Volumetric study on small intestine in newborn rat using design-based stereology technique

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Abstract

The small intestine continues from the pylorus to the cecum, and its main function is digestion and absorption of nutrients. Morphometric data of small intestine structures are related to its function. Therefore, in this study, the volume of the different layers forming the small intestine of the rat at birth in different segments has been calculated with stereology. A total of five newborn rats were used. The total volume of each segment was calculated by dividing the intestinal weight by its specific gravity. After systematic uniform random sampling of each segment of the small intestine, a point counting system place on the images of the tissue sections, then the relative volume of the tunica mucosa, tela submucosa and tunica muscularis was estimated. The total volume of the duodenum jejunum and ileum was 0.0502±0.00497 cm^3, 0.1501±0.01124 cm^3 and 0.0213±0.00279 cm^3, respectively. The fractional volume of the tunica mucosa was estimated as 40.77±2.55% in duodenum, 54.89±2.34% in jejunum and 42.24±1.87 % in ileum. The volume fraction of the tela submucosa was 26.89±1.26% in duodenum, 22.04±1.84% in jejunum and 16.23±1.2 % in ileum which indicated a significant difference between various segments of the small intestine (p<0.05). The tunica muscularis made up 32.33±1.47 %, 23.05±2.11 % and 41.51±1.38 % of the whole intestine in duodenum, jejunum and ileum, respectively. This parameter was also significant between intestinal segments (p<0.05).

These quantitative data may provide a reliable reference value thus contributing to veterinary gastroenterology and to the experimental research on intestinal pathophysiology.

Keywords: Small intestine, Stereology, Volume, Newborn, Rat

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Introduction

The small intestine connects at the pylorus and continues to the cecum and is divided into the duodenum, jejunum and ileum (König and Liebich, 2009). Histologically, the small intestine is composed of four main layers (from the inside to the outside) including tunica mucosa, tela submucosa, tunica muscularis and serosa (Dellman, 1993). The main functions of the small intestine are digestion and absorption. Therefore, the structures of the intestine were adapted to its function (Makanya *et al*., 1995). In many species, such as the rat, the mucosal surface of the small intestine is increased for more digestion and absorption by villi, which can vary in shape and size based on the species, location, age, and disease (Ross and Mayhew, 1984).

In various studies, morphometry of the small intestine has been introduced as an important parameter to understand intestinal function (Nyengaard and Alwasel, 2014; Volk and Lacy, 2017). The morphometry of the small intestine is also affected in various diseases such as diarrhea, dehydratation, and enteric infections (Ginneken *et al*., 2002). The rat is used as a suitable translational model to investigate changes in intestinal morphology and morphometry under pathological conditions in humans (Mayhew *et al*., 1989; Sugimoto *et al*., 2021; Nagar *et al*., 2023). Many quantitative studies on small intestine in rat have been limited to measuring single dimension such as villi height or crypt depth which are varies in size and shape along the small intestine (Vigueras *et al*., 1999; Fatahian Dehkordi, 2021). It should be noted that the measurement of one dimension cannot represent the real three-dimensional characteristics of that structure in the intestine. Therefore, a method that is free assumptions about the sizes and shapes of structures is needed for morphometric analysis (Mayhew and Middleton, 1985).

Design-based stereology allows accurate and precise quantitative estimates on three-dimensional morphometric features of whole organs using statistical sampling and stochastic geometry principles (Boyce *et al*., 2010). This technique converts the 2D data into 3D information and match the characteristics of the original three-dimensional structure, therefore, it has been successfully applied in gastrointestinal tract research (Nyengaard and Alwasel, 2014). Most of the morphometric analyses of small intestine using stereology in rat has been carried out at mature ages (Ross and Mayhew, 1984; Nyengaard and Alwasel, 2014). Meanwhile, most congenital gastrointestinal tract anomalies in human are detected in the newborn period and a delay in diagnosis may cause a significant increase in the morbidity (Lee, 2013). Based on the above-exposed points, this study was designed to estimate the total volume and fractional volumes of different layers of small intestine in various segments including duodenum, jejunum and ileum in newborn rat by using the point counting system. These quantitative data may provide a reliable reference value thus contributing to veterinary

gastroenterology knowledge and to the experimental research on intestinal pathophysiology.

Materials and methods

The male and female adult Wistar rats were purchased from the Pasteur Institute, Tehran, Iran. The rats were allowed to mate overnight. The rats were maintained in a temperature of 23–25°C with a 12-h light/12 h dark cycle provided with food and water ad libitum. The experiment conducted by the standard guide for the care and use of laboratory animals. Five offspring were randomly selected at birth. Each animal was euthanized by cervical dislocation. Then, the abdomen was opened and the small intestinal segments were removed. The duodenum was located between the pylorus and the duodenojejunal flexure. The jejunum ended at the proximal attachment of the ileocecal fold and the ileum terminated at the ileal opening. The mesenteric attachments were cut and the luminal content was washed off with phosphate-buffered saline solution (PBS) 0.01 M. After rising with PBS the segment wet weight was measured using a digital scale. Then, segments were fixed in formalin 10%. A number of 6 to 8 transverse cut samples were prepared from each segment using the systematic uniform random sampling method. The samples were processed after routine histological methods and embedded in paraffin. Five µm thick sections were prepared from each block and stained with hematoxylin-eosin. A microscope (CX40; Olympus) connected to a digital camera (MB-225) was utilized for capturing images from sections in order to estimate the volumes. A point counting system were produced using a dedicated software (ImageJ; https://imagej.nih.gov).

The total volume of each segment was estimated by employing intestinal weight and transforming it into a volume. The fractional volume of intestinal layers including tunica mucosa, tela submucosa and tunica muscularis, was estimated using the following formula (Gundersen *et al*., 1988),

V_v (layer)= ∑p (layer) / ∑P (intestine)

Where, ΣP (layer) is the number of points hitting the mucosa, submucosa, muscular layer, and ΣP (intestine) is the number of points hitting the intestine. Finaly, in order to estimate the total volume of each layer, each volume fraction was multiplied by the total volume of the intestine. One-way analysis of variance followed by Tukey's post hoc test was used for statistical analysis. A value of $p<0.05$ was considered to indicate significance.

Results

The total volume of the duodenum jejunum and ileum, calculated by dividing the intestinal weight by its specific gravity, was 0.0502±0.00497 cm^3, 0.1501±0.01124 cm^3 and 0.0213±0.00279 cm^3, respectively. The differences between segments were statistically significant ($p<0.05$). The fractional volume of the tunica mucosa was estimated as 40.77±2.55% in duodenum, 54.89±2.34% in jejunum and 42.24±1.87% in ileum. The relative volume of the tunica mucous in the

jejunum was higher than in other segments (p<0.05). The volume fraction of the tela submucosa was 26.89±1.26% in duodenum, 22.04±1.84% in jejunum and 16.23±1.2% in ileum which indicated a significant difference between various segments of the small intestine (p<0.05). The tunica muscularis made up 32.33±1.47%, 23.05±2.11% and 41.51±1.38% of the whole intestine in duodenum, jejunum and ileum, respectively. This parameter was also significant between intestinal segments (p<0.05). The fractional and absolute volumes for the different layers constituting the intestinal wall in each segment are presented in Table 1.

Table 1. Stereological data for proportional volume of the tunica mucosa, tela submucosa and tunica muscularis in different segments of small intestine in five newborn rats (Mean ± standard deviation).

Segments	Tunica mucosa volume fraction (%)	Tela submucosa volume fraction (%)	Tunica muscularis volume fraction (%)
Duodenum	40.77±2.55[a]	26.89±1.26[a]	32.33±1.47[a]
Jejunum	54.89±2.34[b]	22.04±1.84[b]	23.05±2.11[b]
Ileum	42.24±1.87[a]	16.23±1.2[c]	41.51±1.38[c]

Different letters in each column indicate the significant difference between segments (p<0.05).

Discussion

This study showed that the fractional volume of each layer of the small intestine varies in different segments in newborn rat. In this research, the stereology technique, which is an unbiased and precise method for 3D calculations, was used for volumetry of layers in small intestine. In fact, with the help of stereology, the spatial features of the structures in the intestine can be estimated, and this information shows a greater relationship with the function of the intestine (Nyengaard and Alwasel, 2014).

The digestive and absorptive functions of the small intestine are facilitated by specialized structures. The mucosa includes the lining epithelium, a lamina propria with glands and a lamina muscularis. The villi are mucosal projections and are the most characteristic feature of the small intestine (Dellman, 1993). In this study, the relative volume of tunica mucosa in various segments of the small intestine showed a significant difference. Jejunum showed the largest volume of mucosa while the ileum had the smallest mucosal volume. In the developmental study of the pig small intestine, the jejunum presented higher and more villi than other parts of the small intestine (Ginneken *et al*., 2002). In fact, the jejunum and duodenum are the main areas of digestion and absorption of nutrients, while the ileum is the main site for water and electrolyte absorption (Tootian *et al*., 2013). Therefore, the considerable amount of tunica mucosa in the jejunum is reasonable. The submucosa a layer of connective tissue was more voluminous in the duodenum in comparison with that of the rest of the segments, which seems to be due to the presence of Brunner's glands in the submucosa of the initial part

of the duodenum. The tunica muscularis of the small intestine consists of inner circular and outer longitudinal smooth muscle layers. The tunica muscularis of the newborn rat was the most voluminous in the ileum, which is consistent with findings obtained from other mammals. The voluminous muscular layer observed in the ileum, which might resemble a long sphincter, indicates a protective role of this tract to the retropulsed content from the cecum (Tootian *et al*., 2013).

To conclude, different layers of the small intestine in newborn rat showed significant changes in different segments. Developmental findings also showed that functionally, these structural changes are related to dietary changes and the functional maturity of the gastrointestinal tract (Ginneken *et al*., 2002).

References

Boyce, R.W., Dorph-Petersen, K.A., Lyck, L. and Gundersen, H.J.G., 2010. Design-based stereology: introduction to basic concepts and practical approaches for estimation of cell number. *Toxicologic Pathology*, 38(**7**), 1011-1025. Doi: 10.1177/0192623310385140

Dellman, H.D., 1993. Textbook of veterinary histology. Lea & Febiger, Philadelphia. 194-198P.

Fatahian Dehkordi, R.A., Norouzi, K. and Habibian Dehkordi, S., 2021. Histometric study the effects of thiamin on the structure of the small intestine in induced *Alloxan diabetic* rats. *Journal of Sabzevar University of Medical Sciences*, 28(*1*), 30-38.

Ginneken, C.V., Meir, F.V., Sys, S. and Weyns, A., 2002. Stereologic characteristics of pig small intestine during normal development. *Digestive diseases and sciences*, 47, 868-878. Doi: 10.1023/a:1014768806773.

Gundersen, H.J., Bendtsen, T.F., Korbo, L., Marcussen, N., Mallei, A,. Nielsen, Nyengaard, J.R., Pakkenberg, B., Sorensen, F.B., Vesterby, A. and West, J., 1988. Some new, simple and efficient stereological methods and their use in pathological research and diagnosis. *Acta Pathologica Microbiologica et Immunologica Scandinavica*, 96, 379–394. Doi: 10.1111/j.1699-0463.1988.tb05320.x.

König, H.E. and Liebich, H.G., 2009. Veterinary anatomy of domestic mammals: Textbook and colour atlas. 4th edition. Schattauer. Germany.

Lee, S.W., 2013. Congenital and neonatal gastrointestinal diseases. In Radiology Illustrated: Pediatric Radiology (pp. 603-627). Berlin, Heidelberg: Springer Berlin Heidelberg.

Makanya, A.N., Mayhew, T.M. and Maina, J.N., 1995. Stereological methods for estimating the functional surfaces of the chiropteran small intestine. *Journal of Anatomy*, 187(**Pt 2**), 361.

Mayhew, T.M. and Middleton, C.A.R.O.L.Y.N., 1985. Crypts, villi and microvilli in the small intestine of the rat. A stereological study of their variability within and between animals. *Journal of anatomy*, 141, 1.

Mayhew, T.M., Carson, F.L. and Sharma, A.K.,1989. Small intestinal morphology in experimental diabetic rats: a stereological study on the effects of an aldose reductase inhibitor (ponalrestat) given with or without conventional insulin therapy. *Diabetologia*. 32(**9**),649-54. Doi: 10.1007/BF00274251.

Nagar, S., Radice, C., Tuohy, R., Stevens, R., Bennyhoff, D. and Korzekwa, K., 2023. The rat continuous intestine model predicts the impact of particle size and transporters on the oral absorption of glyburide. *Molecular Pharmaceutics Journal*, 20(**1**),219-231. Doi: 10.1021/acs.molpharmaceut.2c00597.

Nyengaard, J.R., and Alwasel, S.H., 2014. Practical stereology of the stomach and intestine. *Annals of Anatomy-Anatomischer Anzeiger*, 196(**1**), 41-47. Doi: 10.1016/j.aanat.2013.10.007.

Ross, G.A. and Mayhew, T.M., 1984. Effects of fasting on villi along the small intestine: a stereological approach to the problem of quantifying villus 'shape'. Doi: 10.1007/BF01951993. *Experientia*, 40(**8**), 856-858.

Sugimoto, S., Kobayashi, E., Fujii, M., Ohta, Y., Arai, K., Matano, M., Ishikawa, K., Miyamoto, K., Toshimitsu, K., Takahashi, S., Nanki, K., Hakamata, Y., Kanai, T. and Sato, T., 2021. An organoid-based organ-repurposing approach to treat short bowel syndrome. *Nature*, 592(**7852**),99-104. Doi: 10.1038/s41586-021-03247-2.

Tootian, Z., Sadeghinezhad, J., Sheibani, M.T., Fazelipour, S., De Sordi, N. and Chiocchetti, R., 2013. Histological and mucin histochemical study of the small intestine of the Persian squirrel (*Sciurus anomalus*). *Anatomical science international*, 88, 38-45. Doi: 10.1007/s12565-012-0159-5.

Vigueras, R.M., Rojas-Castaneda, J., Hernandez, R., Reyes, G. and Alvarez, C., 1999. Histological characteristics of the intestinal mucosa of the rat during the first year of life. *Laboratory animals*, 33(**4**), 393-400. Doi: 10.1258/002367799780487814.

Volk, N. and Lacy, B., 2017. Anatomy and physiology of the small bowel. *Gastrointestinal Endoscopy Clinics of North America*, 27(**1**), 1-13. Doi: 10.1016/j.giec.2016.08.001.