56.31 ± 9.71 $g \cdot cm^{-2} \cdot sec^{-1}$ vs. 78.44 ± 11.10 $g \cdot cm^{-2} \cdot sec^{-1}$, $p = .08$). Basal contractile activity, measured 6 hrs after surgery, was significantly less in the RESUS+VH group

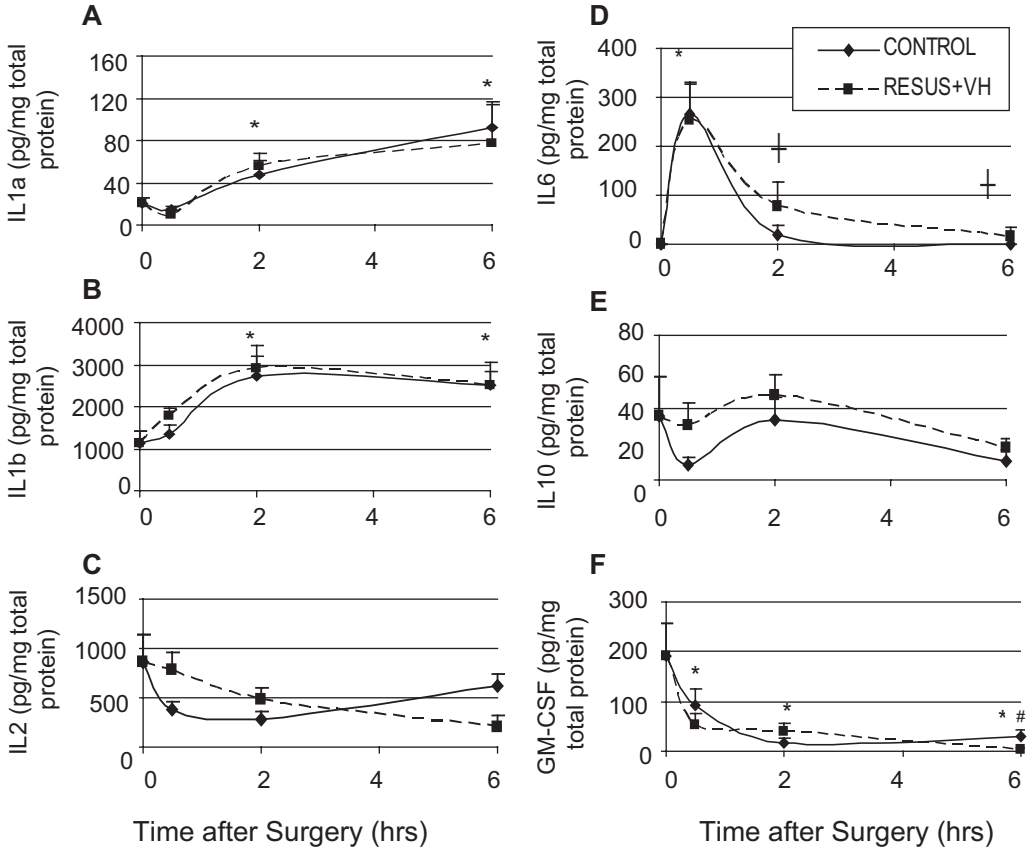

Figure 2. Changes in cytokine levels over time measured in intestinal muscularis cytosolic fractions. The *solid lines* represent the sham operation (*CONTROL*) group, and the *dashed lines* represent the group that received saline infusion and venous hypertension (*RESUS+VH*) group (n = 6 per group). *A–F* show interleukin (*IL*)-1α, IL-1β, IL-2, IL-6, IL-10, and granulocyte–macrophage colony-stimulating factor (*GM-CSF*), respectively. Data are shown as mean ± SE. *$p < .05$ vs. 0 hrs; †$p < .05$ vs. 30 mins; #$p < .05$ CONTROL vs. RESUS+VH.

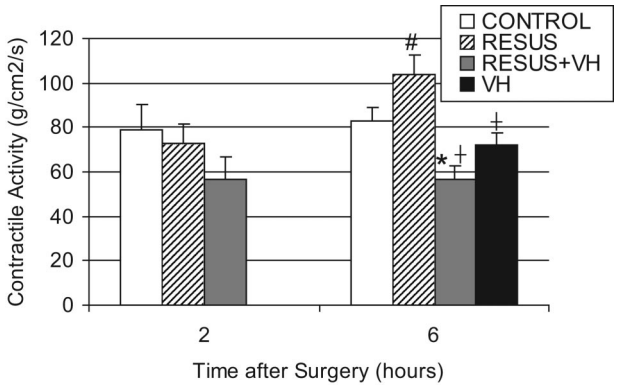

Figure 3. Changes in average basal contractile activity in the rat distal small intestine due to edema development 2 and 6 hrs after surgery. Data are expressed as mean ± SE; n = 6 for each group at 2 hrs. At 6 hrs, n = 10, 12, 9, and 6 for sham operation (*CONTROL*), saline infusion only (*RESUS*), saline infusion and venous hypertension (*RESUS+VH*), venous hypertension only (*VH*) groups, respectively. *$p < .05$ vs. CONTROL; †$p < .05$ vs. RESUS; #$p < .05$ vs. 2 hrs.

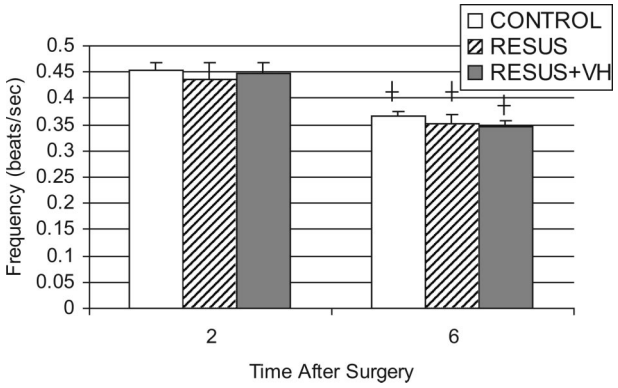

Figure 4. The frequencies of basal contractions are shown at 2 and 6 hrs after surgery in all three groups. Number of animals are the same as in Figure 3. Data are shown as mean ± SE. *CONTROL*, sham operation; *RESUS*, saline infusion only; *RESUS+VH*, saline infusion and venous hypertension; *VH*, venous hypertension only; †$p < .05$ vs. respective 2-hr group.

(56.78 ± 6.19 $g \cdot cm^{-2} \cdot sec^{-1}$) compared with either the RESUS group (103.69 ± 8.71 $g \cdot cm^{-2} \cdot sec^{-1}$) or the CONTROL group (82.99 ± 6.29 $g \cdot cm^{-2} \cdot sec^{-1}$). Although there was an increase in contractile activity in the RESUS group compared with the CONTROL group, this difference was not significant ($p = .08$). Basal contractile activity in the VH group was not significantly different from the CONTROL group.

The frequencies of basal contractile activity were not significantly different among the three groups (Fig. 4). The frequency of contractions in the CONTROL, RESUS, and RESUS+VH groups were 0.3675 ± 0.0078, 0.3521 ± 0.018, and 0.345 ± 0.014 beats/sec, respectively, 6 hrs after surgery. There was a significant decrease in frequency of basal contractions in rats killed 6 hrs after surgery compared with the respective groups killed 2 hrs after surgery.

Maximum Contractile Response. Carbachol induced a tonic contraction of the distal intestinal strip. Dose-response curves to carbachol were generated in the CONTROL, RESUS, and the RESUS+VH groups to determine the dose for maximum contractile response in the rat distal small intestine. As shown in Figure 5*A*, a 10^{-5} M dose of carbachol produced a maximum response in the rat distal small intestine; thus, a carbachol dose of 10^{-5} M was used to calculate maximum contractile response in subsequent experiments. Figure 5*B* shows maximum contractile response in each group 2 and 6 hrs after surgery. At 2 hrs, there were no significant differences in maximum contractile response. However, maximum contractile response was significantly lower in the RESUS+VH group (28.77 ± 4.46 $g \cdot cm^{-2} \cdot sec^{-1}$) compared with the RESUS group (77.99 ± 7.71 $g \cdot cm^{-2} \cdot sec^{-1}$) 6 hrs after surgery. There was no significant difference in maximum contractile response between CONTROL, RESUS, and VH groups.

MLC Phosphorylation. Changes in MLC phosphorylation associated with edema development in the rat distal small intestine are shown in Figure 6. Figure 6*A* and 6*B* show representative blots at 2 hrs and 6 hrs after surgery, respectively. Figure 6*C* shows the quantitation of phosphorylated MLC (MLC-P) to MLC. In the RESUS group, which experienced a transient increase in wet-to-dry ratios, the MLC-P/MLC ratio decreased at 30 mins after surgery and started returning to CONTROL levels at the 2- and 6-hr time points (0.42 ± 0.11, 0.68 ± 0.07, and 0.82 ± 0.15 at 0.5, 2, and 6 hrs, respectively). The fraction of MLC that was phosphorylated was significantly lower in the RESUS+VH group compared with the CONTROL group at all time points (CONTROL: 0.99 ± 0.09, 1.01 ± 0.05, and 0.97 ± 0.08 at 0.5, 2, and 6 hrs, respectively; RESUS+VH: 0.33 ± 0.06, 0.65 ± 0.05, and 0.49 ± 0.02 at 0.5, 2, and 6 hrs, respectively). Furthermore, at 6 hrs after surgery, the MLC-P/MLC ratio in the RESUS+VH group decreased significantly compared with the RESUS group (0.82 ± 0.15).

Figure 7 shows the correlation between MLC phosphorylation and edema development in the distal small intestine 6 hrs after surgery. There is a significant relationship between the wet-to-dry ratio and the MLC-P/MLC ratio ($p = .034$, $R^2 = .23$).

In addition to the decreased ratio of MLC-P/MLC, the total amount of MLC measured by Western analysis and normalized to β-actin changed at the 6-hr time point. As demonstrated in Figure 8, there is no difference in MLC/β-actin among groups 2 hrs after surgery. However, at 6 hrs after surgery, MLC/β-actin decreases significantly in the RESUS+VH group (0.49 ± 0.05) compared with the CONTROL group (1.01 ± 0.13).

The effect of venous hypertension only on the quantity of MLC protein and the fraction of MLC that is phosphorylated is shown in Figure 9. The fraction of MLC that is phosphorylated in the VH only group (MLC-P/MLC) is significantly increased compared with the CONTROL group 6 hrs after surgery. However, the total amount of MLC normalized to β-actin (MLC/β-actin) decreased in the VH only group compared with the CONTROL group. Thus, the combination of increased MLC-P fraction and decreased total MLC resulted in no change in the total amount of MLC-P normalized to β-actin (MLC-P/β-actin) in the VH only group compared with the CONTROL group ($p = .43$).

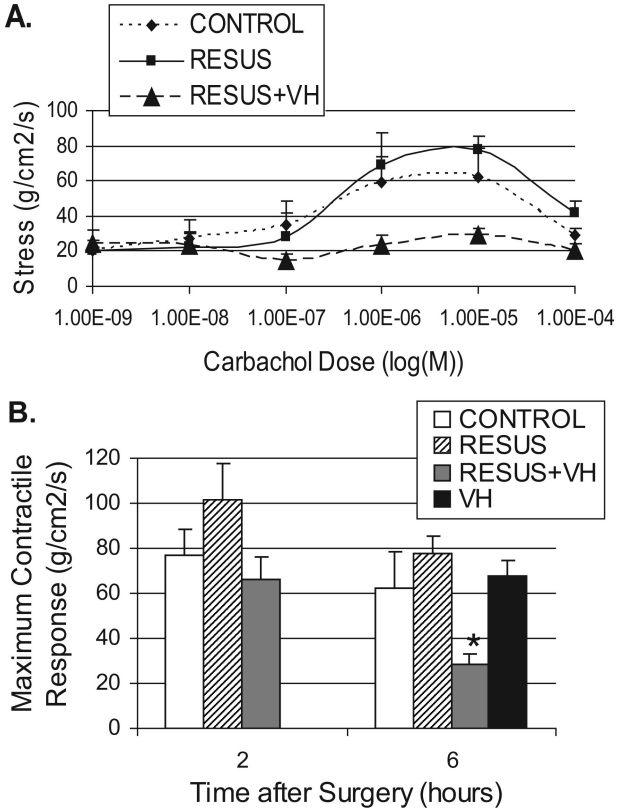

Figure 5. Maximum contractile response in the rat distal small intestine 6 hrs after surgery. *A*, the dose-response curve to carbachol in the sham operation (*CONTROL*), saline infusion only (*RESUS*), and saline infusion and venous hypertension (*RESUS+VH*) groups; *B*, maximum contractile response to 10^{-5} M carbachol in each group 2 and 6 hrs after surgery; n = 6 for each group at both 2 and 6 hrs. Data are shown as mean ± SE. *$p < .05$ vs. RESUS.

DISCUSSION

The results of this study show that a combination of venous hypertension and resuscitation fluids induces a significant amount of edema formation that results in decreased intestinal contractile activity by decreasing MLC phosphorylation. Previously, we have shown that intestinal edema is associated with decreased intestinal transit (5). In this study, we have now shown a potential mechanism by which edema can induce decreased transit.

This model of intestinal edema mimics the clinical situation of damage-control surgical procedures in trauma that result in increased abdominal venous pressures via abdominal packing and decreased plasma oncotic pressure through crystalloid infusion. Furthermore, we are able to examine the effects of edema on the small intestine in this model without the confounding effects of ischemia/reperfusion injury. There is no evidence of ischemia/reperfusion injury in this model as evidenced by unchanged portal venous lactate concentration (5), no significant changes in neutrophil recruitment into the small intestine (5), and no significant differences in either arterial or venous oxygen between the groups (16).

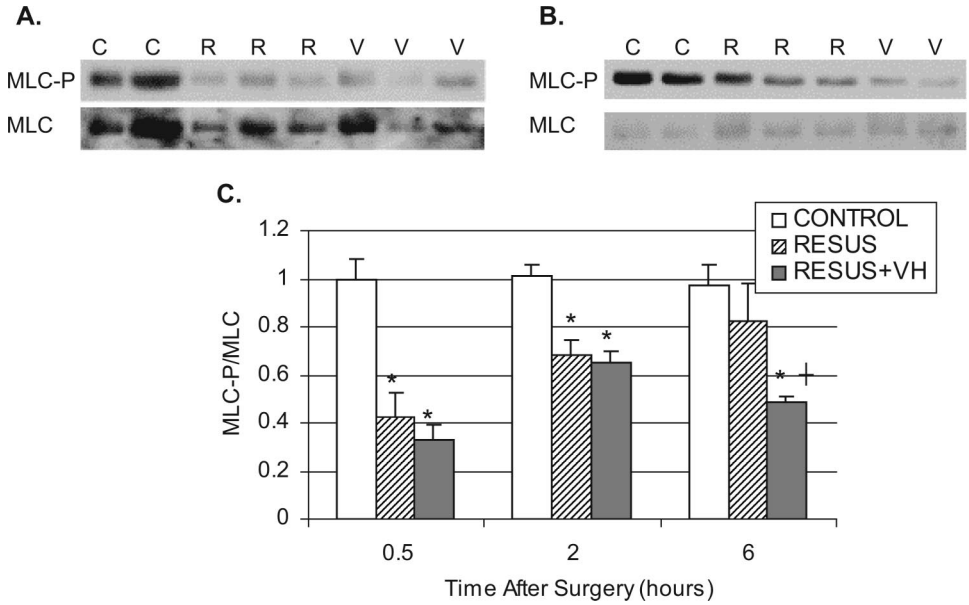

Figure 6. Changes in the ratio of phosphorylated myosin light chain (*MLC-P*) to total myosin light chain (*MLC*) in the rat distal small intestine muscularis layer. *A* and *B* are representative myosin light chain phosphorylation immunoblots at 2 and 6 hrs after surgery, respectively. *C* shows quantitation of these blots. Data are shown as mean ± SE. Definitions: *C*, sham operation group (*CONTROL*); *R*, saline infusion only group (*RESUS*); *V*, saline infusion and venous hypertension group (*RESUS+VH*). *$p < .05$ vs. CONTROL; †$p < .05$ vs. RESUS; n = 4, 6, and 6 at 2 hrs and n = 8, 9, and 6 at 6 hrs in CONTROL, RESUS, and RESUS+VH groups, respectively.

This resuscitation/venous hypertension model produces a significant amount of intestinal edema. As there is no evidence of cellular injury in this model, we assume that the fluid accumulates in the interstitium. In our laboratory, the physiologic/pathologic range of wet-to-dry ratios is approximately 2.5–5 (5, 16). Thus, an intestinal wet-to-dry ratio increase of 0.4, as in the RESUS+VH rats vs. CONTROL rats at 6 hrs after surgery, is a substantial change in the amount of tissue fluid accumulation.

In this model of intestinal edema, edema development was associated with both decreased basal contractile activity and decreased carbachol-induced contraction. Intestinal contractile function was not significantly reduced in the RESUS-only group, which experienced only transient edema development, or in the VH-only group, which experienced a slight increase in edema development ($p = .07$ vs. the CONTROL group) 6 hrs after surgery. Therefore, the decrease in intestinal function could not be attributed to either the fluid resuscitation or the venous hypertension alone. Thus, we conclude from these data that the decreased intestinal contractile activity in this model is due to the edema development.

There were no differences in the contractile function 2 hrs after surgery, although basal contractile activity tended to be lower in the RESUS+VH group compared with the CONTROL group. However, the effects of anesthesia and surgical manipulation of the gut have a large effect on intestinal function so soon after surgery and may blunt any differences in function between the groups. As shown in Figure 3, contractile activity in the CONTROL and RESUS groups tended to be lower at 2 hrs after surgery compared with 6 hrs after surgery ($p = .067$ for combined CONTROL and RESUS groups).

Surgical stress alone has been shown to cause decreased intestinal contractile activity. Manipulation of the small intestine was shown to slow gastrointestinal transit and inhibit colonic contractile activity (20). Evidence suggests that a local inflammatory

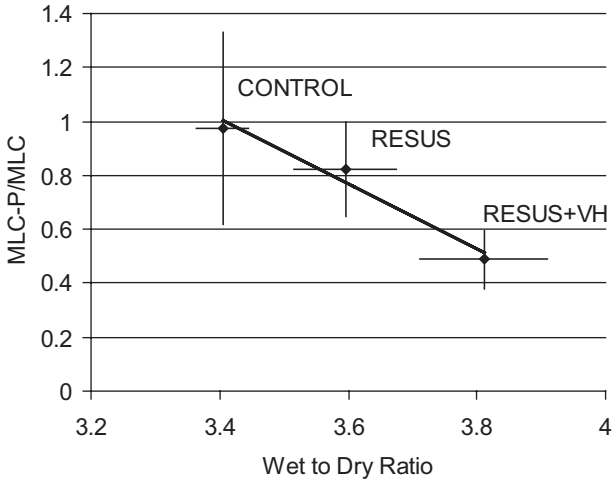

Figure 7. Correlation between wet-to-dry ratios and myosin light chain phosphorylation. The average wet-to-dry ratios vs. the average ratio of phosphorylated myosin light chain (*MLC-P*) to total myosin light chain (*MLC*) at 6 hrs after surgery are shown. Data are shown as mean ± SE; $R^2 = .24$; $p < .05$. *CONTROL*, sham operation; *RESUS*, saline infusion only; *RESUS+VH*, saline infusion and venous hypertension.

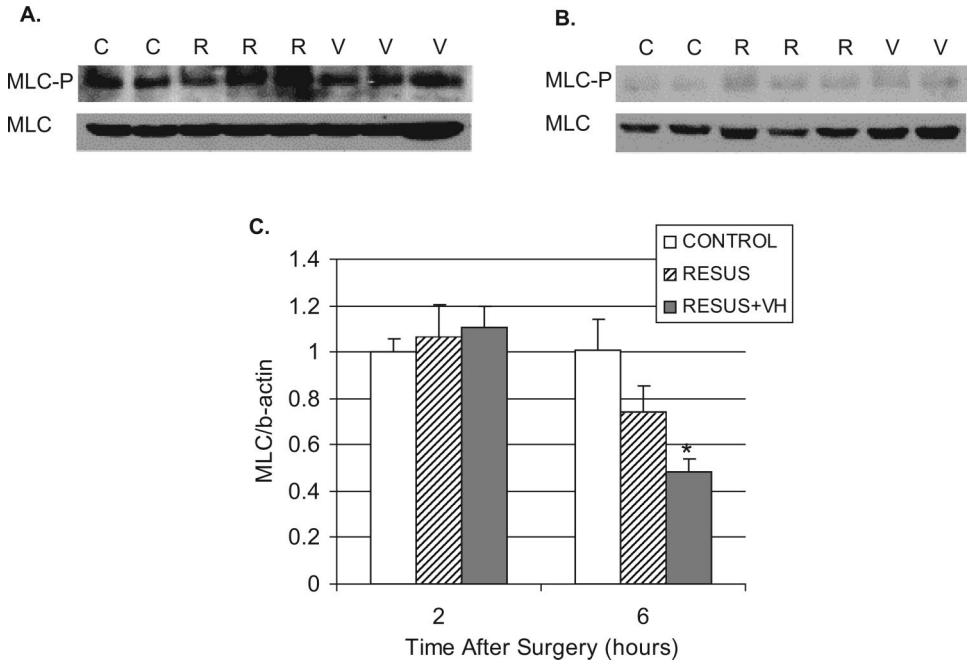

Figure 8. Changes in myosin light chain in rat distal small intestine muscularis. *A* and *B*, representative myosin light chain immunoblots and β-actin immunoblots at 2 and 6 hrs, respectively; *C*, quantitation of these blots. Data are shown as mean ± SE. Definitions: *C*, sham operation group (*CONTROL*); *R*, saline infusion only group (*RESUS*); *V*, saline infusion and venous hypertension group (*RESUS+VH*); *MLC-P*, phosphorylated myosin light chain; *MLC*, myosin light chain. *$p < .05$ vs. CONTROL; n = 4, 6, and 6 at 2 hrs and n = 8, 9, and 6 at 6 hrs in CONTROL, RESUS, and RESUS+VH, respectively.

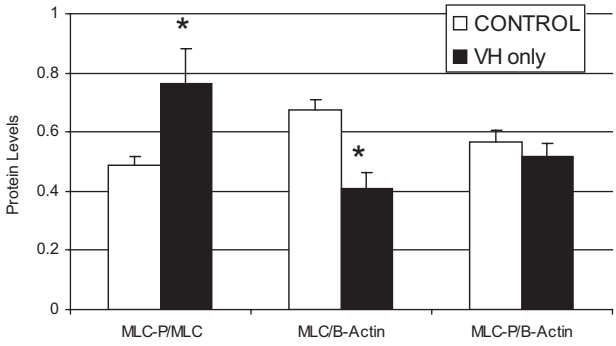

Figure 9. Changes in myosin light chain protein and myosin light chain phosphorylation due to venous hypertension only (*VH*) compared with CONTROL at 6 hrs after surgery. The ratios of phosphorylated myosin light chain (*MLC-P*) to total myosin light chain (*MLC*), MLC to β-actin, and MLC-P to β-actin are shown. *$p < .05$ vs. saline infusion and venous hypertension group, n = 4 for the saline infusion and venous hypertension group and n = 5 for the VH only group.

response triggered by IL-6 participates in the intestinal dysfunction induced by surgical stress (21). In our model of intestinal edema, the gut was manipulated in all four groups, so the effects of surgical stress should be the same in all groups. Thus, differences in contractile activity in the RESUS+VH group compared with the CONTROL group cannot be attributed to manipulation of the gut.

Surgical stress was shown to elicit a local inflammatory response resulting in the release of IL-6 in the small intestine (21). In our model of intestinal edema, we measured nine different cytokines in the distal small intestinal muscularis. Several proinflammatory cytokines were increased after surgery, including IL-6 and IL-1β. These data are similar to the data shown by Schwartz et al. (20) and Wehner et al (21). In addition, the anti-inflammatory granulocyte–macrophage colony-stimulating factor was decreased after surgery. However, we found no differences in cytokine levels between the edematous RESUS+VH group and the CONTROL group. Thus, is seems unlikely that differences in intestinal contractile activity during edema development in our model can be attributed to inflammatory mediators.

Interstitial edema has been shown to cause dysfunction, not only in the intestine, but also in the heart. Myocardial edema was shown to decrease contractile activity and slow isovolumic relaxation (3). However, the mechanism by which edema causes dysfunction in the heart or the intestine is unknown. Edema may induce molecular changes in the cardiac muscle that leads to tissue remodeling, as evidenced by the cardiac fibrosis development associated with myocardial edema (22). Our data suggest that edema also triggers molecular changes in intestinal smooth muscle, including decreased MLC phosphorylation that may lead to tissue remodeling.

The rat small intestinal smooth muscle contracts spontaneously *in vivo* and *in vitro*, even without extrinsic neural input. Thus, the decreased basal contractile activity measured in this study reflects decreased intrinsic contraction of the smooth muscle. Data in the literature indicate that spontaneous intestinal contractions originate from interstitial cells of Cajal in the myenteric plexus between the circular and longitudinal muscle layers (23, 24). Rhythmic oscillation of membrane potentials in interstitial cells of Cajal get transmitted to the surrounding interstitial cells of Cajal and smooth muscle cells through gap junctions to cause spontaneous smooth muscle contraction in the small intestine. As the frequency of contractions was unchanged in our experiments, it is unlikely that edema affects these pacemaker cells. However, changes in the phosphorylation of MLC may account for the edema-induced decrease in intestinal contractile activity.

Edema development in our model was associated with close to a 50% reduction in the ratio of MLC phosphorylation to total MLC (Fig. 6). Furthermore, the total amount of MLC protein was reduced by approximately 50% (Fig. 8). Thus, the total amount of MLC-P was reduced by almost 75%. There was a significant correlation between the level of MLC phosphorylation and the amount of tissue fluid, suggesting a dependent relationship between the two variables. This was true especially for the CONTROL and RESUS+VH groups. As the RESUS group experienced only transient edema development, MLC phosphorylation levels may have been in the process of returning to normal in this group at 6 hrs after surgery. Venous hypertension alone (VH group) did not significantly reduce the fraction of MLC phosphorylated nor was the contractile activity significantly reduced in the VH only group. Thus, change in contractile activity and MLC phosphorylation cannot be attributed to venous hypertension.

As shown in Figure 1, fluid resuscitation alone induced significant fluid accumulation 30 mins after surgery; however, this edema development was resolved by 2 hrs after surgery. In the RESUS group, this transient edema formation caused a significant decrease in MLC phosphorylation at 30 mins after surgery that started to return toward CONTROL group levels by 2 and 6 hrs after surgery (Fig. 6). Thus, although there was a time lag between the resolution of edema and the return of MLC phosphorylation to CONTROL group levels, edema development seemed to be associated with decreased MLC phosphorylation in this group. Edema developed at 30 mins after surgery in the RESUS+VH group did not resolve. The fraction of MLC that was phosphorylated was significantly reduced from 30 mins to 6 hrs after surgery in the RESUS+VH group (Fig. 6). As the edema did not resolve in the RESUS+VH group, the MLC phosphorylation levels were also decreased at each time point in this group. Thus, an inverse correlation exists between the fraction of MLC phosphorylated and the amount of fluid accumulation in the intestine by 6 hrs after surgery (Fig. 7). Neither resuscitation fluid administration alone or venous hypertension alone caused decreased MLC phosphorylation or decreased contractile activity at 6 hrs after surgery. We conclude from this data that the decreased intestinal contractile activity associated with edema development is at least partially due to edema-induced changes in MLC phosphorylation.

Phosphorylation of MLC facilitates interaction of actin and myosin to cause contractions. The highly regulated phosphorylation status of MLC is a result of the balance between MLC kinase and MLC phosphatase. Increased intracellular Ca^{2+}, in response to mechanical or hormonal stimuli, combines with calmodulin to activate MLC kinase. MLC phosphatase activity, on the other hand, is Ca^{2+} independent. MLC phosphatase activity can be regulated through a number of Ca-independent pathways that mainly act through Rho kinase (25). Because we did not measure intracellular calcium levels, it is unclear whether the changes in MLC phosphorylation are due to calcium-dependent or independent mechanisms.

Decreases in the total amount of MLC occur in both the RESUS+VH group and the VH only group. Thus, decreased MLC protein is likely due to the presence of venous hypertension rather than the presence of edema. In the RESUS+VH group, both the fraction of MLC phosphorylated and the total amount of MLC was decreased, resulting in a net decrease in MLC-P. However, in the VH only group, the phosphorylated fraction of MLC was significantly increased, whereas the total amount of MLC was decreased, resulting in no net change in the amount of MLC-P compared with the CONTROL group.

To summarize, edema development induced by a combination of venous hypertension and fluid administration was associated with both decreased basal contractile activity, decreased maximum contractile response induced by cholinergic agonist, and decreased MLC phosphorylation. Resuscitation only or venous hypertension only did not result in increased edema formation, decreased contractile activity, or decreased MLC phosphorylation by 6 hrs after surgery. Thus, we conclude that intestinal edema induces decreased intestinal contractile activity via decreased MLC phosphorylation.

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