Histomorphometric study of the effect of methionine on small intestine parameters in rat: an applied histologic study

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Running head: Effect of methionine on small intestine histomorphology

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Abstract

Assessment of morphological changes has more often been used in the diagnosis and assessment of intestinal pathology and development. Since methionine is widely used in nutritional and sports supplements and also there is not enough information about the effect of this amino acid on the gastrointestinal histomorphometry, the aim of this study was to assess the effect of methionine on the small intestine histomorphometry.

Thirty male Wistar rats were randomly divided to 3 equal groups. Two treatment groups received 100 and 200 mg/kg L-methionine solution respectively via intraperitoneal injection while the control group received normal saline. On day 21, all rats were euthanized and segments from three parts of small intestine were taken to histomorphometrical study. Paraffin sections were stained with haematoxylin & eosin, Alcian blue (AB) and periodic acid Schiff (PAS) methods separately. In order to analyze histomorphometric features of each segment, villus height, width, area, crypt depth, villus height to crypt depth ratio, goblet cell number, and muscle layer thickness were measured.
Obtained results revealed that methionine may change the histomorphometric parameters of small intestine.

**Key words:** histomorphometry, small intestine, methionine, rat

**Introduction**

The small intestine is the long muscular tube of the gastrointestinal tract that is the major site for absorption and digestion of nutrients and drugs. It is made up of three structural segments: duodenum, jejunum and ileum (Huang et al., 1999; Kararli, 1995; Kellow et al., 1986). All of the absorptive and also a part of the digestive capacity of the small intestine occur around and near villi and crypts (Kitt et al., 2001). Actually the crypt-villus is the main functional unit of absorption in the rat’s small intestine. Various substances and also different physiological or pathological conditions may change the proliferation rate of enterocytes in the crypt and affect their migration rate to the top of the villi (Thomson and Keelan, 1986). The relation between small intestine and nutritional factors is very complicated. Nutrients may directly cause changes or adaptations in the intestinal mucosa (Caruso et al., 2012; Thomson et al., 1996).

Methionine, a sulphur-containing essential amino acid, serves as the initiating amino acid in the synthesis of many proteins. It plays an important role in the synthesis of vital molecules such as cysteine, carnitine, taurine, lecithin, phosphotidyl choline and other phospholipids. It has a significant effect on improving cartilage formation and is therefore used in arthritis treatment (Ursini and Pipicelli, 2009).

Histomorphometric analysis is greatly used in studies about gastrointestinal pathophysiology. The availability of animal models and using morphometric analysis as a quantitative assessment have facilitated evaluating the morphological alterations of intestinal mucosa under various experimental conditions such as special diets and drugs (Gulbinowicz et al., 2004).

The laboratory rat, as an animal model, has been used in several histological and histomorphometrical studies (Al-Qudah, 2014; Vigueras et al., 1999). Some of these studies have described the qualitative and quantitative changes that occur in the small
intestine under various physiological and pathological conditions (Gulbinowicz et al., 2004; Habold et al., 2007; Higa et al., 2007; Olaibi et al., 2014a; Olaibi et al., 2014b).

Many studies have investigated the effect of methionine supplementation on the skin (Nazem et al., 2016), teeth (McGrath and Nakamoto, 1985), liver disorders (Lieber, 2002), feather follicles (Nazem et al., 2015), female reproductive system (Berker et al., 2009; Nazem et al., 2017) and neural tube in the embryos (Makhro et al., 2008) but there is not enough information about its effect on the small intestine in the mammals. Methionine is widely used in nutritional and sports supplements and on the other hand the effects of these supplements have not been investigated on the gastrointestinal histomorphometry. So the aim of this study was to assess the effect of injected methionine on histomorphometric aspects of small intestine in the rat.

**Materials and methods**

The experiment in this study was approved by the Animal Ethics Committee, a branch of the Research Council of the Veterinary School in Shahid Bahonar University, Kerman Province, Iran.

A total of thirty male Wistar rats weighing between 190 and 210 g were used for this research. The rats were housed in clean plastic cages and kept under the following conditions: 12 h light/dark cycle, controlled temperature of 22-25°C and humidity of 60-70%. The rats were allowed an adaptation period of 5 days with free access to commercial rodent diet and water *ad libitum*. The rats were randomly divided into three groups containing ten rats. All three groups received intraperitoneal injections for 20 consecutive days. Rats in the group A served as the control group and were administered with normal saline. Group B and C rats were treated with L-methionine (Merck, Germany) 100 and 200 mg/kg BW respectively. Dose regimen, duration and route of administration in this study were based on a previous research that investigated the histomorphometric effect of methionine on the rat’s skin (19). At the end of the treatment period, all rats were euthanized. The abdomen was opened through a midline incision and the small intestine of each rat was gently removed. Then small segments of intestine, about 1cm in size, were
removed from the duodenum (below the pyloro-duodenal junction), jejunum (2cm anterior to Meckel’s diverticulum) and ileum (2cm anterior to ileocecal junction). All intestinal portions were fixed in 10% neutral buffered formalin for 10 days (Sabet Sarvestani et al., 2015; Caruso et al., 2012), dehydrated in graded anhydrous absolute ethanol and xylol, embedded in paraffin blocks and then cut into 5 µm sections. In each animal, one section that represented the best view of villus and crypts was selected to be analysed. The sections were stained using hematoxylin and eosin (H&E) (for standard histological evaluation), periodic acid shiff (PAS) (in order to count the number of neutral goblet cells) and alcian blue (AB) (in order to count the number of acidic goblet cells) techniques. Slides were examined using an Olympus light microscope and photomicrographs were taken by an attached eyepiece camera (Dino-eye, AM-7023, 5Mp, Taiwan). The following intestinal histomorphometric parameters were evaluated in all sections stained with H&E method: villous height, villous width, crypt depth and the muscular layer thickness. These parameters were measured on twenty well-aligned villi and corresponding crypts from each section of all intestinal segments. The heights of the villi were defined from their tip to the base and the widths were measured at the half height point. Calculations using villous height and width at half height gave the villus surface area. The depth of intestinal crypts was measured as the distance from top of villus crypt to muscularis mucosa.

The number of goblet cells per villus was counted in ten well-oriented and adjacent crypt villus on each section stained by PAS and AB histochemical techniques. All measurements were made in µm, at X100 magnification.

Results were expressed as mean values ± standard error. Data were analyzed using one-way analysis of variance (ANOVA) followed by Post Hoc, Tukey’s HSD test. All Statistical analysis was performed using SPSS (SPSS for Windows, version 16.0, SPSS Inc, Chicago, Illinois). Differences were considered statistically significant when the calculated $P$ value was less than 0.05.

**Results**

According to our findings, the villus height and width in the duodenum (Figure 4, Table 1) jejunum (Figure 5, Table 2) and ileum (Figure 6, Table 3) were significantly increased in
both treatment groups compared with controls (P<0.05). However, there was not any significant difference between treatment groups in all intestinal segments (Tables 1,2,3). It was observed that in all small intestine portions, the groups treated with methionine showed significant increase in villus area compared with the control groups (P<0.05). It was noticeable that the difference in the villus area in the jejunum was significant between two treatment groups (Figure 5, Table 2). The results of this study showed that the depth of the crypts in duodenum and jejunum were significantly decreased in the Met 200 group as compared to both Met 100 and control groups (P<0.05), but there was no significant difference between the Met 100 and control groups. However in the ileum, the depth of the crypts of Met 100 group were decreased compared to the other groups (P<0.05) (Figure 1)(Tables 1,2,3).

On histological observation, in the duodenum, significantly increased villus height to crypt depth ratio was observed in methionine treated groups as compared to the control group (P<0.05). It should also be noted that a significant increase in villus height to crypt depth ratio was observed in the Met 200 group compared to Met 100 group (Table 1). In the jejunum, this ratio was greater in the Met 200 group compared with the other groups (P<0.05). No significant difference was observed the Met 100 and the control groups (Table 2). In the ileum, the villus height to crypt depth ratio was significantly increased in treatment groups compared to the control group and also the villus height to crypt depth ratio was greater in Met 100 group compared with Met 200 group (Table 3).

In all small intestine segments, the thickness of the muscular layer was greater in the methionine treated groups than the controls (P<0.05). Also, in the duodenum the Met 200 groups had thicker muscular layer compared with the Met 100 group (P<0.05) (Tables 1,2,3).

In the duodenum, jejunum and ileum the count of goblet cells that were stained by alcian blue and PAS methods, showed a growth in the treatment groups compared to controls. In all small intestine parts, goblet cells in the Met 100 group were more than the other groups and this increase was significant in comparison with the control groups (P<0.05) (Figures 2 &3).

All data are shown in Tables 1-3 and Figures 4-6.
Discussion

The morphology of small intestine is considered as the main indicator of normal gut histology (Laudadio et al., 2012). Absorption of nutrients and drugs is facilitated through the enormous surface area available in this organ (Kararli, 1995). A part of the functional status of the small intestine is defined by villus height and crypt depth (Laudadio et al., 2012). The villi and crypts of the absorptive epithelium play an important role in the final stage of nutrient digestion and assimilation (Wang and Peng, 2008). Alterations in intestinal morphology may affect nutrient metabolizability and performance (Laudadio et al., 2012). For instance, an increase in villi height may result in a growth in total luminal villus absorptive area and leads to satisfactory digestive enzyme action and higher transport of nutrients at the villus surface (Tufarelli et al., 2010).

The development of small intestine can be evaluated through measurements of the crypt depth, villus height and surface area to define the available area for digestion and absorption (Franco et al., 2006; Swatson et al., 2002).

Nowadays methionine supplements is widely used for the treatment of some skin and hair diseases (Tesseraud et al., 2009), arthritis (Ursini and Pipicelli, 2009), tooth growth (McGrath and Nakamoto, 1985) and also protects the kidney from some drugs’ nephrotoxicity (Derakhshanfar et al., 2009). Due to the important side effects of methionine (Peng and Evenson, 1979; Sakino et al., 2006; Sema et al., 2010; Nazem et al., 2017), the consumption of this amino acid must be under controlled conditions. This is the first study to investigate the effect of different methionine dosages on the histomorphometric parameters of the small intestine of rat. Also to the authors’ knowledge, there have been no studies done on the effects of amino acids on the histology and morphology of the rat’s small intestine.

Based on the results of this study, administration of methionine caused an increase in villus height, villus width and finally villus area of all intestinal segments. It has been suggested that increased villi height is correlated with improved normal gut histology and may increase performance by improving nutrient absorption. (Baurhoo et al., 2007; Vaezi et al.,
The measurement of the villus area correlates very well with the total number of epithelial cells in the villus (Hasan and Ferguson et al., 1981). Therefore, it seems that following the injection of methionine could lead to an increase in digestion and absorption of the nutrients in all small intestine parts. The duodenum has the major role in nutrient absorption and the decrease in villi size from the duodenum to ileum is due to the lower absorptive capacity in the last portion of small intestine (Sharp and Villano, 2012). This is consistent with our findings in this study that showed the villi height in the duodenum was greater than those in the jejunum and ileum. It is also noticeable that the villi width was increased from the duodenum, through the jejunum to the ileum. Depth of the crypts was decreased in methionine treated rats compared to the controls. However, the crypt depth of the duodenum in the Met 100 group was not decreased as compared to the control group. Kelly et al. (1991) reported that decrease in the crypt depth caused an increase in the enzymatic activity of the small intestine of pigs. So we suggest that methionine supplementation may increase the intestinal digestion activity. Following the administration of methionine, the ratio of villus height to crypt depth was increased. This parameter was not increased in the jejunum of Met 100 group compared to the control group. A higher villus height to crypt depth ratio results in a decreased turnover of the intestinal mucosa. A slower turnover rate of the intestinal epithelium leads to a lower maintenance requirement and finally can result in a higher growth efficiency of the animal (Van Nevel et al., 2005). Overall it can be said that villus height, crypt depth and the ratio of villus height to crypt depth are considered as a criterion to reflect the small intestine morphology and absorption capacity (Montagne et al., 2003). Therefore, an increase in villus height, villus height to crypt depth ratio or decrease in the crypt depth is correlated with an improvement in the digestion and absorption of nutrients (Hou et al., 2010; Hou et al., 2012; Hou et al., 2013; Yao et al., 2012).

Villus height to crypt depth ratio is one of the most important histomorphometric parameters of the small intestine. Based on the obtained results in the duodenum and jejunum, the villus height to crypt depth ratio was significantly increased in Met 200 group compared to Met 100 group. Since the duodenum and jejunum are the most important absorptive parts of the small intestine, it may be concluded that the 200 mg/kg dosage of
methionine have a better effect on the absorption of the nutrients than the 100 mg/kg dosage. So it can be interpreted that 200 mg/kg dosage does not have toxic effects on the small intestine. This finding is in contrast with a previous study conducted by Nazem et al. (2017) which showed that 200 mg/kg methionine has more toxic effects than 100 mg/kg dosage in rat's uterus.

Unlike the obtained results in duodenum and jejunum, the villus height to crypt depth ratio was greater in Met 100 compare to Met 200 in the ileum. It may be due to an imbalance in amount of amino acids in this small intestine part. The similar pattern between the histomorphometry of the ileum and the two other parts of small intestine was also reported in the pregnant rats (Sabet Sarvestani et al., 2015).

The epithelial cells of the small intestine are covered by a protective mucus blanket composed of high molecular weight glycoproteins known as mucins (Pearson and Brownlee et al., 2005). Mucins which are synthesized and secreted from goblet cells act as the first line of defense against intestinal pathogens and play a significant role in the maintenance of mucosal homeostasis (Dharmani et al., 2009; Montagne et al., 2004; Specian and Oliver, 1991). The mucus layer acts as a medium in which digestion and absorption processes happen close to the brush-border membrane (Smirnov et al., 2004). Several studies have reported the modulation of goblet cell number and mucin secretion under different conditions such as varied diet, surgery and altered microbiota (Dunsford et al., 1991; Olubuyide et al., 1984; Sharma and Schumancher, 1995; Sharma et al., 1995; Vahouny et al., 1985). Obtained results in our study showed that in all intestinal segments, the number of goblet cells that were stained by PAS and AB methods were increased in methionine treated rats compared to controls, which may be indicative of an increase in mucin secretion. The increase in mucin secretion may favor movement of the digesta (Forstner et al., 1995). Also any changes in the thickness of the mucus layer may influence nutrient digestibility processes (Smirnov et al., 2004). So it seems that administration of methionine may affect digestion by an increase in mucin secretion in the small intestine. Muscular layer thickness was also increased after methionine administration. A previous study conducted by Sabet Sarvestani et al. (2015) showed that the duodenum has gained more capacity to digest food intake during pregnancy by increasing villi length and
muscular layer thickness. A positive effect of methionine supplementation on skeletal muscle growth has been also reported in the chickens (Tesseraud et al., 2009). In this study, the tunica muscularis was thicker in all intestinal segments in methionine treated rats, which may increase the contact between the intestinal content and the mucosa. We proposed that methionine may have a positive effect on the growth of the tunica muscularis layer and therefore improves digestion.

Conclusions

In conclusion we have shown that administration of methionine as the first limiting amino acid may affect the digestion and absorption of nutrients in all small intestine segments by an increase in villi length, villi width, villi area, number of goblet cells and finally the muscular layer thickness. Although injection of methionine caused an alteration in small intestine histomorphometric parameters, defining the choice dose of methionine in rat needs further studies. It is noticeable that there is not a direct relation between increased methionine dosages and their effects on some tissues in rats (Nazem et al., 2016).

Acknowledgments

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References


Figure 1. Histological features of the jejunum of control (A), Met 100 (B) and Met 200 (C) groups. Based on these figures, villi height and width in both Met 100 and Met 200 are increased compared to the control group. H&E, 40X.

Figure 2. PAS staining of the jejunum of control (A), Met 100 (B) and Met 200 (C) groups. The greatest number of PAS positive goblet cells is visible in the Met 100 group (B). PAS, 40X.
Figure 3. Alcian blue staining shows an increase in the acidic goblet cells of the jejunum of control (A), Met 100 (B) and Met 200 (C) groups. The greatest number of AB positive goblet cells is visible in the Met 200 group (C). Alcian blue, 40X.
Figure 4. Histomorphometric measurements of the duodenum of control (Control) and treatment groups: 100 (Met 100) and 200 (Met 200). Bars are Mean ± Standard error. The unit of villus height, villus width, crypt depth and muscular layer thickness is µm. The unit of villus area is µm².
Figure 5. Histomorphometric measurements of the jejunum of control (Control) and treatment groups: 100 (Met 100) and 200 (Met 200). Bars are Mean ± Standard error. The unit of villus height, villus width, crypt depth and muscular layer thickness is µm. The unit of villus area is µm².
Figure 6. Histomorphometric measurements of the ileum of control (Control) and treatment groups: 100 (Met 100) and 200 (Met 200). Bars are Mean ± Standard error. The unit of villus height, villus width, crypt depth and muscular layer thickness is µm. The unit of villus area is µm².
Table 1. The effect of intraperitoneal administration of methionine on the histomorphometric parameters of rat duodenum¹.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Met 100</th>
<th>Met 200</th>
</tr>
</thead>
<tbody>
<tr>
<td>VH, µm</td>
<td>389.47±33.84a</td>
<td>533.84±23.05b</td>
<td>525.82±14.61b</td>
</tr>
<tr>
<td>VW, µm</td>
<td>69.47±5.58a</td>
<td>103.22±6.41b</td>
<td>106.66±6.56b</td>
</tr>
<tr>
<td>VA, µm²</td>
<td>27056.65±2350.8a</td>
<td>55102.96±2379.22b</td>
<td>56084.15±1557.7b</td>
</tr>
<tr>
<td>CD, µm</td>
<td>264.28±13.74a</td>
<td>264.32±32.31a</td>
<td>166.72±9.79b</td>
</tr>
<tr>
<td>VH/CD</td>
<td>1.47±0.13a</td>
<td>2.01±0.09b</td>
<td>3.15±0.09c</td>
</tr>
<tr>
<td>Goblet cells (AB)</td>
<td>8.67±1.05a</td>
<td>15.83±1.89b</td>
<td>12.57±2.01ab</td>
</tr>
<tr>
<td>Goblet cells (PAS)</td>
<td>8.9±0.86a</td>
<td>16.11±1.43b</td>
<td>12.13±1.11ab</td>
</tr>
<tr>
<td>Muscle layer thickness, µm</td>
<td>77.94±5.38a</td>
<td>101.26±8.49b</td>
<td>157.17±6.99c</td>
</tr>
</tbody>
</table>

a, b, c Means in the same row with no common superscript differ (P<0.05).

¹ Values are Mean±SE. Abbreviated signs are as follows: VH: villus height; VA; villus width; VA; villus area, CD; crypt depth; AB: alcian blue; PAS; periodic acid schieff.

Table 2. The effect of intraperitoneal administration of methionine on the histomorphometric parameters of rat jejunum¹.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Met 100</th>
<th>Met 200</th>
</tr>
</thead>
<tbody>
<tr>
<td>VH, µm</td>
<td>353.04±10.64a</td>
<td>405.53±8.8b</td>
<td>423.16±16.05b</td>
</tr>
</tbody>
</table>
### Table 3. The effect of intraperitoneal administration of methionine on the histomorphometric parameters of rat ileum

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Met 100</th>
<th>Met 200</th>
</tr>
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<tbody>
<tr>
<td>VH, µm</td>
<td>199.47±5.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>272.2±7.46&lt;sup&gt;b&lt;/sup&gt;</td>
<td>265.56±2.78&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>VW, µm</td>
<td>94.53±2.51&lt;sup&gt;a&lt;/sup&gt;</td>
<td>130.73±3.15&lt;sup&gt;b&lt;/sup&gt;</td>
<td>118.58±4.5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>VA, µm&lt;sup&gt;2&lt;/sup&gt;</td>
<td>18856.89±482.22&lt;sup&gt;a&lt;/sup&gt;</td>
<td>33811.26±2356.84&lt;sup&gt;b&lt;/sup&gt;</td>
<td>31490.1±329.83&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>CD, µm</td>
<td>221.72±5.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>186.87±3.66&lt;sup&gt;b&lt;/sup&gt;</td>
<td>206.68±0.73&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>VH/CD</td>
<td>0.9±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.45±0.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.28±0.01&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Goblet cells (AB)</td>
<td>19.8±2.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>27.67±1.48&lt;sup&gt;b&lt;/sup&gt;</td>
<td>25±1.63&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a, b, c</sup> Means in the same column with no common superscript differ (P<0.05).

Values are Mean±SE. Abbreviated signs are as follows: VH: villus height; VA: villus width; VA: villus area, CD: crypt depth; AB: alcian blue; PAS: periodic acid schieff.
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<tbody>
<tr>
<td><strong>Goblet cells (PAS)</strong></td>
<td>24.86±1.35&lt;sup&gt;a&lt;/sup&gt;</td>
<td>31±1.68&lt;sup&gt;b&lt;/sup&gt;</td>
<td>24.56±1.55&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Muscle layer thickness, µm</strong></td>
<td>55.13±4.54&lt;sup&gt;a&lt;/sup&gt;</td>
<td>66.85±3.94&lt;sup&gt;b&lt;/sup&gt;</td>
<td>72.66±4.15&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a, b, c</sup> Means in the same column with no common superscript differ (P<0.05).

<sup>1</sup> Values are Mean±SE. Abbreviated signs are as follows: VH: villus height; VA: villus width; VA: villus area, CD: crypt depth; AB: alcian blue; PAS: periodic acid schieff.