gastroenterology insights

MDPI

Article

Not All Porcine Intestinal Segments Are Equal in Terms of Breaking Force, but None Were Associated to Allometric Parameters

Tobias Kratz [1], Jan Dauvergne [1], Anne-Sophie Kronberg [1], David Katzer [2], Rainer Ganschow [2], Marit Bernhardt [3], Sarah Westeppe [4], Benjamin Bierbach [5], Joanna Strohm [6] and Christina Oetzmann von Sochaczewski [6,*]

1 Abteilung für Kinderkardiologie, Universitätsklinikum Bonn, 53127 Bonn, Germany
2 Klinik und Poliklinik für Allgemeine Pädiatrie, Universitätsklinikum Bonn, 53127 Bonn, Germany
3 Institut für Pathologie, Universitätsklinikum Bonn, 53127 Bonn, Germany
4 Klinik für Kinder- und Jugendmedizin, Medizinische Fakultät der Universität Witten-Herdecke, Klinikum Dortmund, 44137 Dortmund, Germany
5 Abteilung für Kinderherzchirurgie, Universitätsklinikum Bonn, 53127 Bonn, Germany
6 Sektion Kinderchirurgie der Klinik für Allgemein-, Viszeral-, Thorax- und Gefäßchirurgie, Universitätsklinikum Bonn, 53127 Bonn, Germany
* Correspondence: c.oetzmann@gmail.com

Abstract: Biomechanics are gaining ground in gastroenterology in the creation of educational models and to describe the necessary forces to perforate hallow organs during endoscopy. We thus investigated the breaking forces of porcine intestinal segments and whether they could be predicted based on body weight or crown–rump length. Based on a priori power-analyses, 10 pigs were included. The breaking forces were determined with a motorized test stand. We found that the breaking forces of intestinal segments were different ($H(6) = 33.7$, $p < 0.0001$): Ileal breaking force ($\bar{x} = 24.14$ N) was higher than jejunal ($\bar{x} = 14.24$ N, $p = 0.0082$) and colonic ($\bar{x} = 11.33$ N, $p < 0.0001$) breaking force. The latter was also smaller than cecal breaking force ($\bar{x} = 24.6$ N, $p = 0.0044$). Likewise, rectal ($\bar{x} = 23.57$ N) breaking force was higher than jejunal ($p = 0.0455$) and colonic ($p = 0.0006$) breaking force. Breaking forces were not correlated to body weight or crown–rump length ($R < 0.49$, $p > 0.148$). Intestinal segments differ in their breaking forces. The colon had the least resistance to traction forces. It remains to be determined if similar relationships exist in humans in order to validate porcine models for endoscopy and surgery.

Keywords: swine; experimental gastroenterology; breaking force; allometry; biomechanics

Citation: Kratz, T.; Dauvergne, J.; Kronberg, A.-S.; Katzer, D.; Ganschow, R.; Bernhardt, M.; Westeppe, S.; Bierbach, B.; Strohm, J.; Oetzmann von Sochaczewski, C. Not All Porcine Intestinal Segments Are Equal in Terms of Breaking Force, but None Were Associated to Allometric Parameters. *Gastroenterol. Insights* **2023**, *14*, 475–490. https://doi.org/10.3390/gastroent14040035

Academic Editors: Ludovico Abenavoli and Chun Gao

Received: 30 July 2023
Revised: 23 September 2023
Accepted: 9 October 2023
Published: 11 October 2023

1. Introduction

The biomechanical properties of organs have long been of interest for surgical procedures in particular [1–5]. With the ongoing progress in interventional gastroenterology, regularly replacing surgery [6–9], the biomechanical properties of intestinal organs recently came into focus, too [10]. Cadaveric studies in humans were exceptionally rare in the literature, and all of them were conducted using bursting strength testing [11,12]. It, first described by Chlumsky in the 19th century [13], has several drawbacks. The most relevant is the non-comparability of measurements beyond a single study in terms of quantitative data [14,15]. Breaking force, first described by Howes [16], in contrast, allows for the inter-study comparison of results [14]. This is of particular relevance for gaining a collective level of evidence that allows for the thorough evaluation of an (experimental) technique beyond a single study. Endoscopic procedures have been of interest to gastroenterologists with respect to biomechanical properties, either evaluated in human-like models [17] or in fresh porcine organs [18].

It has been bemoaned that there is considerable variability in the results of biomechanical testing [19]. This is partially attributed to methodological differences, but also to variability in model selection [19,20]. However, detailed knowledge of anatomical and biomechanical parameters is crucial for model selection and the transferability of results to human disease [21]. Another factor for variability might be the size or weight of the investigated species. This can be demonstrated by an example from xenotransplantation: From a surgical point of view, a pig weighing 30 kg was considered to be most suitable for the experiment [22]. However, biomechanical analyses suggested that pigs at the age of five months had tissue properties and vasculature sizes to come as close as possible to the potential human recipient [23].

Swine had long been the model of choice for many areas of experimental research as porcine anatomy and physiology is relatively similar to humans [24]. This is even the case in the most extreme age groups such as in newborns [25]. Consequently, swine had become a standard model in endoscopy research beyond the well-described models of esophageal endoscopic mucosal dissection [26] and esophageal endoscopic submucosal dissection [27]. Their usage as a model organism, for example, now extends to colorectal endoscopic submucosal dissection [28], upper gastrointestinal bleeding [29], or addressing intestinal perforations via endoscopic repair [30]. In addition, simulation models, created to improve training in gastrointestinal endoscopy, utilized porcine intestines to model human intestines and thereby create an adequate training environment. Such a training opportunity contributes towards better training and aid in gaining experience that is required to adequately perform difficult tasks within a procedure [31]. Porcine-based endoscopy simulators have been created for endoscopic submucosal dissection [32], peroral endoscopic myectomy [33], anastomotic strictures in Crohn's disease [34], and colonoscopy [35]. Successful use of these simulators, however, requires that they adequately model the human patient and be consistent models, i.e., in a way that they always have similar tissue parameters and tissue properties. This is necessary to achieve a lasting training effect with respect to the performed interventions.

Designing such a model requires knowledge on the used porcine tissue parameters and a potential starting point to ensure adequate model standardization beyond the single experiment to train interventions using these simulators. In order to investigate whether simple biomechanical properties could be related to allometric parameters, as potential starting points for model standardization, we measured breaking forces in sections of the intestines of German landrace pigs. The intestinal segments were evaluated for a potential relationship to the allometric parameters of crown–rump length and body weight in order to contribute to the further refinement of experimental models of interventions. Our report focused on breaking forces of the intestine, because breaking forces are regularly used to evaluate enterotomy closures [36] and anastomoses [37,38].

2. Materials and Methods

In our study, 10 consecutive German landrace pigs (Sus scrofa domestica), 9 males and 1 female, were included. These pigs were obtained from the Landwirtschaftliche Fakultät der Rheinischen Friedrich-Wilhelms-Universität Bonn, Lehr- und Forschungsstation Frankenforst (Königswinter-Vinxel, Germany) [39]. Each pig was an experimental unit, and only one animal was used per experiment in order to separate them into mini-experiments, because this increases the experiment's reproducibility [40]. Their microbiological status was conventional, and swine had been vaccinated against mycoplasma on day 3 and day 10 of life according to the standard practice of our vendor [41]. Before transfer to our facility, the feeding and husbandry practices adhered to the husbandry regulation and standard guidelines of the Zentralverband der Deutschen Schweineproduktion [41]. Swine had time to acclimatize for three days at our facility [42] before the experiments began in order to ensure that all relevant parameters altered due to transport were back to normal baseline values [43]. The relative humidity at our facility was kept between 50% and 60% at a temperature between 16 °C and 18 °C. The air in the facility was exchanged at

least 8 times per hour. Each swine was housed alone in a box sized 4 m^2 to 6 m^2, which was enriched by chains, balls, and additional play materials for the pig. Each box had an infrared heating lamp available for the swine in order to keep its temperature during resting periods. We used dark–light cycles of 12 h with artificial lighting between 7 am and 7 pm. Drinking water was available ad libitum to the pigs, and they were fed regular chow (Altromin 9023, Altromin Spezialfutter, Lage, Germany) in our facility.

We measured crown–rump length from the top of the skull to the base of the tail [44]. After the pigs were euthanized using T61® (tetracaine/mebenzonium/embutramide, Intervet, München, Germany), we conducted a necropsy as described previously [45]. After removal of the intestinal organ package, we identified the different distinct parts of the intestine, the duodenum, the jejunum, the ileum, the cecum, the colon, and the rectum. In the middle of all these intestinal parts, a representative section of 6 cm length was marked and excised. For the duodenum, this was 10 cm distal to the stomach; for the jejunum, it was 20 cm aboral to the ligament of Treitz; and for the ileum, this was 20 cm oral to the ileo-cecal valve. For the cecum, the representative section was directly adjacent to the aboral part of the ileo-cecal valve; for the colon, it was 20 cm aboral to the ileocecal valve; and for the rectum, it was 10 cm oral to the anus. The excised section was then mounted into a motorized test stand (Sauter THM500N, Kern & Sohn, Balingen, Germany) and subjected to linearly increasing traction forces (Figure 1), which advanced at the standard rate of 10 mm per minute used in such experiments [46–49]. Traction forces were gauged using a tensiometer (FL100, Kern & Sohn, Balingen, Germany) until the muscular layer was disrupted and the mucosal layer was still visible. This was registered as a loss of force by the tensiometer and the maximum value was recorded as the breaking force [50–55]. The experimental setup has been described in detail elsewhere [53,54] and is photographically depicted in Figure 2. We conducted all experiments within two hours after the swine's euthanasia to ensure that our results were not affected by structural changes due to cell death.

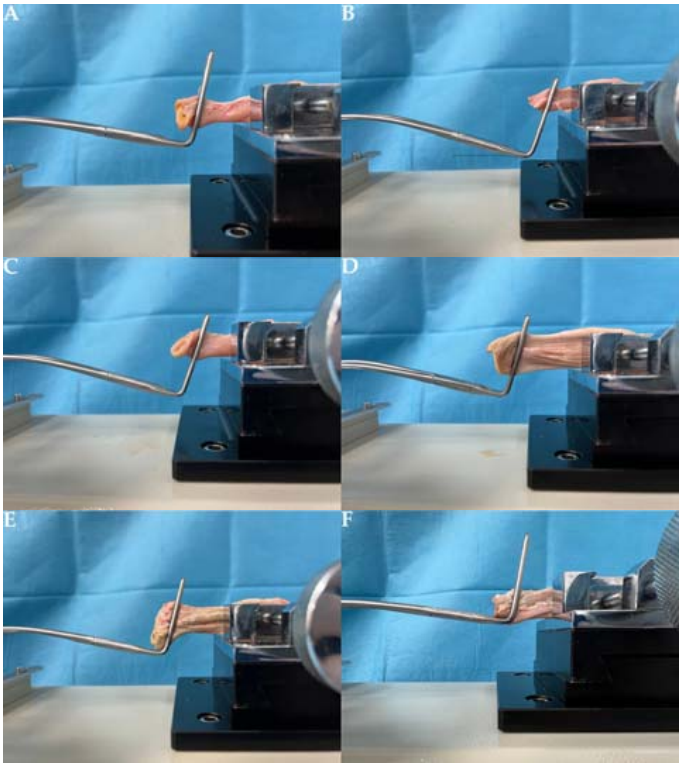

Figure 1. Different intestinal segments within the motorized test stand. (**A**) Duodenum. (**B**) Jejunum. (**C**) Ileum. (**D**) Cecum. (**E**) Colon. (**F**) Rectum.

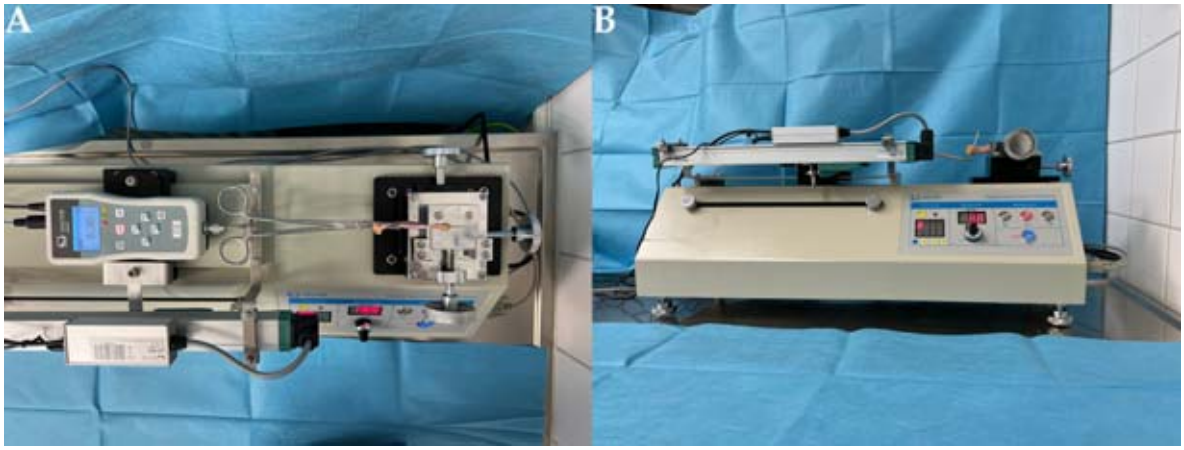

Figure 2. Detailed photographic depiction of the experimental setup. (**A**) Top-view of the experiment at its starting point. (**B**) Corresponding lateral view of the experiment.

Representative specimens were obtained from the site of the tissue rupture as well as 2 cm oral and aboral to it. Specimens were fixed in 4% buffered formaldehyde solution (Sigma Aldrich, Darmstadt, Germany) for 24 h at room temperature. Then, specimens were dehydrated in ascending alcohol concentrations and embedded in Tissue-Tek III paraffin wax (Sakura Finetek, Alphen aan de Rhijn, The Netherlands) to allow further processing for histological staining. Sections of 3 µm size were cut and stained with Hematoxylin-Eosin, Elastica-van Gieson (Medite Tissue Stainer, Medite, Burgdorf, Germany), and Masson's trichrome (Avantor, VWR, Darmstadt, Germany) according to standard protocols [56].

Although data for the breaking force of porcine intestine were not available in the literature, we used the results from dogs [57] to conduct a formal a priori power analysis: With five groups of 10 dogs and the highest standard deviation of 0.56 for the intestinal measurements, the preceding report had an effect size of f = 1.108. Using G*Power 3.1.9.2 [58], we calculated 5 measurements per group to be necessary to achieve a difference in the one-way analysis of variance with $\alpha = 0.01$ and $\beta = 0.9$. In order to correct for uncertainties due to the different species, we doubled the sample size to 10 per group.

For statistical analysis, we used GraphPad Prism 8 (GraphPad Software, San Diego, CA, USA). A Gaussian distribution was checked using the D'Agostino–Pearson test aided by visual analysis of QQ-plots. Homoscedasticity was tested using Levene's test. Correlations were calculated using Pearson's R for breaking force, body weight, and crown–rump length. The correlation between breaking force and intestinal diameters was assessed using Spearman's ρ due to missing normality for some intestinal diameters that precluded parametric statistical testing. The breaking force of different parts of the intestine was compared using the Kruskal–Wallis test due to non-normal distribution of the residuals. Post hoc differences were evaluated exploratorily using the Dunn–Bonferroni test in order to account for alpha inflation.

3. Results

We included 10 consecutive swine, 9 males and 1 female, which had a mean body weight of 21.9 kg (standard deviation of 2). They had a mean crown–rump length of 67.3 cm (standard deviation of 3). The breaking force of the porcine intestine (Table 1) was substantially different between the intestinal segments ($H(6) = 33.7$, $p < 0.0001$).

Post hoc testing revealed that the ileum had a larger breaking force than the jejunum ($\Delta = 9.9$ N, $Z = 3.46$, $p = 0.0082$) and colon ($\Delta = 12.81$ N, $Z = 4.6$, $p < 0.0001$) (Figure 2). Likewise, cecal breaking force was larger than colonic breaking force ($\Delta = 13.27$ N, $Z = 3.62$, $p = 0.0044$) (Figure 3). The breaking force of the rectum was larger than the jejunum's ($\Delta = 9.33$ N, $Z = 2.96$, $p = 0.0455$) and colon's breaking force ($\Delta = 12.24$ N, $Z = 4.11$, $p = 0.0006$)

(Figure 3), whereas differences between the other intestinal segments could not be demonstrated (Figure 3).

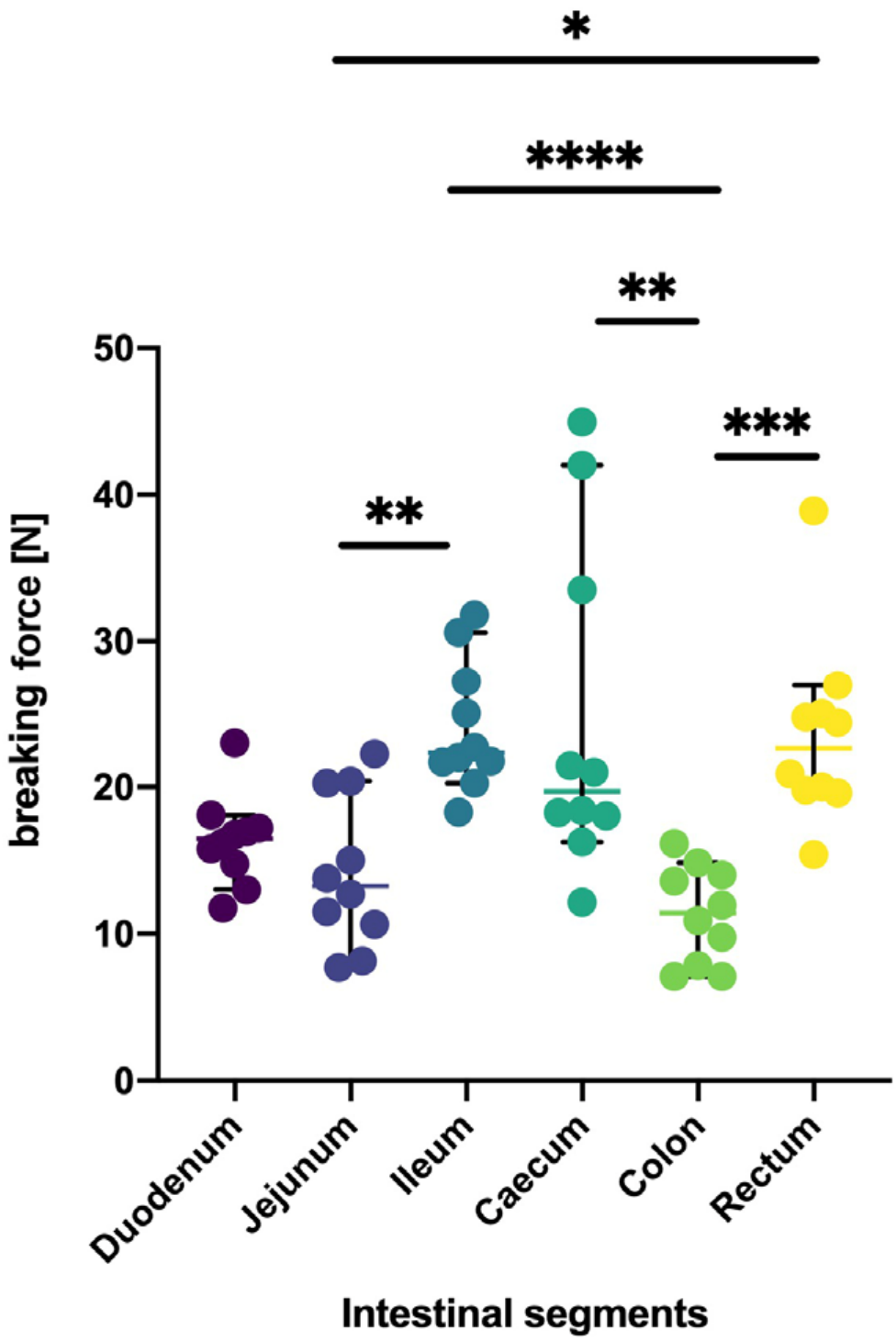

Figure 3. Breaking forces of the different intestinal segments. Individual measurements and median with its 95% confidence intervals are provided to allow interpretation of the Kruskal–Wallis and the subsequent Dunn–Bonferroni-tests. N = 10 for each group. Colors were chosen from the viridis scales in order to be colorblind-friendly. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

Table 1. Breaking forces of the porcine intestinal segments in Newton. As the data were normally distributed and had equal variances, mean and standard deviation are provided. $n = 10$ for each group.

	Duodenum	Jejunum	Ileum	Cecum	Colon	Rectum
Mean breaking force [N]	16.35	14.24	24.14	24.6	11.33	23.57
Standard deviation [N]	3.05	5.18	4.45	11.34	3.31	6.39

Neither body weight nor crown–rump length were correlated to the breaking forces of the intestinal segments (Table 2).

Table 2. Pearson's correlation coefficients for body weight and crown–rump length and breaking force of the porcine intestinal segments.

	Duodenum	Jejunum	Ileum	Cecum	Colon	Rectum
Body weight [*R*] (95% CI)	0.34 (−0.37–0.8)	0.35 (−0.36–0.8)	0.23 (−0.28–0.83)	0.32 (−0.38–0.79)	−0.17 (−0.73–0.51)	0.3 (−0.41–0.78)
p	0.331	0.317	0.422	0.361	0.626	0.398
Crown–rump length [*R*] (95% CI)	−0.16 (−0.72–0.53)	−0.4 (−0.82–0.3)	0.49 (−0.2–0.86)	−0.08 (−0.68–0.58)	0.2 (−0.49–0.74)	0.16 (−0.53–0.72)
p	0.665	0.246	0.148	0.823	0.571	0.669

CI = confidence interval.

We noticed that the diameters of the intestinal segments were similar in the small intestine (Table 3), whereas they varied in the large intestine: The cecum had the largest diameter, which gradually decreased towards its aboral end (Table 3).

Table 3. Intestinal diameters of the intestinal segments measured horizontally and vertically.

	Duodenum	Jejunum	Ileum	Cecum	Colon	Rectum
Horizontal diameter [cm]	1	1.1	1.09	3.45	2.09	1.34
Standard deviation [cm]	0.22	0.17	0.2	0.83	0.19	0.55
Vertical diameter [cm]	0.95	0.93	1.04	3.59	2.48	1.31
Standard deviation [cm]	0.2	0.12	0.42	0.52	0.69	0.3

In order to evaluate whether the diameters of the intestinal segments were linked to breaking force, we conducted a correlation analysis using spearman's ρ, which did not show any associations between breaking force and intestinal diameter, neither for the small nor for the large intestine (Table 4).

Table 4. Spearman's correlation coefficients for breaking forces of the porcine intestinal segments and their diameters.

	Duodenum	Jejunum	Ileum	Cecum	Colon	Rectum
Horizontal diameter [ρ]	−0.04	−0.13	0.2	0.16	−0.4	0.3
(95% CI)	(−0.72–0.69)	(−0.77–0.63)	(−0.59–0.79)	(−0.62–0.78)	(−0.86–0.42)	(−0.51–0.83)
p	0.929	0.752	0.641	0.707	0.324	0.473
Vertical diameter [ρ]	0.02	0.2	0.26	0.54	−0.36	0.17
(95% CI)	(−0.69–0.72)	(−0.59–0.79)	(−0.54–0.82)	(−0.27–0.9)	(−0.85–0.47)	(−0.61–0.78)
p	0.958	0.632	0.531	0.172	0.387	0.694

CI = confidence interval.

We did not detect microstructural alterations in the intestine beyond the damages due to measuring breaking force; neither the small (Figure 4) nor the large intestine (Figure 5) exhibited any changes in standard Hematoxylin-Eosin, Elastica, and Trichrome staining.

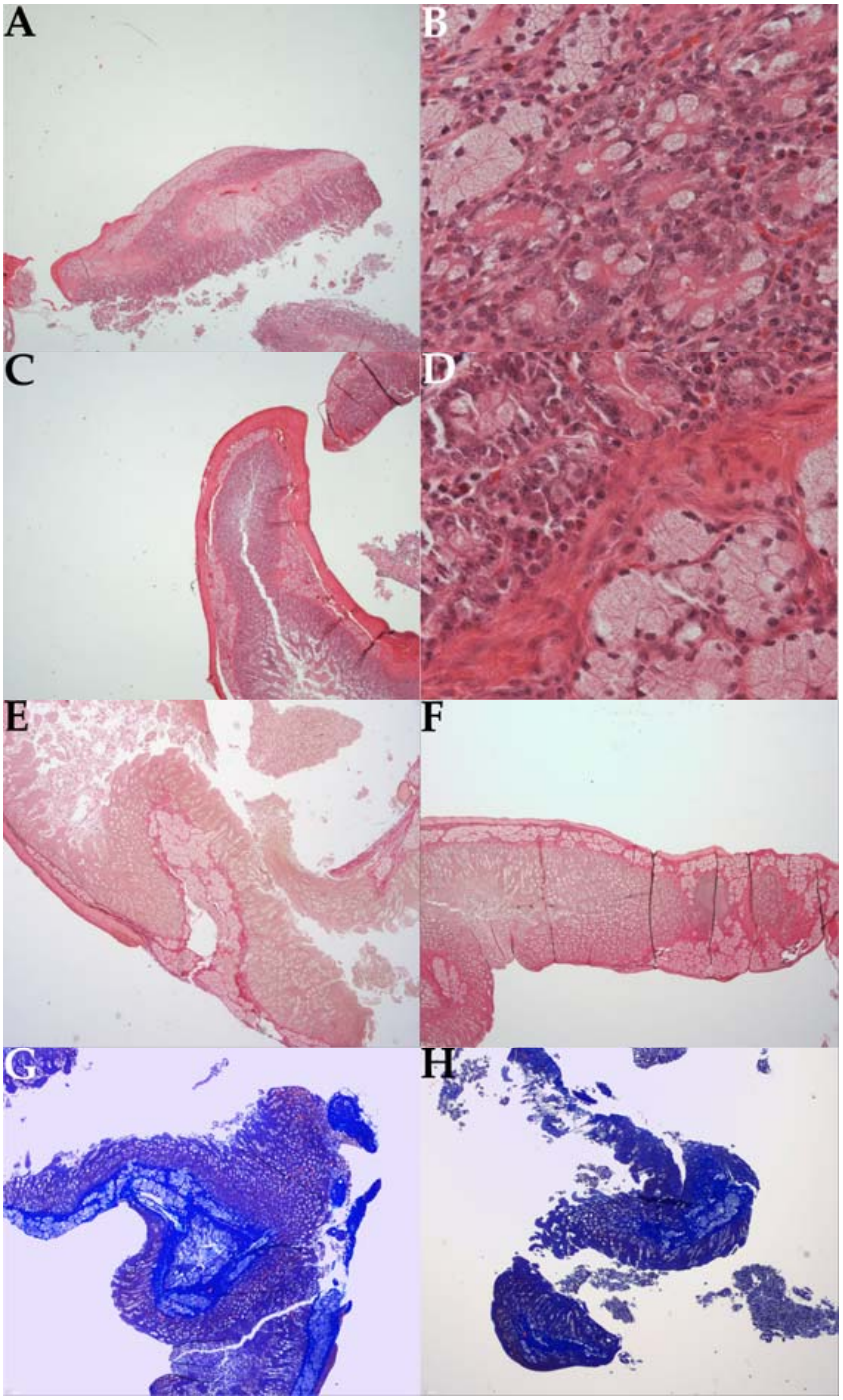

Figure 4. No microstructural alteration beyond the obvious tissue destruction due to measuring breaking force in the duodenum, representative of the small intestine. (**A**) Hematoxylin-Eosin staining of the disrupted tissue at 1.25× magnification. (**B**) Hematoxylin-Eosin staining of the disrupted tissue at 40× magnification. (**C**) Hematoxylin-Esoin staining 2 cm aboral to the site of tissue disruption at 1.25× magnification. (**D**) Hematoxylin-Eosin staining 2 cm aboral to the site of tissue disruption at 40× magnification. (**E**) Elastica-van Gieson staining at the site of tissue disruption at 2.5× magnification. (**F**) Elastica-van Gieson staining 2 cm aboral to the site of tissue disruption at 2.5× magnification. (**G**) Masson's trichrome staining at the site of tissue disruption at 2.5× magnification. (**H**) Masson's trichrome staining 2 cm aboral to the site of tissue disruption at 2.5× magnification.

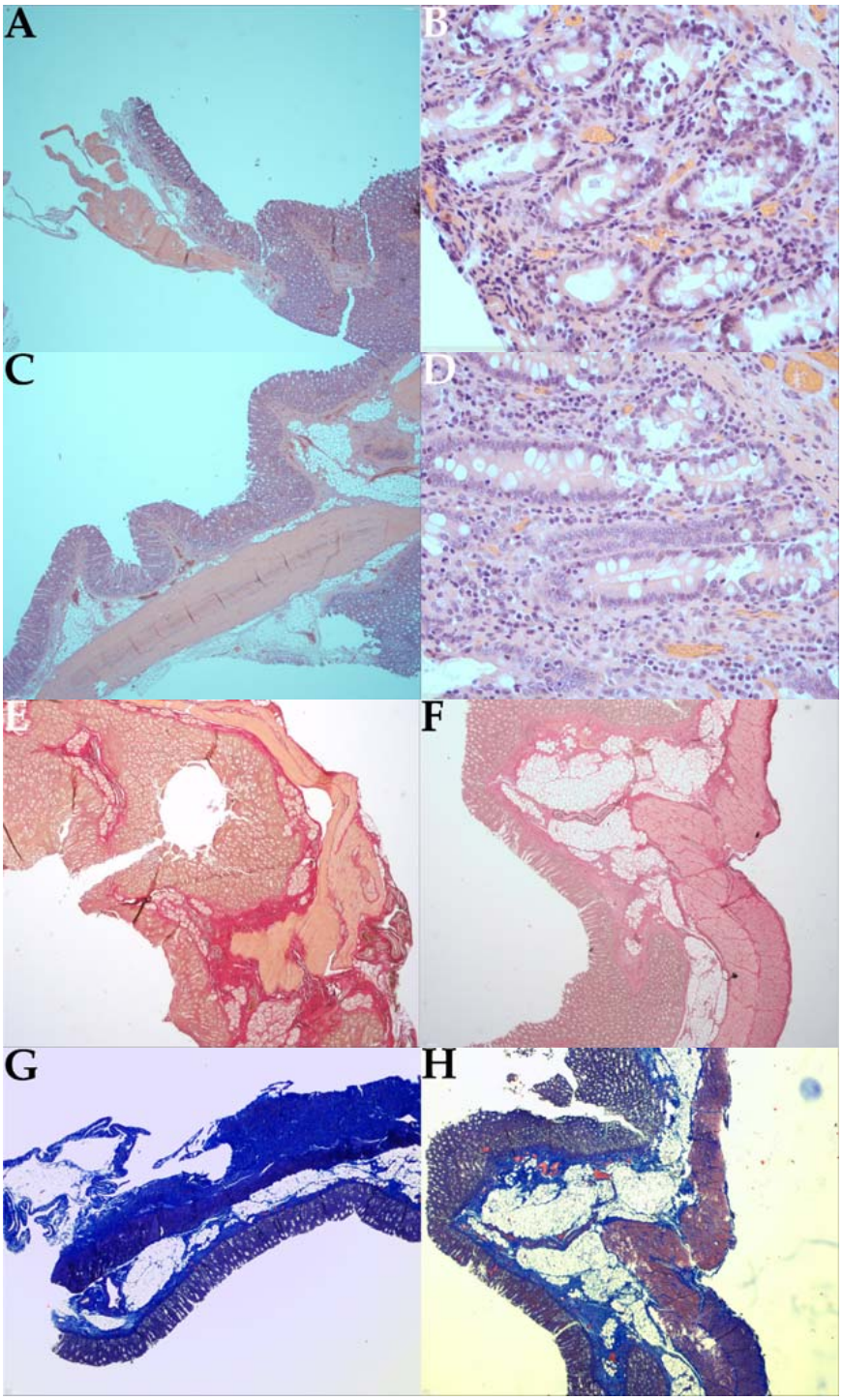

Figure 5. No microstructural alteration beyond the obvious tissue destruction due to measuring breaking force in the cecum, representative of the large intestine. (**A**) Hematoxylin-Eosin staining of the disrupted tissue at 1.25× magnification. (**B**) Hematoxylin-Eosin staining of the disrupted tissue at 40× magnification. (**C**) Hematoxylin-Esoin staining 2 cm oral to the site of tissue disruption at 1.25× magnification. (**D**) Hematoxylin-Eosin staining 2 cm oral to the site of tissue disruption at 40× magnification. (**E**) Elastica-van Gieson staining at the site of tissue disruption at 2.5× magnification. (**F**) Elastica-van Gieson staining 2 cm oral to the site of tissue disruption at 2.5× magnification. (**G**) Masson's trichrome staining at the site of tissue disruption at 2.5× magnification. (**H**) Masson's trichrome staining 2 cm oral to the site of tissue disruption at 2.5× magnification.

4. Discussion

For surgery, biomechanical aspects have long been of importance in the advancement or modification of treatment approaches [1–5]. This has been true to a lesser extent in gastroenterology, which had initially focused on educational models [59,60]. Due to the shift away from surgery to endoscopic interventions in several diseases [6–9], animal models of endoscopic interventions and treatment were gaining ground [27,61]. As it has been the case before in surgical models, swine were the preferred model organism due to its inherent similarity to the human anatomy and physiology [24,62]. Contrary to surgical procedures [4,53,63–65], the biomechanical aspects of interventional procedures were not investigated until this came into focus in the recent years [10,17,18]. We therefore aimed to investigate the breaking forces of porcine intestinal segments. We favored this method, breaking forces instead of burst pressure, because it allows for between-study comparison [14], to assess potential differences between the intestinal segments. In addition, we also tested whether the breaking force could be associated with allometric parameters in order to contribute to experimental standardization.

In liver transplantation research, pigs weighing 30 kg had long been considered to be the ideal model due to surgical feasibility [22]. However, tissue properties of swine aged five months resembled the human situation more closely [23]. Consequently, the biomechanical properties of the bile duct system had been described aiming to gain further insight into training models and potential application in xenotransplantation [66]. These studies emphasize the importance of knowledge in biomechanical tissue properties. They had early been of interest in esophageal atresia, because during its repair, the esophagus has to be frequently anastomosed under tension [2]. Surgically, the biomechanical properties of the intestine were of lesser interest than those of the esophagus, because intestinal anastomoses under tension were rare, but if tension was present, it was a strong predictor for anastomotic stenosis [67].

Differences between the intestinal segments were to be expected in swine, because esophageal segments differed in their ultimate tensile strength [68]. In dogs, the small intestine exhibited smaller breaking forces than the large intestine [57], while substantial variation along the small [69] and large [70] intestine was found, although they were not directly compared. However, there were substantial differences in breaking forces between the different species: In dogs, breaking forces of the small intestinal segments were lower than those of large intestinal segments [57], but we found substantial variation in breaking forces in porcine intestinal segments. We could only assume a difference in breaking forces between the intestinal segments during study planning, but not the direction, i.e., which segments would have lower breaking forces and which would exhibit a larger breaking force. We were thus unable to pre-specify and conduct an a priori power analysis for the comparison of the intestinal segments. Consequently, these were only analyzed exploratorily, whereas the differences between intestinal segments were analyzed confirmatory based on the a priori sample size analysis. We could not assume that porcine biomechanical tissue properties would be similar to human ones described in the literature [71]. Although a certain degree of similarity was described for the infant's esophagus [2], substantial differences between porcine and human intestinal tissue properties had been described for the sigmoid colon before [72]. These preceding data precluded the assumption that porcine and human intestinal breaking forces would be similar.

In order to develop an artificial training simulator for endoscopy [17] or using an animal model [59,60], it is crucial to have similar tissue properties to substitute the patient and aid in the transferability of the learned procedure to the patient [18]. An important aspect in this case is the resistance to traction forces of the organ used to model the patient; it should have properties similar to the human organ. Using breaking force, we systematically described a small proportion of the tissue properties of fresh porcine intestinal segments and were able to describe relationships between the intestinal segments similar to those described in humans, although using a different method [11]. Our results differed from those in dogs [57], which is relevant as dogs had been and still are relevant animal models

of interventions [73–75]. The differing results, compared to Christensen et al. [72], might be attributed to the method or the freeze-thawing used in their work. Freeze-thawing has been shown to affect elastin-containing organs in particular [76], which include the sigmoid colon [77].

Modelling the biomechanics of the living organism is crucial for the acceptance of ex vivo simulators [35]. They have been evaluated and thoroughly tested, including a randomized controlled trial, and found to be helpful in gaining experience, training, and even proficiency, irrespective of the level of experience at the start of the training [32,78]. Trainees and even advanced and experienced endoscopists felt that they gained additional experience and proficiency from using ex vivo simulators, especially if they were using them in a structured manner [79]. Similar effects have been demonstrated for different procedures including foreign body removal [79], endoscopic submucosal dissection [32], and peroral endoscopic myectomy [33]. An additional advantage is that even difficult procedures [32,33,79], or parts of them [31], can be trained before they are first used in patient care, and, thus, the early phase of the learning curve can be avoided in the patient [33].

Such benefit, however, requires that ex vivo simulators and the porcine tissue used in them can adequately model the situation in the human patient. Therefore, we also aimed to investigate whether breaking forces would be associated with allometric parameters in order to improve the standardization of animal models. It seemed plausible to assume this, as breaking forces were correlated to body weight in rats [47]. Moreover, it has been shown that the absolute and relative visceral organ weights of swine were dependent on body weights [80]. Although a certain degree of variation is of particular relevance for the reproducibility of results to avoid idiosyncratic lab-dependent effects [81], a certain level of standardization would be preferable, particularly with the use of swine, as experiments using pigs are rather expensive compared to rodents and, thus, their number will inevitably be smaller due to limited funds. However, in our study, breaking force was not related to body weight or crown–rump length, which suggests that the use of these parameters seems to be unsuitable for model standardization.

We did not aim for a complete and detailed description of porcine intestinal segments with respect to their biomechanical details. At first, this would have required an exact assessment of the specimen thickness, because changes in the sample size would affect the measurements and thus result in an incorrect assessment of the mechanical properties of the specimens [82,83]. Consequently, rectangular specimens cut out of the organs had been used regularly, because they allowed for uni- and biaxial tensile testing with parallel measurement of specimen deformation [84–86], as, for example, depicted in detail by Sommer and colleagues [87]. For intestinal segments that are investigated as a whole specimen, it is unlikely that the assumption of similar specimen thickness is valid. Salvatierra and colleagues investigated, using full-thickness biopsies obtained during colonoscopy, whether colon diameters or wall thicknesses were different between the different anatomical segments of the colon [88]. Moreover, intestinal wall thickness also varies with age and even substantially in the same patient [82]. Salvatierra and co-workers found that while colonic diameters decreased in aboral direction, which also was the case in our study, colonic wall thickness increased, which affected both the mucosal layer and the muscularis propria [88]. On the contrary, investigations in rats showed that the transverse colon had the highest resistance to uniaxial traction forces [70,89], which also was the case in mice [90]. Based on these results, it has been assumed that the longitudinal stiffness decreases both orally and aborally starting from the transverse colon [91], but this was not the case for porcine sigmoidal colon [72]. Consequently, our results were not that unusual, although the used methods differed.

Due to the results reported by Salvatierra and co-workers [88], we assumed a priori that the intestinal segments would have different diameters and wall thicknesses of the intestinal walls. We did not aim, in our simple measurements, to correct for this parameter, as this would not mimic the clinical situation on which we focused in our measurements.

Investigations using breaking forces offer the advantage that the results are comparable between different studies [14]. This is of particular relevance for studies investigating intestinal surgery or their endoscopic counterparts [30], because the surgical procedures, irrespective if conducted openly or laparoscopically, would include stapling of the anastomoses [92]. Although bursting pressure has been used to evaluate staplers [65,93] and anastomoses [57,94–98], this has met criticism [15], because bursting pressures are only comparable within, but not beyond, a single study [14]. Consequently, breaking forces have been the standard to evaluate colorectal surgical stapling [37,38,63,92,99,100] but have also been used to investigate hand-sewn anastomoses [4,101,102]. As stapling is the standard for such colorectal anastomoses, endoscopic interventions that should supplement or even replace surgery in cases of intestinal perforation [30], a comparative assessment of the durability of the anastomoses requires using the same technique to evaluate them. A relevant comparator to anastomotic techniques is the native, surgically untouched, organ, because not including it would limit the internal validity of the study [53]. Our study provides such measurements and thus a potential reference that might be used in sample size calculations, because these measurements are exceedingly rare, exemplified by the fact that we had to use data obtained from investigating dogs [57].

Besides the already mentioned issue of only exploratory investigations in the pairwise-comparison of intestinal segments, our study is also limited by the use of only one pig breed, because only this breed could be supplied in-house. Another limitation is the relatively unequal distribution of sexes despite the use of consecutive swine. Although body weight differences between sexes have been described in terms of agricultural pig fattening, these were limited to swine after they reached sexual maturity [103], which has not occurred in our cohort. Likewise, the issue of non-linear growth in fattening pig breeds, which might distort the results as the predictive value of the allometric parameters, might be even worse due to the non-linear growth. However, this is not a relevant issue in our study, because the pigs were used before the age of seven weeks and thus before the beginning of the non-linear growth [104]. The suitability of landrace breeds instead of minipig breeds has been demonstrated even for complex experiments [105,106], provided they are young enough to avoid the non-linear growth.

Another issue of relevance might be the sample size of our study. Although experimental research might require a smaller sample size than studies involving human subjects, environmental factors might be better controlled and subjects might be more standardized than human participants [107]. Obvious differences, as there are between humans and rats, the latter being rodents instead of omnivores, which results in different macro- and microanatomy due to different modes of feeding [47], are not present between the two omnivores dogs and pigs. However, there are differences in the pH values of intestinal contents, relative lengths, and the relative amounts of intestinal contents between pigs and dogs [108], whose effects on breaking forces of intestinal segments are unknown. Therefore, the sample size calculation that we conducted using the published data from Ogurtan and co-workers [57] might be limited by the transferability between the two species. This limitation might persist despite the correction factor, doubling the calculated sample size, in order to account for the uncertainty associated with data from a different species. For the post hoc tests, we employed the Dunn–Bonferroni test to correct for alpha inflation in order to limit the potential of false-positive results from a statistical point of view. It is puzzling that conducting and reporting a sample size calculation at all is surprisingly uncommon; a recent systematic review found it to have only increased from 5.2% to 7.6% in 2018 [109], despite the methodological drawbacks of not doing so [110]. To have performed a sample size calculation does not, however, support the assumptions that we made, at least some level of transferability from dogs to pigs, but at least provides some support for the reliability of the results, given its assumptions were true, for which there are no data at present.

Taken together, we found substantial differences in the breaking forces of porcine intestinal segments that were not correlated to allometric parameters. Thus, body weight

and crown–rump length are not suitable parameters to standardize in vivo or ex vivo models using porcine tissue for full-scale models of interventions. To assess the similarity of biomechanical properties, as a factor of model suitability, our results need to be compared to measurements in whole human intestinal segments to validate porcine models of endoscopic treatments. In addition, it needs to be investigated what the tissue properties of the porcine intestinal segments are in order to fully characterize them and thereby contribute to model refinement. In particular, his will include the relevant aspect of specimen wall thickness to account for their differences along the intestine. However, our results provide a baseline measurement for porcine intestinal breaking forces in the native organ, which is a suitable comparator for assessing anastomotic devices.

Author Contributions: Conceptualization, T.K. and C.O.v.S.; methodology, D.K. and C.O.v.S.; formal analysis, T.K., D.K. and C.O.v.S.; investigation, T.K., A.-S.K., J.D., D.K., M.B., B.B. and C.O.v.S.; resources, R.G.; writing—original draft preparation, T.K., S.W. and C.O.v.S.; writing—review and editing, J.D., A.-S.K., D.K., R.G., M.B., B.B. and J.S.; visualization, S.W. and C.O.v.S.; supervision, R.G. and J.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The animal study protocol was approved by the Landesamt für Natur, Umwelt und Verbraucherschutz Nordrhein-Westfalen (protocol code 81-02.04.2020.A392).

Data Availability Statement: The data presented in this study are available in Zenodo [111].

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Livaditis, A.; Rådberg, L.; Odensjö, G. Esophageal End-to-End Anastomosis: Reduction of Anastomotic Tension by Circular Myotomy. *Scand. J. Thorac. Cardiovasc. Surg.* **1972**, *6*, 206–214. [CrossRef]
2. Takada, Y.; Kent, G.; Filler, R.M. Circular myotomy and esophageal length and safe esophageal anastomosis: An experimental study. *J. Pediatr. Surg.* **1981**, *16*, 343–348. [CrossRef]
3. Fujiwara, H.; Kuga, T.; Esato, K. High submucosal blood flow and low anastomotic tension prevent anastomotic leakage in rabbits. *Surg. Today* **1997**, *27*, 924–929. [CrossRef]
4. Ekmektzoglou, K.A.; Xanthos, T.; Dontas, I.A.; Zografos, G.C.; Giannopoulos, P.; Pantopoulou, A.; Papanicolopulos, S.A.; Kourkoulis, S.K.; Perrea, D.N. A Research Model of Measuring the Tensile Strength of Colonic Anastomosis in Wistar Rats. *Scand. J. Lab. Anim. Sci.* **2008**, *35*, 313–320.
5. Tagkalos, E.; Lindner, A.; Gruber, G.; Lang, H.; Heimann, A.; Grimminger, P.P.; Muensterer, O.J.; von Sochaczewski, C.O. Using simple interrupted suture anastomoses may impair translatability of experimental rodent oesophageal surgery. *Acta Chir. Belg.* **2020**, *120*, 310–314. [CrossRef]
6. Ell, C.; May, A.; Gossner, L.; Pech, O.; Günter, E.; Mayer, G.; Henrich, R.; Vieth, M.; Müller, H.; Seitz, G.; et al. Endoscopic mucosal resection of early cancer and high-grade dysplasia in Barrett's esophagus. *Gastroenterology* **2000**, *118*, 670–677. [CrossRef]
7. Pech, O.; May, A.; Manner, H.; Behrens, A.; Pohl, J.; Weferling, M.; Hartmann, U.; Manner, N.; Huijsmans, J.; Gossner, L.; et al. Long-term efficacy and safety of endoscopic resection for patients with mucosal adenocarcinoma of the esophagus. *Gastroenterology* **2014**, *146*, 652–660.e1. [CrossRef]
8. Ritz, L.A.; Hajji, M.S.; Schwerd, T.; Koletzko, S.; Von Schweinitz, D.; Lurz, E.; Hubertus, J. Esophageal Perforation and EVAC in Pediatric Patients: A Case Series of Four Children. *Front. Pediatr.* **2021**, *9*, 727472. [CrossRef]
9. Kaczmarek, D.J.; Heling, D.J.; Strassburg, C.P.; Katzer, D.; Düker, G.; Strohm, J.; Müller, A.; Heydweiller, A.; Weismüller, T.J. Management of esophageal perforations in infants by endoscopic vacuum therapy: A single center case series. *BMC Gastroenterol.* **2022**, *22*, 282. [CrossRef]
10. Fontanella, C.G.; Carniel, E.L. Biomechanics of Hollow Organs: Experimental Testing and Computational Modeling. *Bioengineering* **2023**, *10*, 175. [CrossRef]
11. Egorov, V.I.; Schastlivtsev, I.V.; Prut, E.V.; Baranov, A.O.; Turusov, R.A. Mechanical properties of the human gastrointestinal tract. *J. Biomech.* **2002**, *35*, 1417–1425. [CrossRef]
12. Bourgouin, S.; Bège, T.; Masson, C.; Arnoux, P.-J.; Mancini, J.; Garcia, S.; Brunet, C.; Berdah, S.V. Biomechanical characterisation of fresh and cadaverous human small intestine: Applications for abdominal trauma. *Med. Biol. Eng. Comput.* **2012**, *50*, 1279–1288. [CrossRef]
13. Chlumsky, V. Experimentelle Untersuchungen über die verschiedenen Methoden der Darmvereinigung. *Bruns Beitr Klin Chir.* **1899**, *25*, 539–600.
14. Hendriks, T.; Mastboom, W.J.B. Healing of experimental intestinal anastomoses: Parameters for repair. *Dis. Colon Rectum* **1990**, *33*, 891–901. [CrossRef] [PubMed]

15. Juo, Y.-Y.; Dutson, E. Comment on: Improving the side-to-side stapled anastomosis: Comparison of staplers for robust crotch formation. *Surg. Obes. Relat. Dis.* **2018**, *14*, 21–22. [CrossRef] [PubMed]
16. Howes, E.L.; Sooy, J.W.; Harvey, S.C. The Healing of wounds as determined by their tensile strength. *JAMA* **1929**, *92*, 42–45. [CrossRef]
17. Schneider, J.; Duckworth-Mothes, B.; Schweizer, U.; Königsrainer, A.; Fisch, J.; Wichmann, D. Exerting Forces and Wall Load during Duodenoscopy for ERCP: An Experimental Measurement in an Artificial Model. *Bioengineering* **2023**, *10*, 523. [CrossRef]
18. Johnson, S.; Schultz, M.; Scholze, M.; Smith, T.; Woodfield, J.; Hammer, N. How much force is required to perforate a colon during colonoscopy? An experimental study. *J. Mech. Behav. Biomed. Mater.* **2019**, *91*, 139–148. [CrossRef]
19. Bhattarai, A.; Kowalczyk, W.; Tran, T.N. A literature review on large intestinal hyperelastic constitutive modeling. *Clin. Biomech.* **2021**, *88*, 105445. [CrossRef]
20. Bhattarai, A.; May, C.A.; Staat, M.; Kowalczyk, W.; Tran, T.N. Layer-Specific Damage Modeling of Porcine Large Intestine under Biaxial Tension. *Bioengineering* **2022**, *9*, 528. [CrossRef] [PubMed]
21. Lossi, L.; D'Angelo, L.; De Girolamo, P.; Merighi, A. Anatomical features for an adequate choice of experimental animal model in biomedicine: II. Small laboratory rodents, rabbit, and pig. *Ann. Anat.* **2016**, *204*, 11–28. [CrossRef] [PubMed]
22. Chalstrey, L.J.; Parbhoo, S.P.; Tappin, A.; Baker, G.J.; Gracey, L.R.H.; Mullen, P.A.; Lester, R. Technique of orthotopic liver transplantation in the pig. *Br. J. Surg.* **1971**, *58*, 585–588. [CrossRef] [PubMed]
23. Zhang, Y.; Huang, T.; Wang, P.; Li, W.; Yu, M. Comparison of morphology and microstructural components of hepatic portal vein between human and pig. *J. Huazhong Univ. Sci. Technol. Med. Sci.* **2005**, *25*, 419–422. [CrossRef]
24. Livaditis, A.; Jönsson, L. The piglet in experimental pediatric surgery. *Z. Versuchstierkd.* **1979**, *21*, 78–82.
25. Glauser, E.M. Advantages of piglets as experimental animals in pediatric research. *Exp. Med. Surg.* **1966**, *24*, 181–190.
26. Nonaka, K.; Miyazawa, M.; Ban, S.; Aikawa, M.; Akimoto, N.; Koyama, I.; Kita, H. Different healing process of esophageal large mucosal defects by endoscopic mucosal dissection between with and without steroid injection in an animal model. *BMC Gastroenterol.* **2013**, *13*, 72. [CrossRef]
27. Li, L.; Itani, M.I.; Salimian, K.J.; Li, Y.; Gutierrez, O.B.; Hu, H.; Fayad, G.; Donet, J.A.; Joo, M.K.; Ensign, L.M.; et al. A patient-like swine model of gastrointestinal fibrotic strictures for advancing therapeutics. *Sci. Rep.* **2021**, *11*, 13344. [CrossRef]
28. Yamashita, H.; Ikematsu, H.; Murano, T.; Aoyama, N.; Kano, Y.; Mitsui, T.; Sunakawa, H.; Shinmura, K.; Takashima, K.; Nakajo, K.; et al. The usefulness of a double-balloon endolumenal interventional platform for colorectal endoscopic submucosal dissection by non-expert endoscopists in a porcine model (with video). *Surg. Endosc.* **2022**, *36*, 7818–7826. [CrossRef]
29. Ali-Mohamad, N.; Cau, M.F.; Zenova, V.; Baylis, J.R.; Beckett, A.; McFadden, A.; Donnellan, F.; Kastrup, C.J. Self-propelling thrombin powder enables hemostasis with no observable recurrent bleeding or thrombosis over 3 days in a porcine model of upper GI bleeding. *Gastrointest. Endosc.* **2023**, *98*, 245–248. [CrossRef]
30. Cai, Q.; Fu, H.; Zhang, L.; Shen, M.; Yi, S.; Xie, R.; Lan, W.; Dong, W.; Chen, X.; Zhang, J.; et al. Twin-grasper assisted mucosal inverted closure achieves complete healing of large perforations after gastric endoscopic full-thickness resection. *Dig. Endosc.* **2023**, *35*, 736–744. [CrossRef]
31. Soeder, M.; Turshudzhyan, A.; Rosenberg, L.; Tadros, M. High-Quality Colonoscopy: A Review of Quality Indicators and Best Practices. *Gastroenterol. Insights* **2022**, *13*, 162–172. [CrossRef]
32. Huang, J.; Du, B.; Qiao, W.; Huang, S.; Xue, L.; Deng, L.; Liang, J.; Wang, J.; Li, J.; Chen, Y. Endoscopic submucosal dissection training: Evaluation of an ex vivo training model with continuous perfusion (ETM-CP) for hands-on teaching and training in China. *Surg. Endosc.* **2023**, *37*, 4774–4783. [CrossRef]
33. Gonzalez, J.-M.; Meunier, E.; Debourdeau, A.; Basile, P.; Le-Mouel, J.-P.; Caillo, L.; Vitton, V.; Barthet, M. Training in esophageal peroral endoscopic myotomy (POEM) on an ex vivo porcine model: Learning curve study and training strategy. *Surg. Endosc.* **2023**, *37*, 2062–2069. [CrossRef] [PubMed]
34. Lukas, M.; Kolar, M.; Ryska, O.; Juhas, S.; Juhasova, J.; Kalvach, J.; Pazin, J.; Kocisova, T.; Foltan, O.; Kristianova, H.; et al. Novel porcine model of Crohn's disease anastomotic stricture suitable for evaluation and training of advanced endoscopic techniques. *Gastrointest. Endosc.* **2021**, *93*, 250–256. [CrossRef] [PubMed]
35. Steger, J.; Kwade, C.; Berlet, M.; Krumpholz, R.; Ficht, S.; Wilhelm, D.; Mela, P. The colonoscopic vacuum model–simulating biomechanical restrictions to provide a realistic colonoscopy training environment. *Int. J. Comput. Assist. Radiol. Surg.* **2023**, *18*, 105–116. [CrossRef] [PubMed]
36. Erridge, S.; Payne, C.J.; Sodergren, M. Novel clip applicator for minimally invasive surgery. *Surg. Endosc.* **2019**, *33*, 2710–2718. [CrossRef]
37. Giaccaglia, V.; Antonelli, M.S.; Addario Chieco, P.; Cocorullo, G.; Cavallini, M.; Gulotta, G. Technical characteristics can make the difference in a surgical linear stapler. Or not? *J. Surg. Res.* **2015**, *197*, 101–106. [CrossRef]
38. Giaccaglia, V.; Antonelli, M.S.; Franceschilli, L.; Salvi, P.F.; Gaspari, A.L.; Sileri, P. Different characteristics of circular staplers make the difference in anastomotic tensile strength. *J. Mech. Behav. Biomed. Mater.* **2016**, *53*, 295–300. [CrossRef]
39. Vilz, T.O.; Sommer, N.; Kahl, P.; Pantelis, D.; Kalff, J.C.; Wehner, S. Oral CPSI-2364 Treatment Prevents Postoperative Ileus in Swine without Impairment of Anastomotic Healing. *Cell. Physiol. Biochem.* **2013**, *32*, 1362–1373. [CrossRef]
40. von Kortzfleisch, V.T.; Karp, N.A.; Palme, R.; Kaiser, S.; Sachser, N.; Richter, S.H. Improving reproducibility in animal research by splitting the study population into several 'mini-experiments'. *Sci. Rep.* **2020**, *10*, 16579. [CrossRef]

41. Yang, Q.; Pröll, M.J.; Salilew-Wondim, D.; Zhang, R.; Tesfaye, D.; Fan, H.; Cinar, M.U.; Große-Brinkhaus, C.; Tholen, E.; Islam, M.A.; et al. LPS-induced expression of CD14 in the TRIF pathway is epigenetically regulated by sulforaphane in porcine pulmonary alveolar macrophages. *Innate Immun.* **2016**, *22*, 682–695. [CrossRef] [PubMed]
42. Kratz, T.; Ruff, R.; Koch, T.; Kronberg, A.-S.; Breuer, J.; Asfour, B.; Herberg, U.; Bierbach, B. Proof of concept of an accelerometer as a trigger for unilateral diaphragmatic pacing: A porcine model. *Biomed. Eng. OnLine* **2023**, *22*, 55. [CrossRef] [PubMed]
43. Obernier, J.A.; Baldwin, R.L. Establishing an Appropriate Period of Acclimatization Following Transportation of Laboratory Animals. *ILAR J.* **2006**, *47*, 364–369. [CrossRef]
44. Poore, K.R.; Fowden, A.L. Insulin sensitivity in juvenile and adult Large White pigs of low and high birthweight. *Diabetologia* **2004**, *47*, 340–348. [CrossRef]
45. Thacker, H.L. Necropsy of the Feeder Pig and Adult Swine. *Vet. Clin. N. Am. Food Anim. Pract.* **1986**, *2*, 173–186. [CrossRef] [PubMed]
46. Cui, Y.; Urschel, J.D. Comparison of anastomotic suturing techniques in the rat esophagus. *J. Cardiovasc. Surg.* **1999**, *40*, 613–614.
47. Oetzmann von Sochaczewski, C.; Tagkalos, E.; Lindner, A.; Baumgart, N.; Gruber, G.; Baumgart, J.; Lang, H.; Heimann, A.; Muensterer, O.J. Bodyweight, Not Age, Determines Oesophageal Length and Breaking Strength in Rats. *J. Pediatr. Surg.* **2019**, *54*, 297–302. [CrossRef]
48. Saxena, A.K.; Biro, E.; Sommer, G.; Holzapfel, G.A. Esophagus stretch tests: Biomechanics for tissue engineering and possible implications on the outcome of esophageal atresia repairs performed under excessive tension. *Esophagus* **2021**, *18*, 346–352. [CrossRef]
49. Toczewski, K.; Gerus, S.; Kaczorowski, M.; Kozuń, M.; Wolicka, J.; Bobrek, K.; Filipiak, J.; Patkowski, D. Biomechanics of esophageal elongation with traction sutures on experimental animal model. *Sci. Rep.* **2022**, *12*, 3420. [CrossRef]
50. Behrend, M.; Kluge, E.; Schüttler, W.; Klempnauer, J. Breaking strength of native and sutured trachea. An experimental study on sheep trachea. *Eur. Surg. Res.* **2001**, *33*, 255–263. [CrossRef]
51. Karliczek, A.; Zeebregts, C.J.; Benaron, D.A.; Coppes, R.P.; Wiggers, T.; Van Dam, G.M. Preoperative irradiation with 5 × 5 Gy in a murine isolated colon loop model does not cause anastomotic weakening after colon resection. *Int. J. Colorectal Dis.* **2008**, *23*, 1115–1124. [CrossRef] [PubMed]
52. Karliczek, A.; Benaron, D.A.; Zeebregts, C.J.; Wiggers, T.; Van Dam, G.M. Intraoperative Ischemia of the Distal End of Colon Anastomoses as Detected With Visible Light Spectroscopy Causes Reduction of Anastomotic Strength. *J. Surg. Res.* **2009**, *152*, 288–295. [CrossRef] [PubMed]
53. Oetzmann von Sochaczewski, C.; Tagkalos, E.; Lindner, A.; Lang, H.; Heimann, A.; Schröder, A.; Grimminger, P.P.; Muensterer, O.J. Esophageal biomechanics revisited: A tale of tenacity, anastomoses, and suture bite lengths in swine. *Ann. Thorac. Surg.* **2019**, *107*, 1670–1677. [CrossRef] [PubMed]
54. Oetzmann von Sochaczewski, C.; Tagkalos, E.; Lindner, A.; Lang, H.; Heimann, A.; Muensterer, O. Technical Aspects in Esophageal Lengthening: An Investigation of Traction Procedures and Suturing Techniques in Swine. *Eur. J. Pediatr. Surg.* **2019**, *29*, 481–484. [CrossRef]
55. Oetzmann von Sochaczewski, C.; Tagkalos, E.; Lindner, A.; Lang, H.; Heimann, A.; Muensterer, O.J. A Continuous Suture Anastomosis Outperforms a Simple Interrupted Suture Anastomosis in Esophageal Elongation. *Eur. J. Pediatr. Surg.* **2020**, *31*, 177–181. [CrossRef]
56. Kratz, T.; Ruff, R.; Bernhardt, M.; Katzer, D.; Herberg, U.; Asfour, B.; Breuer, J.; Oetzmann Von Sochaczewski, C.; Bierbach, B. A porcine model of postoperative hemi-diaphragmatic paresis to evaluate a unilateral diaphragmatic pacemaker. *Sci. Rep.* **2023**, *13*, 12628. [CrossRef]
57. Ogurtan, Z.; Gezici, M.; Kul, M.; Ceylan, C.; Alkan, F. Compararative study of bursting and tensile strengths of digestive tract in the dog. Application to esophago-intestinal sutures. *Rev. Méd. Vét.* **2001**, *152*, 491–494.
58. Faul, F.; Erdfelder, E.; Lang, A.-G.; Buchner, A. G*Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behav. Res. Methods* **2007**, *39*, 175–191. [CrossRef]
59. Noar, M.D. An Established Porcine Model for Animate Training in Diagnostic and Therapeutic ERCP. *Endoscopy* **1995**, *27*, 77–80. [CrossRef]
60. Hochberger, J.; Maiss, J.; Magdeburg, B.; Cohen, J.; Hahn, E.G. Training Simulators and Education in Gastrointestinal Endoscopy: Current Status and Perspectives in 2001. *Endoscopy* **2001**, *33*, 541–549. [CrossRef]
61. La Francesca, S.; Aho, J.M.; Barron, M.R.; Blanco, E.W.; Soliman, S.; Kalenjian, L.; Hanson, A.D.; Todorova, E.; Marsh, M.; Burnette, K.; et al. Long-term regeneration and remodeling of the pig esophagus after circumferential resection using a retrievable synthetic scaffold carrying autologous cells. *Sci. Rep.* **2018**, *8*, 4123. [CrossRef]
62. Swindle, M.M.; Smith, A.C.; Hepburn, B.J.S. Swine as Models in Experimental Surgery. *J. Investig. Surg.* **1988**, *1*, 65–79. [CrossRef] [PubMed]
63. Khoorjestan, S.M.; Rouhi, G.; Toolabi, K. Experimental Investigations on Intestinal Anastomosis—A Comparison between Automatic and Hand Suturing Techniques. *J. Mech. Med. Biol.* **2016**, *16*, 1650056. [CrossRef]
64. Carniel, E.L.; Frigo, A.; Fontanella, C.G.; De Benedictis, G.M.; Rubini, A.; Barp, L.; Pluchino, G.; Sabbadini, B.; Polese, L. A biomechanical approach to the analysis of methods and procedures of bariatric surgery. *J. Biomech.* **2017**, *56*, 32–41. [CrossRef] [PubMed]

65. Kimura, M.; Takahashi, H.; Tanaka, T.; Sagawa, H.; Takeyama, H. Profiling Surgical Staplers: Does Staple Direction Affect the Strength of the Anastomosis? *Dig. Surg.* **2015**, *32*, 454–458. [CrossRef] [PubMed]
66. Li, W.C.; Zhang, H.M.; Li, J.; Dong, R.K.; Yao, B.C.; He, X.J.; Wang, H.Q.; Song, J. Comparison of Biomechanical Properties of Bile Duct Between Pigs and Humans for Liver Xenotransplant. *Transplant. Proc.* **2013**, *45*, 741–747. [CrossRef]
67. Morse, B.C.; Simpson, J.P.; Jones, Y.R.; Johnson, B.L.; Knott, B.M.; Kotrady, J.A. Determination of independent predictive factors for anastomotic leak: Analysis of 682 intestinal anastomoses. *Am. J. Surg.* **2013**, *206*, 950–956. [CrossRef]
68. Stavropoulou, E.A.; Dafalias, Y.F.; Sokolis, D.P. Biomechanical behavior and histological organization of the three-layered passive esophagus as a function of topography. *Proc. Inst. Mech. Eng. Part H* **2012**, *226*, 477–490. [CrossRef]
69. Dou, Y.; Zhao, J.; Gregersen, H. Morphology and Stress-Strain Properties Along the Small Intestine in the Rat. *J. Biomech. Eng.* **2003**, *125*, 266–273. [CrossRef]
70. Gao, C.; Gregersen, H. Biomechanical and morphological properties in rat large intestine. *J. Biomech.* **2000**, *33*, 1089–1097. [CrossRef]
71. Watters, D.A.; Smith, A.N.; Eastwood, M.A.; Anderson, K.C.; Elton, R.A.; Mugerwa, J.W. Mechanical properties of the colon: Comparison of the features of the African and European colon in vitro. *Gut* **1985**, *26*, 384–392. [CrossRef] [PubMed]
72. Christensen, M.B.; Oberg, K.; Wolchok, J.C. Tensile properties of the rectal and sigmoid colon: A comparative analysis of human and porcine tissue. *SpringerPlus* **2015**, *4*, 142. [CrossRef]
73. Vegesna, A.; Korimilli, A.; Besetty, R.; Bright, L.; Milton, A.; Agelan, A.; McIntyre, K.; Malik, A.; Miller, L. Endoscopic pyloric suturing to facilitate weight loss: A canine model. *Gastrointest. Endosc.* **2010**, *72*, 427–431. [CrossRef]
74. Luo, H.; Pan, Y.; Min, L.; Zhao, L.; Li, J.; Leung, J.; Xue, L.; Yin, Z.; Liu, X.; Liu, Z.; et al. Transgastric endoscopic gastroenterostomy using a partially covered occluder: A canine feasibility study. *Endoscopy* **2012**, *44*, 493–498. [CrossRef]
75. Park, S.J.; Lee, K.Y.; Choi, S.I.; Kang, B.M.; Huh, C.; Choi, D.H.; Lee, C.K. Pure NOTES Rectosigmoid Resection: Transgastric Endoscopic IMA Dissection and Transanal Rectal Mobilization in Animal Models. *J. Laparoendosc. Adv. Surg. Tech.* **2013**, *23*, 592–595. [CrossRef] [PubMed]
76. Nguyễn, N.H.; Dương, M.T.; Trần, T.N.; Phạm, P.T.; Grottke, O.; Tolba, R.; Staat, M. Influence of a freeze–thaw cycle on the stress–stretch curves of tissues of porcine abdominal organs. *J. Biomech.* **2012**, *45*, 2382–2386. [CrossRef] [PubMed]
77. Whiteway, J.; Morson, B.C. Elastosis in diverticular disease of the sigmoid colon. *Gut* **1985**, *26*, 258–266. [CrossRef]
78. Cassidy, D.J.; Coe, T.M.; Jogerst, K.M.; McKinley, S.K.; Sell, N.M.; Sampson, M.; Park, Y.S.; Petrusa, E.; Goldstone, R.N.; Hashimoto, D.A.; et al. Transfer of virtual reality endoscopy training to live animal colonoscopy: A randomized control trial of proficiency vs. repetition-based training. *Surg. Endosc.* **2022**, *36*, 6767–6776. [CrossRef] [PubMed]
79. VanDruff, V.N.; Wong, H.J.; Amundson, J.R.; Wu, H.; Campbell, M.; Kuchta, K.; Hedberg, H.M.; Linn, J.; Haggerty, S.; Denham, W.; et al. "Into the fire" approach to teaching endoscopic foreign body removal using a modular simulation curriculum. *Surg. Endosc.* **2023**, *37*, 1412–1420. [CrossRef]
80. Elefson, S.K.; Lu, N.; Chevalier, T.; Dierking, S.; Wang, D.; Monegue, H.J.; Matthews, J.C.; Jang, Y.D.; Chen, J.; Rentfrow, G.K.; et al. Assessment of visceral organ growth in pigs from birth through 150 kg. *J. Anim. Sci.* **2021**, *99*, skab249. [CrossRef]
81. Von Kortzfleisch, V.T.; Ambrée, O.; Karp, N.A.; Meyer, N.; Novak, J.; Palme, R.; Rosso, M.; Touma, C.; Würbel, H.; Kaiser, S.; et al. Do multiple experimenters improve the reproducibility of animal studies? *PLoS Biol.* **2022**, *20*, e3001564. [CrossRef]
82. Hosseini, H.S.; Dunn, J.C.Y. Biomechanical Force Prediction for Lengthening of Small Intestine during Distraction Enterogenesis. *Bioengineering* **2020**, *7*, 140. [CrossRef]
83. Bonaldi, L.; Berardo, A.; Pirri, C.; Stecco, C.; Carniel, E.L.; Fontanella, C.G. Mechanical Characterization of Human Fascia Lata: Uniaxial Tensile Tests from Fresh-Frozen Cadaver Samples and Constitutive Modelling. *Bioengineering* **2023**, *10*, 226. [CrossRef] [PubMed]
84. Yang, W.; Fung, T.C.; Chian, K.S.; Chong, C.K. Directional, Regional, and Layer Variations of Mechanical Properties of Esophageal Tissue and its Interpretation Using a Structure-Based Constitutive Model. *J. Biomech. Eng.* **2006**, *128*, 409. [CrossRef] [PubMed]
85. Jia, Z.G.; Li, W.; Zhou, Z.R. Mechanical characterization of stomach tissue under uniaxial tensile action. *J. Biomech.* **2015**, *48*, 651–658. [CrossRef] [PubMed]
86. Dou, Y.; Fan, Y.; Zhao, J.; Gregersen, H. Longitudinal residual strain and stress-strain relationship in rat small intestine. *Biomed. Eng. OnLine* **2006**, *5*, 37. [CrossRef]
87. Sommer, G.; Schriefl, A.; Zeindlinger, G.; Katzensteiner, A.; Ainödhofer, H.; Saxena, A.; Holzapfel, G.A. Multiaxial mechanical response and constitutive modeling of esophageal tissues: Impact on esophageal tissue engineering. *Acta Biomater.* **2013**, *9*, 9379–9391. [CrossRef]
88. Salvatierra, E.; Spataro, T.; Thomas, J.; Sheth, A.; Traylor, J.; Jin, L.; Minoch, A.; McLarty, J. W1445: Regional Differences in Colon Circumference and Wall Thickness. *Gastrointest. Endosc.* **2010**, *71*, AB330. [CrossRef]
89. Sokolis, D.P.; Orfanidis, I.K.; Peroulis, M. Biomechanical testing and material characterization for the rat large intestine: Regional dependence of material parameters. *Physiol. Meas.* **2011**, *32*, 1969–1982. [CrossRef]
90. Siri, S.; Maier, F.; Chen, L.; Santos, S.; Pierce, D.M.; Feng, B. Differential biomechanical properties of mouse distal colon and rectum innervated by the splanchnic and pelvic afferents. *Am. J. Physiol.-Gastrointest. Liver Physiol.* **2019**, *316*, G473–G481. [CrossRef]
91. Siri, S.; Zhao, Y.; Maier, F.; Pierce, D.M.; Feng, B. The Macro- and Micro-Mechanics of the Colon and Rectum I: Experimental Evidence. *Bioengineering* **2020**, *7*, 130. [CrossRef] [PubMed]

92. Caulk, A.W.; Chatterjee, M.; Barr, S.J.; Contini, E.M. Mechanobiological considerations in colorectal stapling: Implications for technology development. *Surg. Open Sci.* **2023**, *13*, 54–65. [CrossRef] [PubMed]
93. Kimura, M.; Kuwabara, Y.; Taniwaki, S.; Mitsui, A.; Shibata, Y.; Ueno, S. Improving the side-to-side stapled anastomosis: Comparison of staplers for robust crotch formation. *Surg. Obes. Relat. Dis.* **2018**, *14*, 16–21. [CrossRef] [PubMed]
94. Ikeuchi, D.; Onodera, H.; Aung, T.; Kan, S.; Kawamoto, K.; Imamura, M.; Maetani, S. Correlation of Tensile Strength with Bursting Pressure in the Evaluation of Intestinal Anastomosis. *Dig. Surg.* **1999**, *16*, 478–485. [CrossRef]
95. Oetzmann von Sochaczewski, C.; Lindner, A.; Heimann, A.; Balus, A.; Patel, V.H.; Harrison, M.R.; Muensterer, O.J. Beyond Magnamosis: A Method to Test Sutureless Esophageal Anastomotic Devices in Living Swine by Creating an Esophageal Bypass Loop for Natural Oral Nutrition. *J. Laparoendosc. Adv. Surg. Tech.* **2019**, *29*, 852–855. [CrossRef]
96. Muensterer, O.J.; Sterlin, A.; Oetzmann von Sochaczewski, C.; Lindner, A.; Heimann, A.; Balus, A.; Dickmann, J.; Nuber, M.; Patel, V.H.; Manfredi, M.A.; et al. An Experimental Study on Magnetic esophageal compression Anastomosis in Piglets. *J. Pediatr. Surg.* **2020**, *55*, 425–432. [CrossRef]
97. Paral, J.; Lochman, P.; Blazej, S.; Pavlik, M. Glued versus stapled anastomosis of the colon: An experimental study to determine comparative resistance to intraluminal pressure. *Asian J. Surg.* **2014**, *37*, 154–161. [CrossRef]
98. Müller, G.; Kieninger, G.; Breucha, G.; Bustamante, I.; Neugebauer, W. Vergleichende Untersuchungen ein-und zweireihiger Anastomosen am Schweinedünndarm. *Langenbecks Arch. Chir.* **1978**, *346*, 37–45. [CrossRef]
99. Sánchez-De Pedro, F.; Moreno-Sanz, C.; Morandeira-Rivas, A.; Tenías-Burillo, J.M.; Alhambra-Rodríguez De Guzmán, C. Colorectal anastomosis facilitated by the use of the LigaSure® sealing device: Comparative study in an animal model. *Surg. Endosc.* **2014**, *28*, 508–514. [CrossRef]
100. Larsen, K.D.; Westerholt, M.; Madsen, G.I.; Le, D.Q.S.; Qvist, N.; Ellebæk, M.B. Poly-ε-caprolactone scaffold for the reinforcement of stapled small intestinal anastomoses: A randomized experimental study. *Langenbecks Arch. Surg.* **2019**, *404*, 1009–1016. [CrossRef]
101. Van Der Vijver, R.J.; Van Laarhoven, C.J.H.M.; De Man, B.M.; Lomme, R.M.L.M.; Hendriks, T. The effect of fibrin glue on the early healing phase of intestinal anastomoses in the rat. *Int. J. Colorectal Dis.* **2012**, *27*, 1101–1107. [CrossRef]
102. Khoorjestan, S.M.; Rouhi, G.; Toolabi, K. An investigation of the effects of suture patterns on mechanical strength of intestinal anastomosis: An experimental study. *Biomed. Tech.* **2017**, *62*, 429–437. [CrossRef]
103. Stupka, R.; Šprysl, M.; Pour, M. The impact of sex on the economics of pig fattening. *Agric. Econ. Czech* **2004**, *50*, 217–222. [CrossRef]
104. Köhn, F.; Sharifi, A.R.; Simianer, H. Modeling the growth of the Goettingen minipig. *J. Anim. Sci.* **2007**, *85*, 84–92. [CrossRef]
105. Oetzmann von Sochaczewski, C.; Deigendesch, N.; Lindner, A.; Baumgart, J.; Schröder, A.; Heimann, A.; Muensterer, O.J. Comparing Aachen Minipigs and Pietrain Piglets as Models of Experimental Pediatric Urology to Human Reference Data. *Eur. Surg. Res.* **2020**, *61*, 95–100. [CrossRef]
106. Baumgart, J.; Deigendesch, N.; Lindner, A.; Muensterer, O.J.; Schröder, A.; Heimann, A.; Oetzmann von Sochaczewski, C. Using multidimensional scaling in model choice for congenital oesophageal atresia: Similarity analysis of human autopsy organ weights with those from a comparative assessment of Aachen Minipig and Pietrain piglets. *Lab. Anim.* **2020**, *54*, 576–587. [CrossRef]
107. Stallings, E. Biostatistics for gastroenterologists. Part II ? Rethinking sample size. *Rev. Esp. Enfermedades Dig.* **2023**, *115*, 411–413. [CrossRef]
108. Kararli, T.T. Comparison of the gastrointestinal anatomy, physiology, and biochemistry of humans and commonly used laboratory animals. *Biopharm. Drug Dispos.* **1995**, *16*, 351–380. [CrossRef]
109. Kousholt, B.S.; Præstegaard, K.F.; Stone, J.C.; Thomsen, A.F.; Johansen, T.T.; Ritskes-Hoitinga, M.; Wegener, G. Reporting quality in preclinical animal experimental research in 2009 and 2018: A nationwide systematic investigation. *PLoS ONE* **2022**, *17*, e0275962. [CrossRef]
110. Festing, M.F. On determining sample size in experiments involving laboratory animals. *Lab. Anim.* **2018**, *52*, 341–350. [CrossRef]
111. Kratz, T.; Dauvergne, J.; Kronberg, A.-S.; Katzer, D.; Ganschow, R.; Bernhardt, M.; Westeppe, S.; Bierbach, B.; Strohm, J.; Oetzmann von Sochaczewski, C. Allometric Parameters and Linear Breaking Strength of Intestinal Segments. Available online: https://zenodo.org/record/8177381 (accessed on 24 July 2023).