Small Intestinal Ischemia and Reperfusion – Bioimpedance measurements

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Abstract

Objective: Trans-intestinal bioimpedance measurements have previously been used to investigate changes in electrical parameters during 6 hours of ischemia in the small intestine. Knowledge is lacking regarding the time course of trans-intestinal bioimpedance parameters during reperfusion. As reperfusion is an important part in the clinical treatment of intestinal ischemia, we need to know how it affects the bioimpedance measurements.

Approach: We performed bioimpedance measurements, using a two-electrode setup on selected segments of the jejunum in 15 pigs. A controlled voltage signal was applied while measuring the resulting current. In each pig, five or six 30-cm segments of the jejunum were made ischemic by clamping the mesenteric arteries and veins creating segments with ischemia from 1-16 hours duration. Reperfusion was initiated at selected time intervals of ischemia, and measured for 5-15 hours afterwards.

Main results: The $\tan \delta$ parameter (loss tangent) was different ($p < 0.016$) comparing ischemic and control tissue for the duration of the experiment (16 hours). Comparing the control tissue 30 cm from the ischemic area with the control tissue 60 cm from the ischemic tissue, we found that the mean $\tan \delta$ amplitude in the frequency range (3900-6300 Hz) was significantly higher ($p < 0.036$) in the proximal control after 10 hours of experiment duration. After reperfusion, the time development of $\tan \delta_m$ (loss tangent maximum over a frequency range) amplitude and frequency overlapped and periodically increased above the $\tan \delta_m$ in the ischemic intestine. Dependent on the ischemic duration pre-reperfusion, the initial increase in $\tan \delta$ stabilizes or increases drastically over time, compared to the $\tan \delta$ amplitude of the ischemic tissue.

Significance: As during ischemia, the electrical parameters during reperfusion also follow a characteristic time-course, depending on the ischemic exposure before pre-reperfusion. The temporal changes in electrical parameters during small intestinal ischemia followed by reperfusion provides important information for assessment of tissue injury.

Keywords

Bioimpedance, reperfusion, ischemia, small intestine

1 Introduction

Ischemia/reperfusion injury (IRI) in the intestines is a life-threatening condition (Bulkley et al., 1981; Clair & Beach, 2016), where evaluation of intestinal viability is an essential factor influencing the outcome in patients (Horgan & Gorey, 1992; Tilsed et al., 2016). The standard clinical method for assessment of intestinal viability perioperatively is still visual inspection and palpation (Wyers, 2010). This method is not very specific and requires a high level of clinical experience (Urbanavicius, Pattyn, de Putte, & Venskutonis, 2011), as the appearance of the ischemic/reperfused intestine can be deceptive (Urbanavicius et al., 2011).

Two different mechanisms mediate injury related to ischemic tissue (Haglund, Bulkley, & Granger, 1987). The first is the hypoxic injury that occurs as a direct effect of ischemia. The second is the process related to reperfusion of previously hypoxic tissue. While there are several different causes of intestinal ischemia, the clinical treatment in all cases is focused on the restoration of perfusion (Kalogeris, Baines, Krenz, & Korthuis, 2012). However, while reperfusion is essential to prevent further tissue damage, reperfusion itself may cause both local and systemic responses, potentially creating damage far beyond the direct ischemic injury (Parks & Granger, 1986; Yandza et al., 2012). The extent of IRI is variable and dependent on the underlying mechanisms, the duration of ischemia, and the size and hypoxic tolerance of the affected tissue (Kalogeris et al., 2012).

Over the years, various experimental methods of assessing intestinal viability during laparotomy have been tested. Visible light spectrophotometry and laser doppler flowmetry have been most frequently used in humans (Urbanavicius et al., 2011). Horgan and Gorey defined criteria for the ideal bowel viability test (Horgan & Gorey, 1992), but so far, no method has surpassed palpation and visual inspection performed by an experienced clinician, followed by second look laparotomy, as the gold standard. One of the experimental approaches that has been suggested, is the use of electrical tissue properties to assess intestinal viability (Beltran & Sacristan, 2015; Carey, Kayser, Ellison, & Lepley, 1964; C. A. Gonzalez, Villanueva, Othman, Narvaez, & Sacristan, 2003; Matsuo et al., 2004).

We have previously described how the electrical parameters of the in-vivo small intestine change during 6-hour full mesenteric ischemia (Strand-Amundsen et al., 2016). By placing opposing electrodes on each side of the intestine and measuring trans-intestinal electrical parameters, we found that the time course of ratio parameters like the loss tangent ($\tan \delta$) and the phase angle (ϕ) had significant correlations with the time course of ischemia. With trans-intestinal measurements the electric current will, dependent on the frequency, pass along certain layers or pass through the walls of the small intestine to reach the other electrode (Strand-Amundsen et al., 2016). We estimate that the pathological changes to the intestinal wall during reperfusion will affect the electrical properties.

To the best of our knowledge, no studies have so far been published demonstrating trans-intestinal measurements of electrical parameters during reperfusion in the ischemic small intestine. Closest to doing so, are those of Beltran et al. and Gonzalez et al. who performed measurements on the mucosal side of the intestine during ischemia/reperfusion, using a 4-electrode probe inside the intestinal lumen in pigs and rabbits (Beltran & Sacristan, 2015; C. A. Gonzalez et al., 2003). In our experimental model for assessment of intestinal viability using bioimpedance, we have so far investigated the development of trans-intestinal electrical parameters only in the fully ischemic intestine.

The purpose of the present study, was to assess the development of electrical parameters in reperfused porcine jejunum compared to ischemic jejunum and control jejunum, while using trans-intestinal measurements. As reperfusion is an important part in the clinical treatment of intestinal ischemia (Kalogeris et al., 2012), we need to know how it affects the development of the electrical parameters. This is an important step in the overall aim to develop methods and equipment that can assist the surgeon in the assessment of intestinal viability with improved sensitivity and specificity.

2 Materials and methods

2.1 Animals and experimental design

We conducted the study on 15 Norwegian Landrace pigs, weight range 44.3-58.6 kg, 11 females. Food was withheld 12 hours prior to surgery. 30 cm segments of the jejunum were selected, starting 30 cm distal from the duodenum. More than 30 cm intervals were used between the segments. Local ischemia was induced by clamping the arteries and veins of the jejunal mesentery on the selected segments, resulting in a 20 cm central zone of warm ischemia and two surrounding 5 cm edge zones of marginal tissue hypoxia (Noer & Derr, 1949). Reperfusion was initiated by releasing the clamps and verified by observing the return of colour in the previously ischemic segments. We conducted two measurement series (A, B) with ischemia/reperfusion at selected intervals (Table 1). In each series (A/B), parallel ischemia/reperfusion cases (A1-A4 or B1-B5) was conducted on the jejunum of each pig. The use of parallel cases was based on the need to reduce the number of animals, and we treated each segment as an isolated case. Measurements were performed before the initiation of ischemia, and then every hour for the duration of the experiment.

Table 1. Experimental protocol with time intervals. Each series (A/B) of cases was run in parallel on 30 cm segments of jejunum in the same pig model. Green = normal perfusion, red = ischemia, blue = reperfusion.

A-Series													B-Series																				
#	Case:	Experimental duration [hrs]:											# Pigs:	#	Case:	Experimental duration [hrs]:																# Pigs:	
A-C	Control	0	1	2	3	4	5	6	7	8	9	10	11	B-C	Control	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	4
A1	1hr	0	1	1	2	3	4	5	6	7	8	9	11	B1	1hr	0	1	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	4
A2	3hr	0	1	2	3	1	2	3	4	5	6	7	11	B2	4hr	0	1	2	3	4	1	2	3	4	5	6	7	8	9	10	11	12	4
A3	5hr	0	1	2	3	4	5	1	2	3	4	5	11	B3	6hr	0	1	2	3	4	5	6	1	2	3	4	5	6	7	8	9	10	4
A4	Ischemia	0	1	2	3	4	5	6	7	8	9	10	11	B4	8hr	0	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8	4
														B5	Ischemia	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	4

The selection of time intervals was based on the reported time dependence of tissue damage in the porcine ischemic intestine (Chan, Chan, & Tam, 1998). We selected 2 groups of control tissue in each of the series, the first being 30 cm distal to the ischemic area, and the second being 60 cm distal to the ischemic area. The model creates full mesenteric occlusion in a segment of jejunum, similar to what is caused by events like strangulation/volvulus, and produces more uniform ischemic damage to the small intestine, than using a superior mesenteric artery (SMA) occlusion model (L. M. Gonzalez, Moeser, & Blikslager, 2015). At the end of the experiment, the animals were sacrificed by a lethal dose of potassium chloride (100 mmoles). The experiment was approved by the Norwegian Food Safety Authority and conducted in accordance with national animal welfare guidelines.

2.2 Anaesthesia and monitoring

Anaesthesia was induced with intramuscular ketamine (Warner Lambert, Morris Plains, NJ) 15 mg/kg, azaperone (Janssen-Cilag Pharma, Austria) 1 mg/kg, and atropine (Nycomed Pharma, Asker, Norway) 0.02 mg/kg. Tracheotomy was performed, and anaesthesia was maintained with isoflurane (Abbott Scandinavia AB, Kista, Sweden) (1–1.5%) and a mixture of air and O₂ to obtain an FIO₂ of 30 %. Morphine (Alpharma, Oslo, Norway) 0.4 – 0.7 mg/kg/h was administered as a continuous intravenous infusion. Ventilation was adjusted to a pCO₂ of 5–6 kPa (37.5–45.0 mm Hg). A continuous infusion of Ringer acetate 10 - 30 mL/kg/h was administered as fluid replacement.

2.3 Surgery

Surgery was performed under sterile conditions. Tracheostomy was performed initially for mechanical ventilation. The internal jugular veins were cannulated for blood sampling, measuring of central venous pressure and infusion of fluids. Arterial pressure was measured through a catheter placed in a carotid artery, the urinary bladder temperature was measured with a thermistor probe. Arterial and venous blood gases were regularly measured throughout the experimental period. The jejunum was made accessible through midline laparotomy in the abdominal cavity.

2.4 Impedance measurements

A Solartron® 1260 Impedance/gain-phase analyser (Solartron Analytical, United Kingdom) was used with a 1294A interface. The Solartron was controlled, data logged, and measurements acquired by the Zplot® software (Copyright © 1990-2015 Scribner Associates, USA). A 2-electrode setup (each electrode acting both as stimulating and pickup electrode), with 9 mm diameter Quickels® (Quickels System AB, Sweden) Ag/AgCl electrocardiography disc electrodes (intrinsic R = 0,18 ohm, X = 0 ohm), was used for the measurements (Strand-Amundsen et al., 2016). During measurement, the intestinal area under investigation was placed between the two opposing electrodes with a light pressure (around 0.2N) to exclude air between the intestinal surface and the electrodes. As a voltage was applied across the electrodes and a small current pass through the intestinal tissue between the electrodes, the measurements are trans-intestinal. The jejunum was placed inside the abdominal cavity between the measurements to maintain a stable temperature and reduce exposure to the environment and loss of humidity. A 50mV AC RMS signal was applied across the electrodes while sweeping the frequencies from 1 Hz to 1 MHz in 41 steps. The resulting current was measured, and the impedance Z calculated. Using the real (R) and imaginary components (X) of the impedance, we calculated the loss tangent Tan δ (Surowiec & Stuchly, 1986).

$$|Z| = \sqrt{(R^2 + (X_L - X_C)^2)} \quad [\text{Ohm}] \quad (1)$$

$$\text{Tan } \delta = \frac{R}{X} \quad [\text{Dimensionless}] \quad (2)$$

Z = Impedance

|Z| = Impedance modulus

R = Real part of the impedance, resistance

X = Imaginary part of the impedance, reactance (Inductance – X_L and Capacitance – X_C)

Tan δ = Loss tangent/dissipation factor

Tan δ m = maximum tan δ amplitude over a frequency range

The resistance (R) is an equivalent series resistance, representing the sum of in-phase AC resistance. Tan δ can be viewed as a unitless frequency dependent ratio between the resistance (R) and reactance (X). Increasing tan δ indicates that electrical power is consumed at increasing rate. It has been suggested that tan δ is a good parameter for the evaluation of the electric properties of a tissue, as it is not dependent on the distance between the electrodes (two-electrode setup), assuming homogeneity of the tissue (Heo & Jung, 2011). In the small intestine, the tissue is structured in circular layers, and to achieve near-homogeneity by averaging over a relatively large area, we use relatively large electrodes (9 mm diameter) and trans-intestinal measurements.

2.5 Statistical analysis

The data was analysed for distribution, skewness, kurtosis and homogeneity of variance. Comparisons between the two control groups in each pig was made using a paired t-test, with Holm-Sidak's multiple comparisons correction. Comparison of the control, ischemia and reperfusion data from the two series (A, B) at hourly intervals was made using two-way repeated measures analysis of variance (RM ANOVA), with Holm-Sidak's multiple comparisons correction. For the ANOVA, the responses of interest were tan δ m (maximum tan δ amplitude over a frequency range) and "tan δ m frequency", and the factors used were "case" (control, ischemic, reperfusion) and "time duration [min]". The ANOVA were run using GraphPad Prism version 7.00 (GraphPad Software, USA).

3 Results

3.1 Impedance measurements

3.1.1 Control

We compared the development of electrical parameters within the control group, to assess if there was a difference between the controls depending on the proximity to the ischemic areas, with the null hypothesis that there was no difference (in means) in the development between the $\tan \delta$ parameter at the two control measurement sites (30 and 60 cm proximity to the ischemic area). Overall there was a small drift in the time development of $\tan \delta$ in the control tissue. When comparing the controls based on the proximity to the ischemic tissue (30 cm versus 60 cm), we found that there was a significant difference ($p < 0.036$) between the two at frequency range (3900-6300 Hz), after 10 hours duration of the experiment. As shown in Figure 1, the mean $\tan \delta$ amplitude in the frequency range (3900-6300 Hz) was higher in the control proximal to the ischemic area.

Figure 1. Plots shows the mean $\tan \delta$ over the measured frequency range, at hourly time intervals over 10 hours, on perfused control jejunum (A series, $n = 11$ pigs). The confidence bands show the 95% CI of the mean. **Top:** Control 30 cm distal of full ischemia. **Bottom:** Control 60 cm distal of full ischemia. The frequency range where there was a significant difference between the two control groups after 10 hours of duration, is marked with a black clamp. *** ($p < 0.036$).

3.1.2 Ischemia

Figure 2 shows the time development of $\tan \delta$ during at hourly intervals (10-hour duration) of mesenteric full occlusion warm ischemia (A4 series).

Figure 2. Tan δ measured on ischemic jejunum over a duration of 10 hours. Plot shows the median ($n = 11$ pigs). The black dotted arrows show the general time development direction of the maximum tan δ amplitude (tan δ m). The confidence bands show the 95% CI of the median.

The frequency of the maximum tan δ value (tan δ m) changes over time. When comparing the tan δ m of full-ischemia (A4) and the control over the 16-hour experiment duration, we observed that the frequency of tan δ m of the control remained in the 10-20 kHz range, while the frequency of tan δ m in the ischemic jejunum ranged from 1 kHz – 100 kHz (Figure 3). The amplitude of tan δ m was significantly different from the control in the 4-hour interval ($p < 0.016$) and for the duration of the experiment. The tan δ m frequency in the ischemic tissue was significantly different from the control tissue ($p < 0.001$) in the 1-3 hour interval, and in the 11-16 hour interval.

Figure 3. Mean $\tan\delta$ values (0-10 hrs n=15 pigs, 11-16 hrs, n= 4 pigs) of ischemic and control jejunum, plotted versus frequency. The starting point at 0 hours is marked by a black dotted circle. The general direction of the time development is plotted as black dotted arrows. Solid lines are intended only as guides. The error bars show the 95% CI of the $\tan\delta$ max amplitude.

3.1.3 Reperfusion

In Figure 4 we compared the development of $\tan\delta$ amplitudes versus time for the two series (A/B). We observed a small gradual increase in the $\tan\delta$ amplitude of the control over time. In the ischemic intestine (A4/B5) there was a large increase over time. In the reperfused intervals we observed an abrupt increase in $\tan\delta$ for some hours after reperfusion. The tendency was that a longer period of ischemia followed by reperfusion resulted in a larger initial increase in $\tan\delta$ amplitude. When ischemia for 8 hours was followed by reperfusion, there was a very large increase in $\tan\delta$ amplitude.

Figure 4. Mean $\tan\delta$ values plotted versus time. Solid lines are intended only as guides. **Top:** A-series (n=11 pigs). **Bottom:** B-series (n=4 pigs). Coloured arrows show time points for start of reperfusion. The confidence band show the 95%

CI of the mean. For better readability, we scaled the Y-axis without showing the top parts of the CI band from the last two hours of the 8-hour ischemic/8-hour reperfusion, as well as the last hour of the 16-hour ischemic tissue.

The reperfused tissue appeared edematous and swollen compared to the control and ischemic tissue (Figure 5).

Figure 5. Images of small intestine (jejunum) at selected time periods of experiment duration. **C:** Perfused control tissue. **I-1:** Ischemic for 1 hours. **I-6:** Ischemic for 6 hours. **I-5 R-5:** Ischemic for 5 hours, and reperfused for 5 hours.

4 Discussion

In the present study, the aim was to evaluate how reperfusion affected the electrical parameters of the jejunum compared to the ischemic and normally perfused state (control), using trans-intestinal measurements. When comparing data at one-hour intervals of ischemia with the control, the combination of $\tan\delta_m$ (maximum $\tan\delta$ amplitude over a frequency range) amplitude and frequency allowed us to significantly determine if the tissue was ischemic or not for the duration of the experiment. The time course of $\tan\delta$ after reperfusion differed both from the control and from the ischemic tissue.

Matsuo et al. used $\tan\delta_m$ as a parameter to assess ischemic intestine using a coaxial probe (Matsuo et al., 2004). We adopted the approach, as we found that the $\tan\delta$ parameter was sensitive to ischemic effects on the small intestine. Overall, there was a small increase in $\tan\delta_m$ in the control tissue during the experimental period. We suggest the cause to be injury due to proximity to the ischemic and reperfused segments, combined with response to our handling of the tissue. The largest part of the change in the control measurements occurred in the 100 Hz to 10 kHz region, following the late reperfusion intervals. In these intervals we observed gradually increased edema and visible irritation of the intestinal tissue close (15-30 cm) to the reperfused areas.

When comparing $\tan\delta$ in the control areas with different proximity (30 cm vs 60 cm) to the ischemic area after 10 hours of experiment duration (Figure 1), we found that there was a significant difference ($p=0.036$) between the two at frequency range 3900-6300 Hz. This correlated with the observation of increased edema and irritation over time in the tissue segments close to the ischemic/reperfused areas. In a previous article (Strand-Amundsen et al., 2016) we reported on using COMSOL Multiphysics to create a model of the small intestine. In the model the current in the range of 3900-6300 Hz passes mainly through the outer layers on the small intestine. We estimate that the observable irritation with inflammation and edema is affecting the outer layers, and that this leads to the observable change in the abovementioned frequency range. It indicates that when using a trans-intestinal

2-electrode setup on the small intestine, the $\tan \delta$ parameter is sensitive to effects like edematous/inflammatory bowel.

We used several ischemia/reperfusion models in one pig to reduce the overall number of pigs. We suggest that our use of a combination of ischemia and reperfusion models with various time durations in one single pig caused some interference between the samples due to the cascading effects after reperfusion (Grootjans et al., 2010), potentially resulting in larger measurement variation than if we had limited the procedure to one in each pig. Each time we initiate reperfusion in a segment, a series of mediators are activated, including proinflammatory cytokines. Previous studies have reported that the level of cytokines is reduced when continuing to reperfuse segments in the same pig, due to increasing tolerance levels (Cavaillon, Adrie, Fitting, & Adib-Conquy, 2003; Ruud et al., 2007).

This study is restricted to investigating the effects of full occlusion mesenteric ischemia followed by reperfusion in the jejunum. While there are several other methods of creating ischemia modelling other clinical settings, resulting in a different course of pathological changes (L. M. Gonzalez et al., 2015), we selected the segmental mesenteric occlusion model as it provides a well-defined area of ischemic damage affecting the whole intestinal wall. The commonly used ischemia model of occluding the superior mesenteric artery (SMA), produces a gradient of ischemic damage along the intestine, with local variation (L. M. Gonzalez et al., 2015; Megison, Horton, Chao, & Walker, 1990). In addition, SMA occlusion allows for some minimal perfusion through collaterals, resulting in better perfusion to the muscularis/serosa than the mucosa/submucosa (Kalogeris et al., 2012).

In the ischemic jejunum, we observed that the frequency of the maximum amplitude of $\tan \delta$ changes as a function of time compared to the control (Figure 2/Figure 3). Thus, we agree with the suggestion of Matsuo et al. that $\tan \delta_m$ can be useful for assessing the state of small intestine during ischemia (Matsuo et al., 2004). By comparing the information from the amplitude and frequency of $\tan \delta_m$, the ischemic tissue differed significantly from the control at all time intervals (hrs) after the initiation of ischemia ($p < 0.016$). In consequence, bioimpedance measurement is a suitable method for detecting ischemia in small intestinal tissue, confirming our earlier results (Strand-Amundsen et al., 2016). This method could potentially be useful during diagnostical laparoscopy.

When comparing the changes in electrical parameters with the reported time development of injury in the ischemic intestine (Cerqueira, Hussni, & Yoshida, 2005; Chan et al., 1998), we made several observations. While the frequency of $\tan \delta_m$ changes significantly during the first hour of ischemia, the reported ischemic injury is the beginning of loss of the surface epithelium of the mucosa (Chiu, McArdle, Brown, Scott, & Gurd, 1970). The gradual loss of the barrier layer of epithelium increases the ion permeability of the tissue, affecting the trans-intestinal impedance. Another early change in ischemic tissue is the swelling of cells as the function of the ionic pumps is reduced, resulting in a shift in the ratio between extracellular and intracellular liquid (Grimnes & Martinsen, 2015). This factor leads to increased low frequency resistance. This is probably the dominating phenomenon explaining why the $\tan \delta_m$ is shifted to a significantly lower frequency after 1 hour of ischemia.

As the duration of ischemia increases, the surface epithelium is lost and deeper layers of the intestinal wall are damaged, increasing the ion permeability in the tissue (Beltran & Sacristan, 2015). The correlating effect on electrical properties is a decreasing overall impedance (Strand-Amundsen et al., 2016), where the reactance is decreasing more relative to resistance, observable as an increased amplitude of $\tan \delta_m$. After an initial large decrease in $\tan \delta_m$ frequency after one hour of ischemia, the $\tan \delta_m$ frequency starts to gradually increase. We estimate the cause to be increased liquid in the interstitial space due to increased permeability, reducing the initial increase in low frequency resistance caused by the swelling of the cells.

After reperfusion, the trend for the time course of $\tan \delta_m$ is an initial increase in the amplitude for some hours, above the $\tan \delta_m$ amplitude of the ischemic tissue, with higher increases for reperfusion at later stages (Figure 4). Following the initial changes, the trend for the time development of the reperfused tissue was different than for the ischemic tissue. In the 10-hour experiment window in the A-series, the $\tan \delta_m$ amplitude of the all reperfusion series (A1-A3) initially rose above the $\tan \delta_m$ amplitude of the ischemic tissue. A1 and A2 stabilised after 3 hours of reperfusion, while the experiment duration was not long enough to observe if A3 would stabilize. In the 16-hour experiment window of

the B-series the $\tan\delta$ amplitude of B1 and B2 stabilized respectively after 3 and 4 hours of reperfusion, while the B3 series increased for 6 hours before starting a trend of decrease/stabilization. The $\tan\delta$ amplitude of the B4 series rose above the amplitude of the ischemic tissue for most of the remaining experiment duration.

Granger et al. reported a doubling of vascular permeability during ischemia and a fourfold increase in vascular permeability after reperfusion (Granger, 1988). Following reperfusion, increasing vascular permeability and effects caused by the immune response leads to the formation of edema (Kalogeris et al., 2012), allowing for increased ion mobility. Radhakrishnan et al. reported a correlation between increasing tissue water concentration and increasing tissue capacitance (Radhakrishnan et al., 2007). Edema reduces both the resistance and reactance, and the ratio is affected because reactance decreases more than the resistance, increasing $\tan \delta$. 30-60 minutes after reperfusion, the reperfused segments appear edematous (Figure 5), with more edema in the segments reperfused following longer ischemia intervals. We estimate that the formation of edema is a major cause for the increase in $\tan\delta$ following reperfusion.

The results in the present study, with significant differences between the development of electrical parameters in the control tissue compared to the ischemic and reperfused tissue, indicates that the relation between electrical parameters and ischemia/reperfusion is complex, but valuable information for viability assessment could be provided by looking at the time-development of the $\tan \delta$ parameter.

5 Conclusion

The $\tan \delta$ parameter was different ($p<0.016$) comparing ischemic and control tissue for the duration of the experiment (16 hours), showing that trans-intestinal bioimpedance measurements can be used to assess if small intestine is ischemic or not. Comparing the control tissue 30 cm from the ischemic area with the control tissue 60 cm from the ischemic tissue, we found that the mean $\tan \delta$ amplitude in the frequency range (3900-6300 Hz) was significantly higher ($p< 0.036$) in the control tissue closer to the ischemic area after 10 hours of experiment duration. This indicates that bioimpedance measurements has a potential usefulness in assessing if small intestinal tissue experiences inflammation. After reperfusion, the time development of $\tan\delta$ amplitude and frequency overlapped and periodically increased above the $\tan\delta$ in the ischemic intestine, showing that not only does reperfusion cause changes in the electrical parameters, but that these changes can be larger than the changes caused by ischemia itself. Valuable information for viability assessment could be provided by looking at the time-development of the $\tan \delta$ parameter following reperfusion.

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7 Conflict of interest

The authors do not have any potential conflicts of interest to declare.

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