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Mechanical analysis of intestinal contractility in a neonatal maternal deprivation irritable bowel syndrome rat model

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ABSTRACT

The aims of the present study are to investigate biomechanical properties and provide mechanical analysis of contractility in ileum and colon in a neonatal maternal deprivation (NMD) irritable bowel syndrome (IBS) rat model. Mechanical testing was done on segments from ileum and colon in 25 IBS rats and 13 Control rats. Morphometric data were obtained from digitized images of the segments at no-load and zero-stress states. Pressure and diameter changes were measured during flow and ramp distensions under active and passive experimental conditions. Circumferential stresses (force per area) and strains (deformation) were computed with referenced to the zero-stress state. The contraction frequency was analyzed. Contraction thresholds and maximum contraction amplitude were calculated in terms of mechanical stress and strain. Compared with controls, the IBS rats had lower body weight ($P < 0.01$), smaller colonic opening angle ($P < 0.05$), higher colonic contraction frequency ($P < 0.05$ and $P < 0.01$) and lower contraction thresholds of pressure, stress and strain in both ileum and colon ($P < 0.05$ and $P < 0.01$). The maximum contraction pressure, stress and strain did not differ between IBS and Control groups ($P > 0.05$). In conclusion, the pressure, stress, and strain to evoke contractility in ileum and colon were lower whereas the frequency of induced colon contractions was higher in NMD IBS rats compared to normal rats. Furthermore, zero-stress state remodeling occur in colon in NMD IBS rats. Further studies on the association between intestinal biomechanical properties, hypersensitivity and afferent signaling in the IBS animal models are warranted.

1. Introduction

Irritable bowel syndrome (IBS) is one of the most common functional gastrointestinal disorders worldwide (Simrén et al., 2018). According to Rome IV Criteria, IBS is diagnosed on basis of recurrent abdominal pain related to defecation or in association with a change in stool frequency or form (Ford et al, 2017). It affects people of all ages and both sexes though it is more frequent in women. Increased visceral sensitivity is observed in up to 60% of IBS patients (Mujagic et al., 2017). Visceral sensitivity is the term used to describe the experience of sense within the inner organs (viscera) in responses to different stimuli. However, causes and mechanisms of visceral hypersensitivity in IBS patients are not well understood.

Structural and biomechanical properties of the intestines are important for intestinal sensory-motor functions (Zhao et al., 2017). Structural changes may alter the relative positions and activation properties of the mechanosensitive afferents. Furthermore, mechanical wall remodeling affect tension and stress distributions that act on the mechanosensitive afferents. Consequently, the afferent sensitivity to stimulations may change. Evidence suggests that the primary abnormality in IBS patients occurs at the level of mechanosensitive intestinal afferents (Accarino et al., 1995; Azpiroz, 1999). Therefore, it is important to study whether IBS is associated with biomechanical remodeling in the intestine. Decreased compliance of colon and rectum has been demonstrated in IBS patients (Prior et al., 1990; Zar et al., 2006; Park et al., 2008; van der Veek et al., 2008; Törnblom et al., 2014). However, evidence of biomechanical remodeling has not been shown in human IBS intestine. Furthermore, to the best of our knowledge, biomechanical properties in the intestine in IBS animal models have not been studied so far.

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The contraction threshold during stimulation is a proxy of intestinal sensitivity (Brock et al., 2009) and that contraction amplitude reflects contractile strength. The maximum frequency of intestinal motility reflects slow wave frequency (Sanders et al., 2006). We have previously studied intestinal contractility in humans and in various animal models and developed analysis for such assessments based on mechanical stress and strain calculations (Gregersen et al., 2007; Zhao et al., 2008, 2013; Liu et al., 2019). Such contractility parameters have presumably not been reported for IBS-affected intestine.

When human studies cannot be carried out, an appropriate animal model must be selected. The neonatal maternal deprivation (NMD) rat model results in permanent visceromotor and somatic alterations associated with neurochemical changes, altered hypothalamic pituitary adrenal responsiveness to stressors, and increased risk of developing depression-like behavior. Hence, it mimics all the main features of human IBS (Deiteren et al., 2016). Using this model, Ren et al. (2007) studied neurochemical and sensory responses to colonic distension and demonstrated that NMD rats exaggerated neurochemical responses and visceral hyperalgesia in colon. Therefore, NMD constitutes a valuable experimental model to study IBS pathophysiology. In this study, the passive biomechanical properties of ileum and colon were studied in the NMD IBS rat model. Distension-induced contraction thresholds and frequencies and maximum contraction amplitude of flow-induced contractions in terms of stress and strain were analyzed. We hypothesized that the passive stress-strain relation of the ileum and colon will reflect increased stiffness and that the threshold to evoke contractility decreased in NMD IBS rats.

2. Material and methods

2.1. Animals and groups

Approval of the protocol was obtained from the Danish Committee for Animal Experimentation (2008-561-1530). The NMD model was adopted since it mimics human IBS (Barreau et al., 2007). Wistar neonates from postnatal day 1 were used. After delivery (day 1), litters were culled for the separation group and control group. Maternal deprivation was done daily from 9:00am to 12:00noon between postnatal days 2–14 where pups were removed from their home cage and kept in temperature-controlled cages at 28 °C. During maternal deprivation, pups were individually isolated. Control pups were left undisturbed with their dam. From days 15–22, all control and maternally deprived pups were maintained with their dam. Weaning was performed on day 22, the siblings were housed in the same cage until experiments were done at age 12 weeks. Bedding was changed every day. Twenty-five IBS rats (17 females and 8 males) were compared to a sex-matched group of control rats (9 females and 4 males). The sex ratio and matching reflects that IBS in humans occurs more frequently in females than in males

2.2. In vivo intestinal preparation

At the termination of experiments, the rats were anesthetized with Hypnorm (fentanyl/fluanisone) 0.05 mg kg^{-1} and Dormicum (midazolam) 0.025 mg kg^{-1}. The abdominal cavity was opened with careful dissection of colon and ileum. The middle part of colon and ileum starting from 5 cm proximal to the end of ileum were used for quantitative assessment of contractility and for passive distension experiments. Short colon and ileum segments proximal and distal to above segments were used for no-load and zero-stress state analysis.

2.3. The intestinal no-load and zero-stress state

The intestinal segments were cut into short ring-shaped segments (1–2 mm) for the no-load state and zero-stress state analysis (Gregersen, 2002; Zhao et al., 2003). Morphometric data were obtained from digitized photographs of these segments in the zero-stress and no-load state. The following data were measured from each specimen using image analysis software (Sigmascan Pro 4.0): the circumferential length (C), wall thickness (h), wall area (A), and opening angle at the zero-stress state (α). The mucosa and serosal boundaries were measured at the inner and outer circumferential lengths. The resolution of the measuring system was less than 0.05 mm $pixel^{-1}$.

2.4. Experimental set up and procedures

The experimental set-up is illustrated in Fig. 1. Two cannulas were fixed on two sides of the inner small organ bath. The proximal and distal end of each intestinal segment were tied on the two cannulas with silk threads. The cannulas connected via a tube to a syringe (proximal end) and a reservoir (distal end). The inner organ bath, syringe and reservoir contained Krebs solution (mmol L^{-1}): NaCl, 118; KCl, 4.7; $NaHCO_3$, 25; NaH_2PO_4, 1.0; MgCl, 1.2; $CaCl_2$-H_2O, 2.5; Glucose, 11; ascorbic acid, 0.11). The Krebs solution was aerated with a gas mixture (95% O_2 and 5% CO_2, pH 7.4). A pump was used to circulate the water in the outer bath for maintaining the temperature of the solution in the inner organ bath at 37 °C. The luminal flow was applied to ileal segments (rate 0.5 ml min^{-1}) and colonic segments (rate 1.0 ml min^{-1}) using a pump (Genie Programmable Syringe Pump, World Precision Instrument, Stevenage, UK). The prepared segments were immediately transferred to the small organ bath. Thirty minutes equilibration time was needed for recovery of the motility before the experiments started.

For the flow test, the distal end of the segment was opened to connect to the reservoir. The outlet resistance (pressure) was modulated by changing the height of the reservoir. The outlet resistance was varied from 0, 2.5, 5, 7.5, and 10 cmH_2O for ileum and 0, 5, 10, 15, and 20 cmH_2O for colon. Each flow test at a selected outlet resistance lasted three minutes. Afterwards three ramp distensions (0–10 cmH_2O for ileum and 0–20 cmH_2O for colon) were done with closed outlet. For passive distension, the Krebs solution was replaced by calcium-free Krebs solution with added 0.4% ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA) and 2 mg papaverine in order to abolish smooth muscle contractility (Papaverine inhibits enzyme phosphodiesterase causing elevation of cyclic AMP levels, altering mitochondrial respiration, and inhibition of calcium influx). Three ramp distensions were done with closed outlet. The recovery time between two tests was at least 2 min. For measuring lumen pressure, a catheter was inserted into the lumen through the proximal cannula. The outer diameter of the segments was videotaped by using a CCD camera (Sony, Japan) through a stereomicroscope and recordings were aligned with pressure recordings. The sampling frequency of pressure and diameter was 10 Hz.

2.5. Analysis of contractions from pressure and diameter curves

The pressure changes during flow and ramp distension tests were recorded in real time. Data were exported to Excel. The diameter changes during the distensions were measured by analyzing the video clips (Zhao et al., 2009). Examples of pressure-diameter curves of distension-induced and flow-induced contractions from ileal and colonic segments are presented in Fig. 2. The frequency and maximal amplitude of contractions were analyzed for the flow experiments. The contraction threshold was obtained from disten-

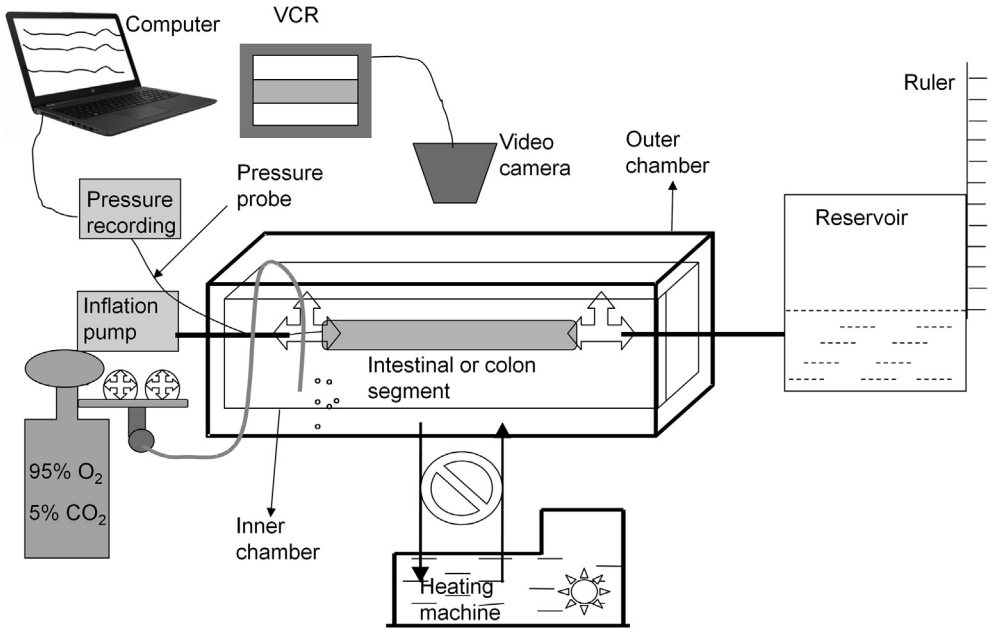

Fig. 1. Experimental set-up for flow-induced motility and ramp distension experiments. The organ bath is composed of an inside chamber and an outside chamber. The Krebs solution contained in the inner chamber is maintained constant at 37 °C by circulating hot water in the outer chamber using a heater and circulation pump. The intestinal segment was placed in the Krebs solution in the inner organ bath. Flow and distension were applied by a pump. The pressure probe was used to measure pressures. The diameter changes of the segments are videotaped through a stereomicroscope. Diameters of the segment and pressures were recorded simultaneously.

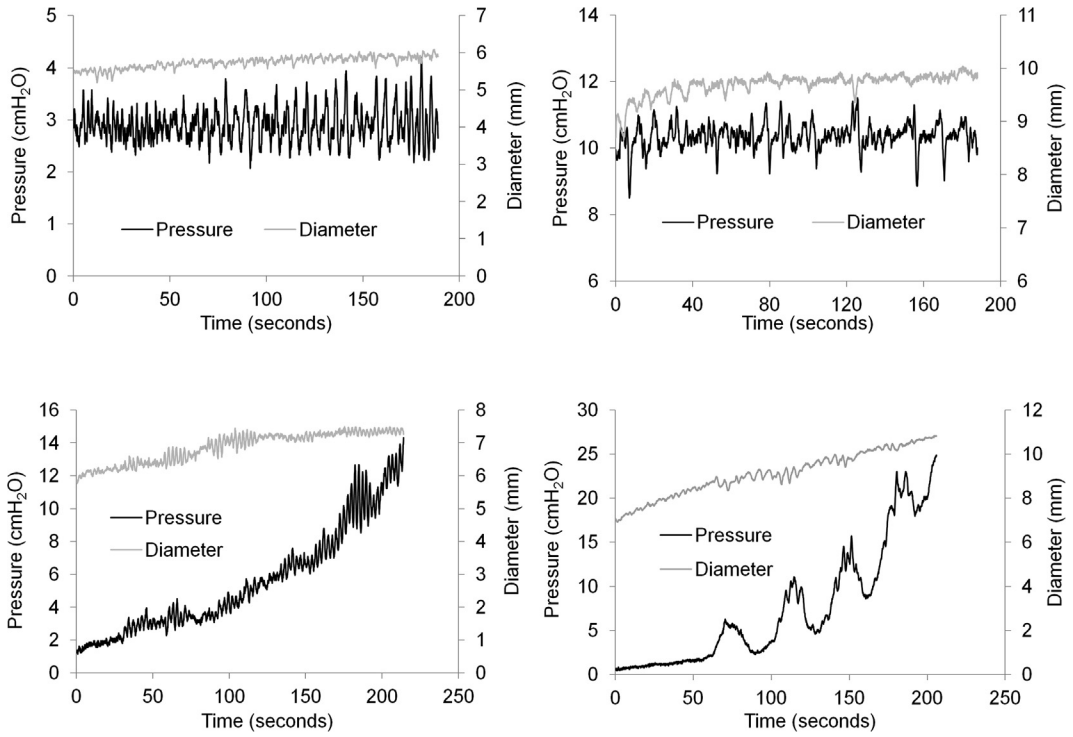

Fig. 2. Examples of pressure-diameter curves of flow-induced contraction (upper row) and distension-induced contraction (lower row) from normal ileal (left) and colonic (right) segments. The contraction frequency and the maximum amplitude of contraction pressure can be obtained from flow-induced contraction curves, whereas the pressure threshold to evoke contraction can be obtained from distension-induced contraction curves.

sion experiments. Since the intestinal mechano-sensory receptors likely respond to changes in mechanical stress and strain, distension-induced contraction thresholds and maximal contractions of flow-induced contractions were calculated as stress and strain with reference to the zero-stress state.

2.6. Stress and strain calculation

The following morphometric data were measured from the segments in the zero-stress and no-load states as mentioned above: circumferential length (C), wall thickness (h), and wall area (A) at

no-load and zero-stress state. Furthermore, the outer diameter (D) was measured from the pressurized segments by using a house made software subroutine (Zhao et al., 2009). The intestinal segments in the pressurized state were assumed thin-wall circular cylindrical. Hence, the circumferential Kirchhoff stress was derived as:

$$S_\theta = \frac{\Delta \mathrm{Pr}_{i-p}}{h_p \lambda_\theta^2} \quad (1)$$

The circumferential Green strain was computed from the circumferential stretch ratio λ_θ as:

$$E_\theta = \frac{1}{2}(\lambda_\theta^2 - 1) \quad (2)$$

r_{i-p}, h_p, λ_θ, and P are the luminal radius, the wall thickness, the circumferential stretch ratio and the intraluminal pressure. Calculations of r_{i-p}, h_p and λ_θ have been described in detail previously (Gregersen, 2002; Zhao et al., 2003). S_θ is circumferential 2nd Piola-Kirchhoff's stress and E_θ is circumferential Green strain. These mechanical parameters were selected because intestinal tissue expresses large deformation properties.

The stress and strain immediately before distension-induced contractions (stress and strain thresholds) and at the maximum flow-induced contraction level were determined.

2.7. Statistical analysis

The results were expressed as means ± SD. Differences in morphometry data, passive stress-strain curves (stiffness) and contraction thresholds between IBS and Control groups were statistically analyzed using *t*-test. If data were not normal distributed, Mann–Whitney *U* test was used. The maximum contraction amplitudes and contraction frequencies were statistically analyzed using two-way ANOVA with factors 1: groups and 2: outlet pressures. The Tukey test was used for post hoc analysis. For comparing the

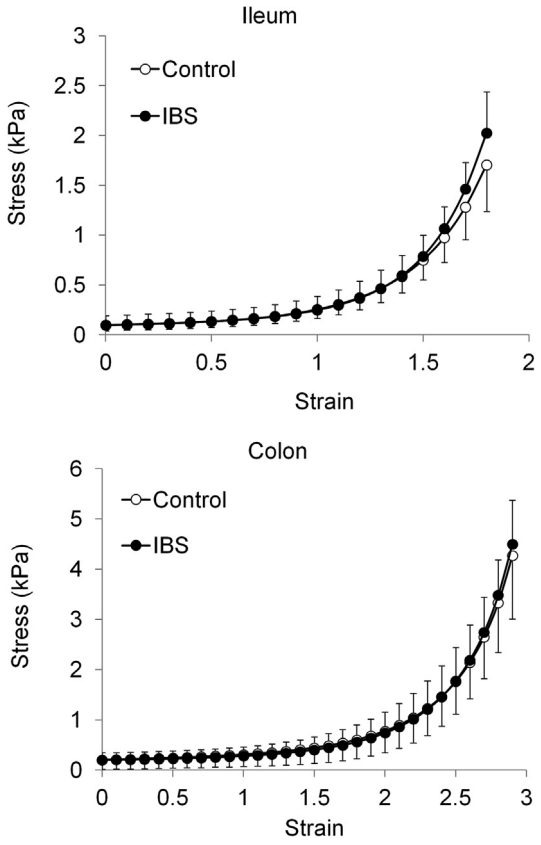

Fig. 4. Passive stress-strain relationship (top, ileum; bottom, colon). The stress-strain curves show an exponential pattern with a slight left shift of the IBS curve at high loads. Analysis of material constant *a* (passive wall stiffness) did not show difference between IBS and Control groups for both ileum and colon, though it was borderline significant for ileum.

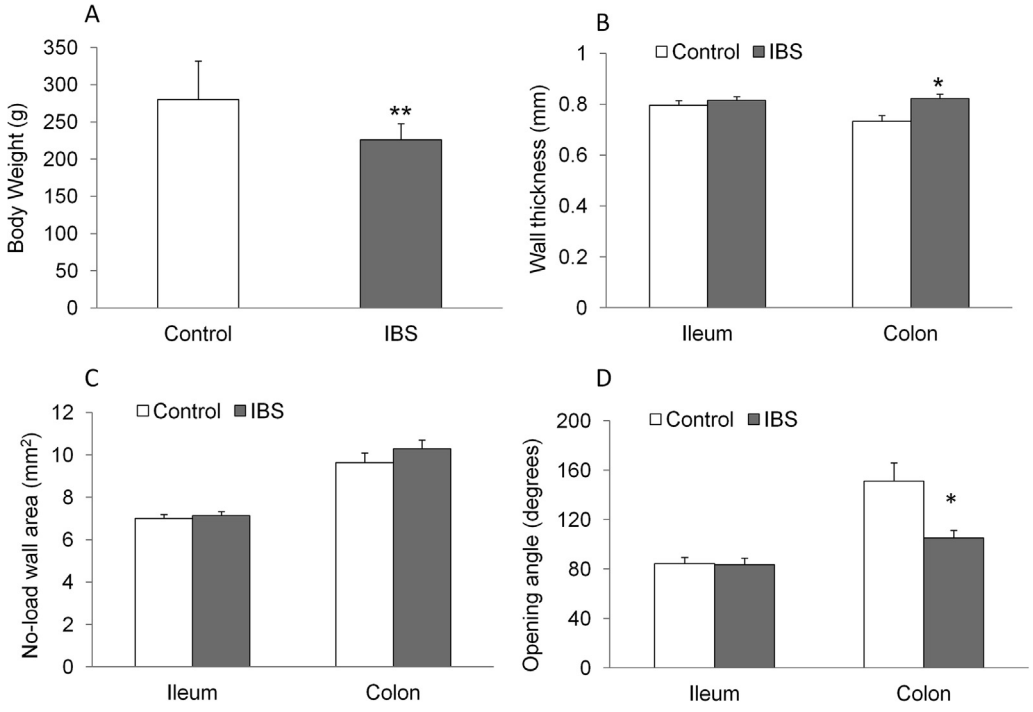

Fig. 3. The body weight (A), wall thickness (B), wall area (C) and opening angle (D). Compared with the Control group, IBS rats had lower body weight, bigger wall thickness of colon and smaller colonic opening angle. Wall thickness and opening angle of ileum and the wall area for both intestinal segments did not differ between IBS and Control groups. Compared with Control group: *P < 0.05, **P < 0.01.

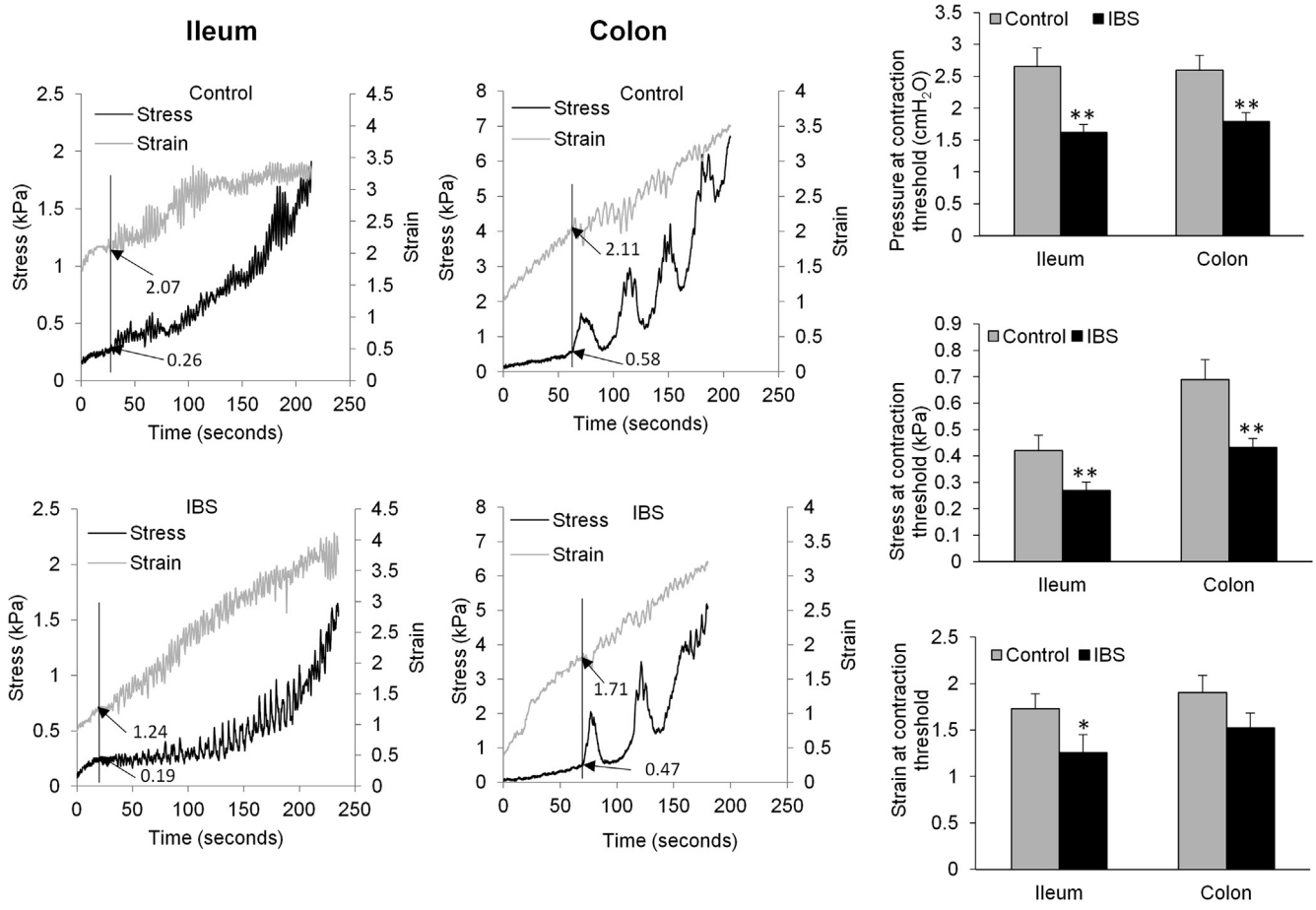

Fig. 5. Examples of stress-strain curves during distension-induced contraction and average contraction thresholds. The contraction waves are clearly visible, and stress and strain values at contraction thresholds can be easily determined. The average pressure (top), stress (middle) and strain (bottom) at the contraction threshold (Right column) were smallest in the IBS group for both ileum and colon segments. Compared with Control group: *P < 0.05, **P < 0.01.

stiffness of colon and ileum between IBS and Control groups, the stress-strain data were curve-fitted to the function $S = (S^* + b)\, e^{a\,(E-E^*)} - b$, where S is stress, E is strain, and S^* and E^* are the stress and the strain at an arbitrary point on the stress-strain curve; a and b are constants. In this context, a represents the slope of the curve which expresses wall stiffness change as function of stress. The results were regarded as significant when $P < 0.05$.

3. Results

We did not find sex-related differences in either of the two groups.

3.1. Body weight and morphometry data

At week 12, the body weight was significantly smaller in the IBS group compared to the Control group (Fig. 3A, Mann-Whitney U test, P = 0.001). The colonic wall thickness was biggest in the IBS group (Fig. 3B, t = 3.315, P = 0.002). The ileum wall thickness and wall area of both segments did not differ between groups (Fig. 3B, 3C, P > 0.25). The colonic opening angle was smallest in the IBS group (Fig. 3D, Mann-Whitney U test, P = 0.015). The opening angle in ileum did not differ between groups (Fig. 3D, t = 0.105, P = 0.917).

3.2. Passive stress-strain relations

The passive stress-strain curves for ileum and colon showed non-linear exponential pattern in both groups (Fig. 4). At high stress and strain levels, the curve for IBS ileum shifted towards left (Fig. 4 top) whereas the colon curve did not differ between groups (Fig. 4 bottom). The material constant a obtained by curve fitting the stress-strain curves was borderline significant between IBS and Control groups for ileum ($0.1 > P > 0.05$).

3.3. Pressure, stress and strain at contraction thresholds

Examples of intestinal stress-strain curves during distension-induced contraction are shown in Fig. 5. Stress and strain values at the contraction thresholds are shown. The averaged contraction threshold data (Fig. 5 right) show that the pressure (top, Ileum: Mann-Whitney U test, P < 0.001; Colon: Mann-Whitney U test, P = 0.007), stress (middle, Ileum: Mann-Whitney U test, P = 0.002; Colon: Mann-Whitney U test, P = 0.007) and strain (bottom, Ileum: t = 2.7, P = 0.01; Colon: t = 1.772, P = 0.084) were smallest in the IBS group.

3.4. Contraction frequencies and maximum contraction amplitudes

Fig. 6 shows the stress and strain changes in an ileum segment during flow-induced contraction for both IBS and Control groups for outlet resistance between 2.5 and 10cmH_2O. Fig. 7 shows changes in a colon segment for outlet resistance between 5 and

20cmH_2O. The pressure and diameter changes exhibited similar patterns as those for stress and strain (data not shown). From the flow-induced contraction curves, the number of contractions and the contraction frequency (cpm, cycles per minute) was counted and calculated. Comparing Figs. 6 and 7, the contraction frequencies was smaller in colon than in ileum (two-way ANOVA: $q = 76.23$, $P < 0.001$).

Fig. 8 shows the averaged contraction frequency (AB), the maximum contraction pressure (CD), the maximum contraction stress (EF) and the maximum contraction strain (GH) for flow-induced contractions at different outlet resistances. Ileum contraction frequencies did not differ between IBS and Control groups (Fig. 8A, two-way ANOVA: $0.202 < q < 1.258$, $0.377 < P < 0.989$). The colonic contraction frequency (Fig. 8B) was highest in the IBS group at most outlet pressure levels (two-way ANOVA, $3.434 < q < 4.491$, $0.002 < P < 0.017$). The maximum contraction pressure, stress and strain amplitudes did not differ between groups for both ileum and colon (two-way ANOVA: $0.295 < q < 2.474$, $0.08 < P < 0.983$).

4. Discussion

The main findings in the present study were that the NMD-IBS rats had: (1) the smallest pressure, stress, and strain at the contraction threshold for distension-induced contractions in ileum and colon; (2) highest contraction frequency for flow-induced contractions in colon and (3) highest wall thickness and smallest opening angle in colon. The data indicate that ileum and colon in IBS rats were hypersensitive to distension-evoked contractions. Furthermore, the IBS colon had higher contraction frequencies for flow-induced contraction. However, the maximum contractility of flow-induced contractions did not change for ileum and colon in IBS. Wall thickness and opening angle changes in colon indicate that histomorphometric remodeling occurs in IBS colon. However, the wall stiffness of ileum was borderline significant whereas colon did not change in IBS rats.

4.1. Intestinal biomechanical properties in IBS

To date only a few studies have been done to study the distensibility of rectum and colon in IBS patients (Drewes et al., 2001, Steens et al., 2002; Lee et al., 2006, Zar et al., 2006; Park et al., 2008). IBS patients show decreased rectal compliance during rapid intermittent distension and increased rectal perception of pain (Steens et al., 2002). Decreased rectal compliance in the fasting state were observed in 52% of IBS patients. Diarrhea-predominant IBS (D-IBS) patients showed significant postprandial

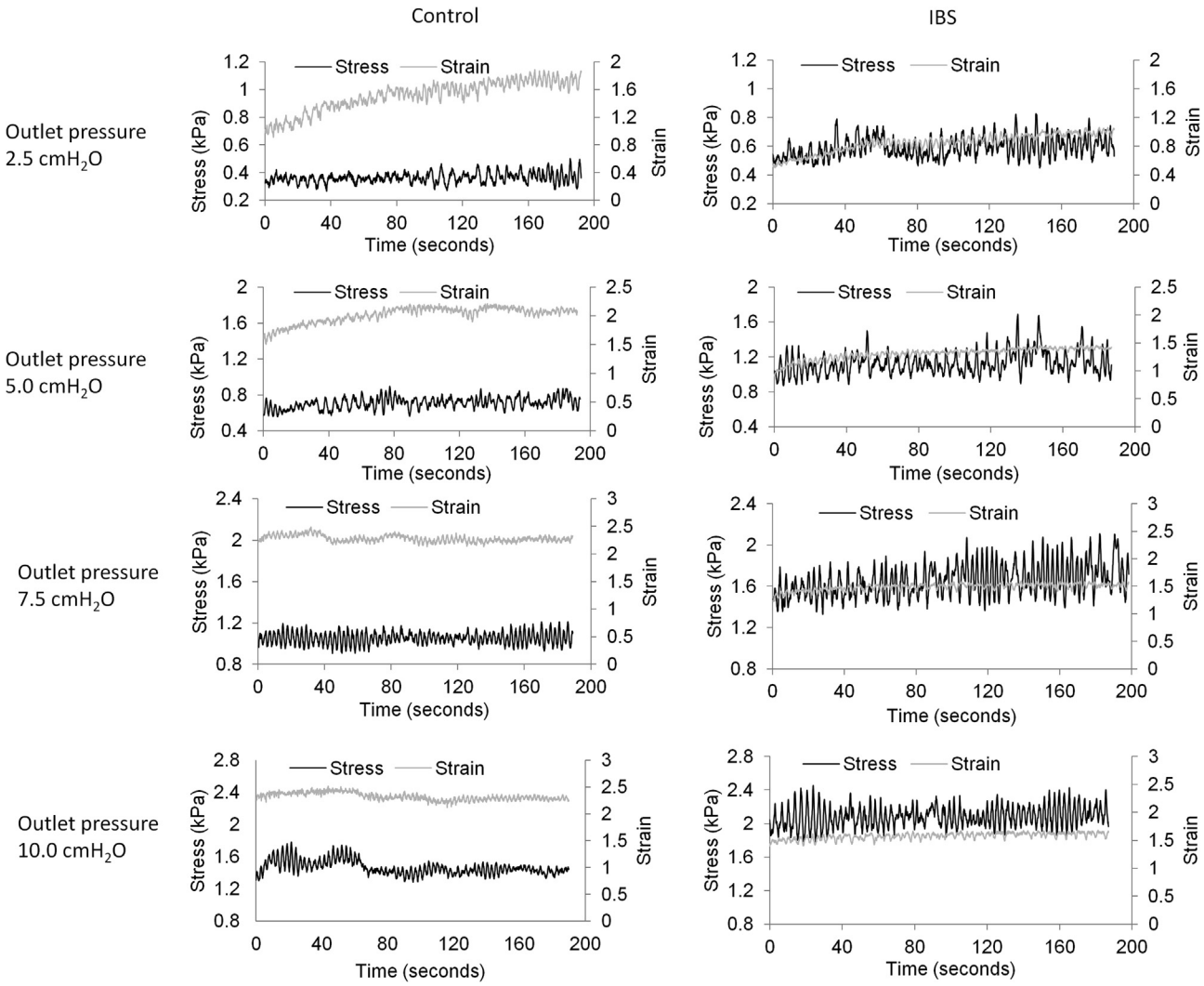

Fig. 6. Examples of ileum stress and strain curves during flow-induced contraction. The contraction cycles are clearly visible. The number of contraction cycles and the maximum stress and strain are determined from the curves.

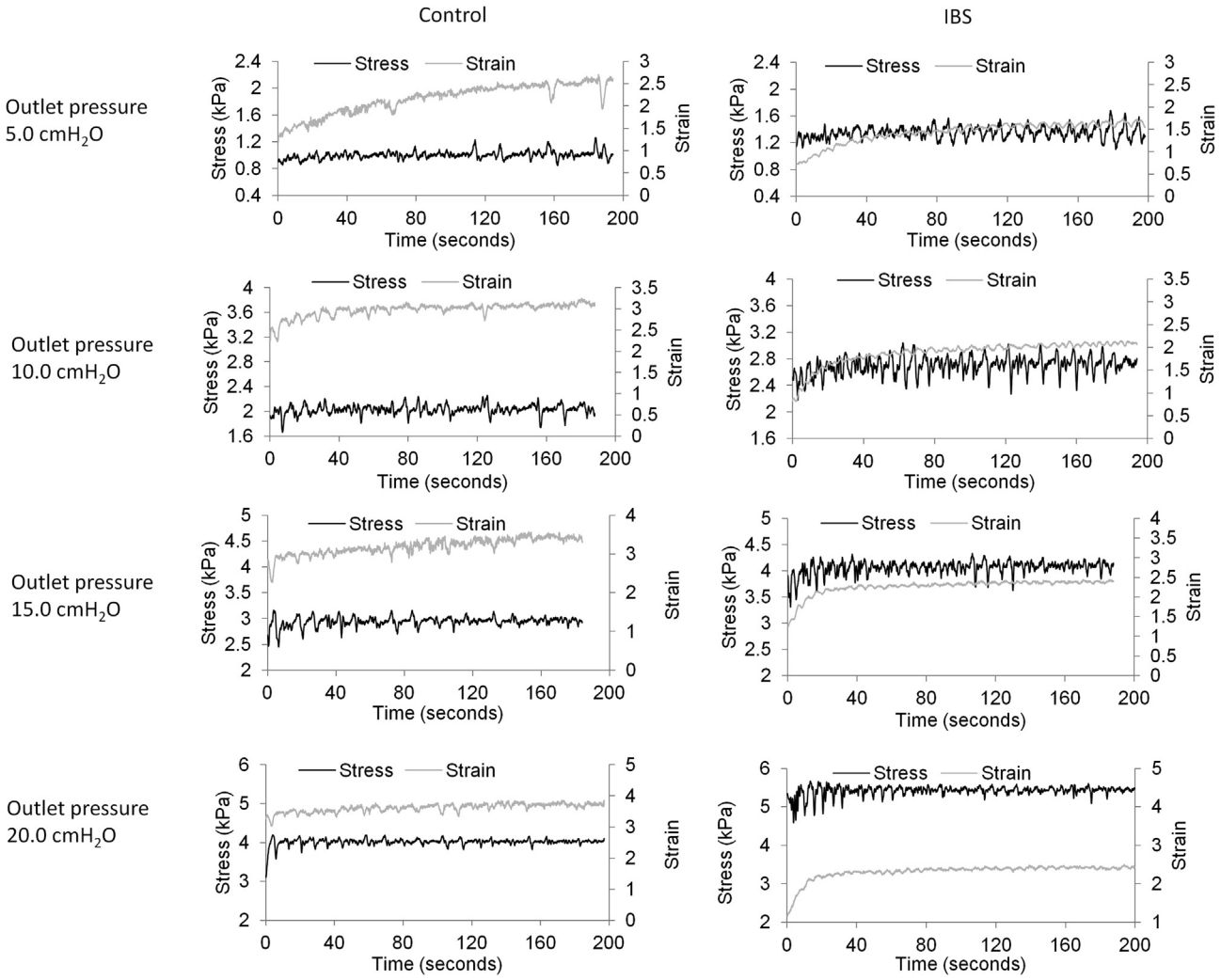

Fig. 7. Examples of colonic stress and strain curves during flow-induced contraction. The colonic contraction frequency was biggest in the IBS group.

decrease in rectal compliance whereas the constipation-predominant IBS (C-IBS) group did not (Lee et al., 2006). Postprandial decreased rectal compliance was associated with a sense of incomplete evacuation and increased bowel movements (Lee et al., 2006). Another study showed that sensory thresholds for urge-to-defecate and rectal compliance were significantly lower in D-IBS compared with C-IBS and controls (Zar et al., 2006). Significant differences in static compliance of anorectum were found between normal subjects and IBS patients indicating intestinal biomechanical properties in IBS patients may have remodeled (Park et al., 2008). However, in another study, strain and tension in rectum and sigmoid colon did not differ between IBS patients and controls (Drewes et al., 2001). Despite the incomparable results obtained from human studies, it seems that structural and biomechanical remodeling may occur in IBS.

The zero-stress state is sensitive to remodeling by disease, growth or degeneration (Gregersen, 2002). The change of the opening angle is a result of non-uniform tissue remodeling of the organ wall (Fung, 1993; Gregersen et al., 2000) or changes of tissue biomechanical properties (Gregersen, 2002). The present study presents the opening angle in ileum and colon in an IBS animal model. The opening angle decreased in colon but not in ileum. This suggested that the outer part of the colon outgrows the inner part in the NMD IBS rats, which is consistent with the observed wall thickness increase in colon. However, the stress-strain relationship of ileum was only borderline significant and did not differ in colon between normal and IBS rats. Hence, the effect of IBS on intestinal elasticity was minor in this IBS animal model. Besides changes of colonic wall thickness in IBS rats, we did not find differences of other morphometry data between IBS and controls. It is well known that IBS is a functional gastrointestinal disorder without obvious organic histological changes of the intestinal wall. The passive biomechanical properties of the intestine is a response of the structural properties of the intestinal wall (Gregersen et al., 2000). The lack of significant tissue changes in this IBS model may explain the lack of difference for the passive stress-strain relationships for ileum and colon in IBS rats.

4.2. Distension-induced contraction thresholds

Continued flow and distention evoke intestinal contraction through neurogenic and myogenic pathways (Costa et al., 2013). When a threshold is reached, contractions are evoked. The sensory neurons in the intestine can either enhance their response (Sengupta and Gebhart, 1994) (hypersensitivity) or reduce their response to subsequent mechanical stimulations (Xue et al., 2009) (hyposensitivity). Since the intestinal mechanosensitive receptors likely respond to changes in mechanical force and deformation, it is important to compute contraction thresholds as stress and strain with reference to the zero-stress state (Gregersen et al.,

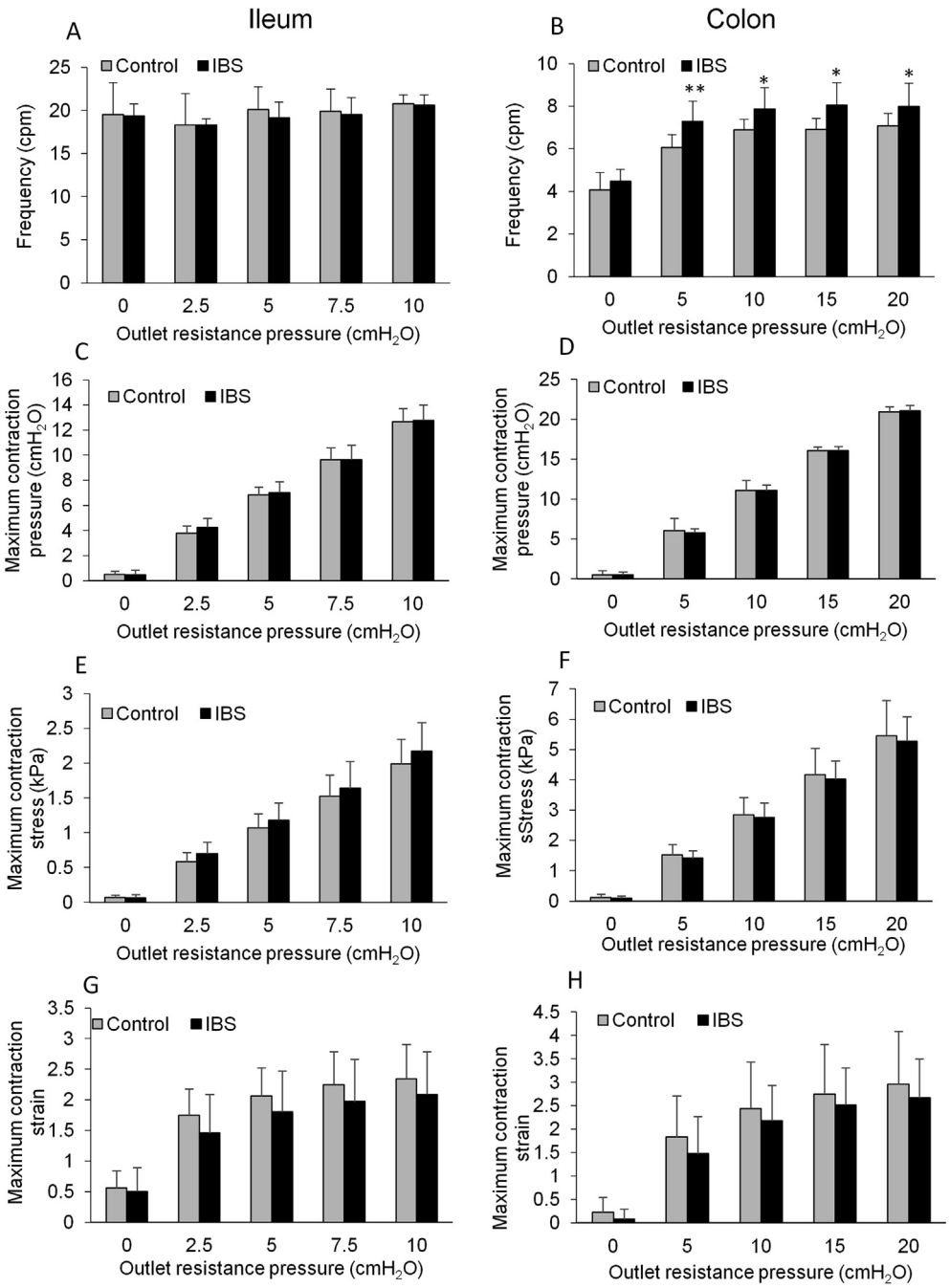

Fig. 8. Averaged contraction frequencies and maximum contraction amplitudes. The contraction frequency of ileum (A) did not differ between IBS and Control groups. The contraction frequency of colon (B) was biggest in the IBS group. The maximum contraction pressure (C&D), stress (E&F) and strain (G&H) of ileum and colon did not differ between groups. Compared with Control group: *P < 0.05, **P < 0.01.

2000). We demonstrated that the pressure, stress and strain threshold decreased in ileum and colon of NMD IBS rats. This indicates that sensitivity to distension-induced contraction in NMD IBS rats increased. This is consistent with studies in IBS patients who were more sensitive to distension of the sigmoid colon and ileum than healthy controls (Ritchie, 1973; Drewes et al., 2001). Visceral hypersensitivity is a biomarker of IBS (Mertz et al., 1995; Thompson et al., 1999), although visceral perception is not abnormal in all IBS patients (Kuiken et al., 2005; Sabate et al., 2008). The GI tract contains intrinsic and extrinsic enteric neurons, smooth muscle cells and interstitial cells of Cajal (ICC), which respond to mechanical deformations by altering transmembrane ionic currents (Mazzuoli-Weber and Schemann, 2015; Alcaino et al., 2017). Comparison of response of patients and controls to jejunal distension and electrical stimulation of primary afferents suggests that the primary abnormality is at the mechanoreceptor level (Accarino et al., 1995). Although the passive stress-strain distribution during distension did not differ between IBS and normal rats, zero-stress state remodeling happened in colon of IBS rats. Therefore, changes in the structural environment of the mechanoreceptors may affect the sensitivity of afferents. Possible mechanisms of pressure, stress and strain threshold changes in IBS rat intestine were not studied in detail in the present study and needs further attention in future studies.

4.3. Maximum contractions and contraction frequency

IBS was for long time considered a gastrointestinal motility disorder. During prolonged recordings of small intestinal motility in IBS, abnormalities of migrating motor complex (MMC) cycle length and presence of abnormal motor patterns were described (Kellow and Phillips, 1987; Hellow et al., 1990; Pimentel et al., 2002). In IBS patients with distinct constipation, increased motor activity was seen in the colon, irrespective of colon transit times (Hasler et al., 2009). In this study, we analyzed the contraction amplitude of pressure, stress, and strain in ileum and colon. Differences were not found between IBS and normal rats. Intestinal contraction amplitudes reflect the contractile strength of intestinal smooth muscle. Therefore, smooth muscle contractile strength was not changed in this IBS model. However, we found increased frequency of flow-induced contraction in colon of NMD IBS rats. This finding is consistent with previous reports (Ritsema and Thijn, 1991; Clemens et al., 2003). The frequency of intestinal contractions is determined by the propagation of slow waves and the accompanying spike potentials and myoelectrical activity (Subramanya et al., 2015). Slow waves are determined by pacemaker cells (ICCs), especially those located in the myenteric plexus (Sanders et al., 2006). Early studies in IBS patients reported abnormal slow wave activity in rectal and rectosigmoid myoelectrical recordings (Snape et al., 1976, 1977; Taylor et al., 1978) and rectal and colonic motor hyperactivity during baseline recording (Chaudhary and Truelove, 1968), after food intake (Connell et al., 1965), injection of neostigmine (Chaudhary and Truelove, 1968), and rectal distension (Whitehead et al., 1980). The increased frequency of contraction in the colon of IBS patients and NMD rats may indicate disorders of ICC and myoelectrical activity.

4.4. Limitations of the study

Firstly, although the NMD rat model is a well-known IBS animal model that are mimicking all the main features of human IBS (Deiteren et al., 2016), we did not monitor the consistency of feces and whether the rats had diarrhea or were constipated in this study. This would have provided information about the type of IBS, though we believe, due to the hypersensitivity, that it would tend to be IBS-D. We did not find such information in the literature either. Secondly, tissue blocks were accidently lost and therefore the histological features of the intestine were not analyzed in the study. Future studies must remedy this shortcoming. Finally, detailed mechanisms behind intestinal hypersensitivity such as intestinal afferent signaling to various types of stimulations and contributions from individual components of the neuromuscular system were not studied and need further attention in future studies.

4.5. Conclusions and perspectives

Our data indicate that the NMD IBS model is a model of intestinal hypersensitivity of mechanosensitive receptors and intrinsic neural circuits to mechanical stimulation. The frequency of flow-induced contraction was higher in colon of IBS rats indicating location-dependent alteration of ICC and myoelectrical activity in the intestines. Furthermore, zero-stress state remodeling occurred in colon of IBS rats, indicating tissue remodeling in NMD IBS rats. The study adds new knowledge from a biomechanical point of view to the field of IBS-induced intestinal contractility changes. Further studies on the relation between intestinal biomechanical properties, hypersensitivity and afferent signaling in IBS animal models are warranted.

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Declaration of Competing Interest

The authors declare no conflict of interest.

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