Original Article

# Effect of Protein Deficiency Diet on Gastric Histology and Histomorphometric Indices in Mice Animal Model

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## ABSTRACT

**Background:** In the long run, diets can affect the histological structure and histomorphometry of the gastrointestinal tract in order to adapt to the diet and provide its primary role of absorbing the necessary materials and energy for the organism's survival.

**Objectives:** This study aims to investigate the effect of dietary protein deficiency on the histology and histomorphometric indices of stomach and blood chemical parameters and liver enzymes.

**Methods:** A total of 12 immature female Balb/C mice at the age of three weeks were divided into a control group that received a complete protein diet and an experimental group that received a protein-deficient diet. Three months later, the serum levels of calcium, phosphorus, glucose, cholesterol, triglycerides, urea, creatinine, and liver enzymes were evaluated, and the thickness of the layers of the stomach wall, as well as pit depth, and the number of parietal cells were measured in stomach tissue.

**Results:** Comparing the thickness of stomach layers showed that the thickness of the mucosa, muscle layer, depth of pits, and the number of parietal cells of the stomach wall increased significantly in the experimental group ($P<0.05$). Also, the serum levels of phosphorus, glucose, cholesterol, triglyceride, urea, creatinine, aspartate transaminase (AST) and alanine transaminase (ALT) in the experimental group showed a significant decrease. Still, the serum level of alkaline phosphatase in the experimental group increased significantly ($P<0.05$).

**Conclusion:** The results of the present study showed that changes in diet in the long term can alter the histology and histomorphometry of the stomach wall as well as blood parameters, which may be unfavorable for people's health in some parameters.

**Keywords:** Histology, Histomorphometry, Stomach, Protein deficiency, Mice

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## Introduction

The human digestive system consists of the alimentary canal (oral cavity, esophagus, stomach, small and large intestines, and anus) and its attached glands (salivary glands, liver and pancreas). It is responsible for providing energy and ensuring the survival of living organisms from the consumed materials. In the digestive process, proteins, complex carbohydrates, nucleic acids and fats are broken into smaller molecular subunits and absorbed by the small intestine. In addition, along the entire length of the alimentary canal, the inner layer forms a protective barrier between the contents of the alimentary canal and the internal environment of the connective tissue and vascular system of the body (Li et al., 2020; Mackie et al., 2020; Mescher, 2013). The present research was conducted on mice that have a stomach almost similar to the human digestive tract, with the difference that the mouse stomach consists of two parts, non-glandular and glandular, and has four regions: Cardia, fundus, body, and pylorus and two small and big curvatures. From the small curvature, the lesser omentum connects the stomach to the visceral surface of the liver, and from the greater curvature, the greater omentum, which has two layers, connects the stomach to the spleen and intestines. In mice, the border between non-glandular and glandular parts is clearly defined (Scudamore, 2014; Yang et al., 2022; Amalia et al., 2023).

Undoubtedly, the histological characteristics and histomorphometric indicators of the digestive system, especially the mucous layer of the stomach, adapt in the long term according to the type of food consumed by each animal so that it can fulfill its role in providing the materials and energy needed for the survival of the animal (Fazelipour et al., 2016a). So far, a lot of research has been done to know the components, physiology, and histology of the digestive system, the function and the effect of diet on its development and growth (Fazelipour et al., 2016a; Amer et al., 2021; Choudhury et al., 2021; Ravindran & Abdollahi, 2021). A large part of the research is related to the effect of diet on the digestive system and aims to answer the questions raised in meat animal breeding centers. In these centers, which are economic enterprises, most of the current cost is providing feed for livestock, poultry, fish, etc. Therefore, there is always this concern about how you can get the most profit by changing the composition of diet ingredients and using cheaper ingredients while maintaining efficiency as much as possible. To answer this question, it is necessary to know the effect of increasing and decreasing the different foods in the animals' feed in these centers. Therefore, much research has been done in this direction, mainly related to meat-breeding animals (Vermeulen et al., 2020; Erickson et al., 2020; Te Pas et al., 2021; Morach et al., 2021). Although most research results have generally agreed with each other, sometimes differences are also seen. In some cases, the reasons for the changes in the digestive system are unknown.

In addition to the changes in the histology and histomorphometry of digestive tract tissues, blood parameters, and liver enzymes are also affected by diet (Kozeniecki et al., 2020; İçil et al., 2020; Ahmed & Ahmad, 2020; Traub et al., 2021; Kalas et al., 2021; Wang et al., 2023). Considering that conducting research in this field on humans faces many obstacles and problems, the effect of diet on the mouse animal model was chosen due to its similarity to humans and the lack of research records for it. This research is focused on the effect of reducing protein in the diet on the histology and histomorphometric indices of the stomach, as well as the chemical parameters of mice's blood and liver enzymes.

## Materials and Methods

### Animal model

This experimental research was conducted on 12 immature female Balb/C mice weighing 10 to 12 g. The animals were obtained from Razi Vaccine and Serum Production Research Institute and were kept in suitable living conditions for one week in the university's animal house. Optimal conditions included a 12-hour light/dark cycle at an ambient temperature between 20 ˚C and 24 ˚C with relative humidity between 40% and 60%. After the adaptation period, the mice were randomly divided into the control group, which received a complete protein diet (23%) and the experimental group, which received a protein-deficient diet (13.5%). The nutritionist prepared the diet.

### Preparing animal diet

A nutritionist prepared the diet for protein deficiency and complete protein. Then, the food items, according to Table 1, were purchased and transferred to the Faculty of Veterinary Medicine of University of Tehran. The food ingredients were weighed according to the Table and turned into powder by the mill, and then it was made into a paste, made into a plate, dried, and given to the animals.

**Table 1.** Diet in 1000 g of mouse food

| Food Items | Protein Deficiency (13.5% Protein) (g) | Complete Protein (23% Protein) (g) |
|---|---|---|
| Corn | 700 | 430 |
| Rapeseed meal | 150 | 420 |
| Sunflower meal | 112 | 112 |
| Oyster powder | 17 | 17 |
| Dicalcium phosphate | 15 | 15 |
| Mineral supplement | 2.5 | 2.5 |
| Vitamin supplement | 2.5 | 2.5 |
| Salt | 1 | 1 |
| Total | 1000 | 1000 |

### Implementation of the plan

Mice were kept under the determined dietary conditions of each group for three months according to the period of growth and development of the mice. After the treatment, the animals were anesthetized and blood was taken from their hearts to determine the serum levels of calcium, phosphorus, glucose, cholesterol, triglycerides, urea, creatinine, and liver enzymes. The sera were sent to the Pathobiology Laboratory, and the mentioned cases' serum levels were measured by the radioimmunoassay method (Badi et al., 2022; Chahnaz Hamza et al., 2024).

Then, the abdominal cavity of the animals was dissected, and the stomachs of the animals were removed, and after washing with normal saline, they were placed in 10% formalin. From each sample, serial sections with a thickness of 5 µm were prepared and stained by the hematoxylin-eosin (H & E) method (Badi et al., 2022; Chukwu et al., 2023). Four sections of each sample and four fields of view were taken using a photomicroscope equipped with AxioVision and DinoCapture software. The required images were prepared, and the histological and histomorphometric features (the thickness of the layers of the stomach wall, as well as pit depth, the number of parietal cells and the coordinates of the villi) were analyzed according to the parameters of the research variable table (Vajed Ebrahimi et al., 2024).

### Data analysis

SPSS software, version 26 was used for data analysis in this study, and quantitative data results were presented as Mean±SD. Data analysis was done by one-way analysis of variance (ANOVA) to compare the average data between the control and experimental groups. The significance level was set at $P<0.05$.

## Results

### Histological findings

The results showed that the stomach consists of the non-glandular and glandular parts. The non-glandular part, which is located immediately after the esophagus, has a squamous type of mucosal epithelium with fine keratin layers in the most superficial part. The thickness of the epithelium is higher in some areas and less in some, including several layers of cells from the basement membrane to the surface. It consists of a basal layer of cubic to short cylindrical cells. On this layer, there are one to three spherical to polyhedral cell layers with large spherical and euchromatic nuclei. Then, a layer of granular cells and thin keratin sheets form the epithelium's most superficial part. The mucous lining in the non-glandular part is not very thick because it has no gastric glands. The mucosa and submucosa lining consists of loose to semi-loose connective tissue, and the muscular layer consists of two to three thin layers of smooth muscle, which is surrounded by a thin serous layer from the outside (Figures 1 and 2).

The gastric mucosa comprises three components: An epithelial tissue that lines the internal cavity, a connective tissue (lamina propria) and smooth muscle layers forming the mucosa. A simple cylinder composed of surface lining cells is furnished, which produces a thick mucus layer. In the histological examination of the stomach, no abnormal histological observations were observed between the control and experimental groups (Figures 1 and 2).

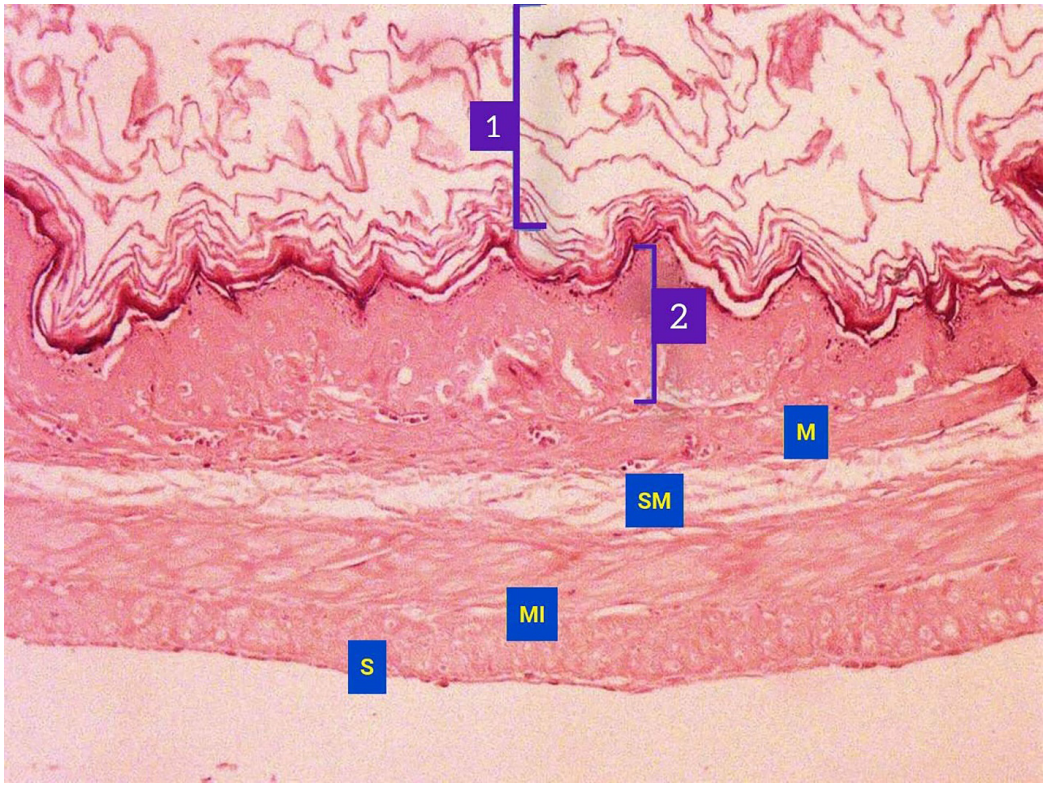


**Figure 1.** Photomicrograph of the non-glandular part of the stomach in the experimental group

Note: In this image, the non-glandular part of the stomach can be seen with its different layers: 1) Keratinized stratified squamous epithelium; 2) Mucous lining: Connective tissue; M: Mucous muscle, smooth muscle; SM: Submucosa layer; MI: Muscular layer, visible inside the circular muscles and outside the longitudinal muscles and S: Serous (hematoxylin-eosin stain, ×100 magnification).

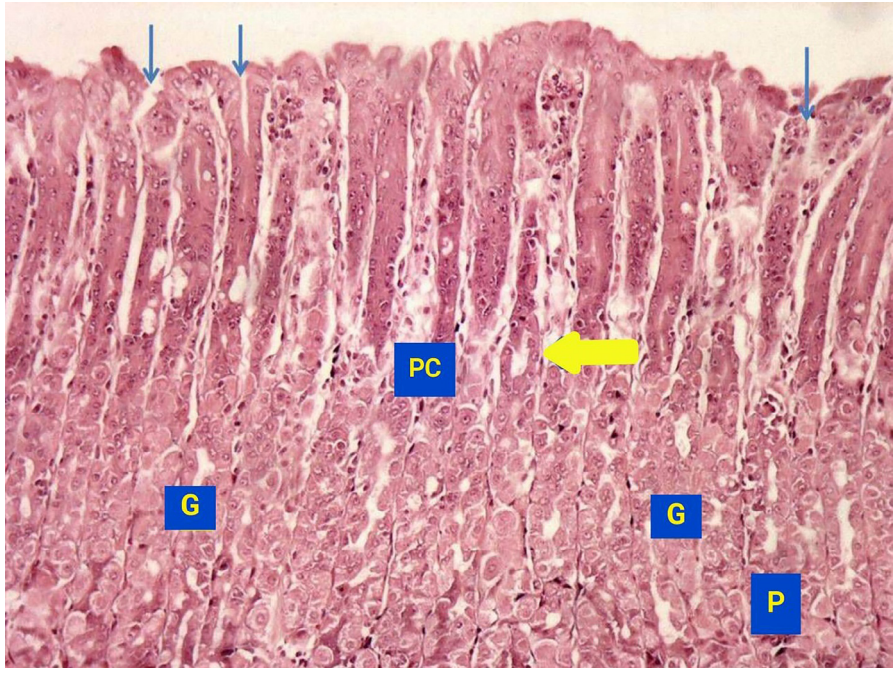


**Figure 2.** Photomicrograph of the glandular part of the stomach in the experimental group

Note: In this picture, blue arrows are pits and yellow arrows are mucous lining. Also, PC refers to parietal cells, G to gastric glands, and P to zymogenic cells (hematoxylin-eosin stain, ×100 magnification).

**Table 2.** The thickness of the mucous and submucous layers of the glandular and non-glandular stomach parts in the control and experimental groups

| Variables | Mean±SD | | | |
|---|---|---|---|---|
| | Mucous Layer Thickness (µm) | | Submucous Layer Thickness (µm) | |
| | Glandular | Non-glandular | Glandular | Non-glandular |
| Control | 615.29±75.90[a] | 427.16±70.78[a] | 93.42±7.93[a] | 100.64±7.59[a] |
| Experimental | 1298.03±144.97[b] | 859.65±79.52[b] | 114.47±16.68[b] | 126.72±13.26[b] |
| P | 0.003 | 0.009 | 0.029 | 0.01 |

Note: Dissimilar letters in each parameter indicate significant differences between the groups.

## Histomorphometric findings

### The thickness of the mucous layer of the stomach wall

The mouse stomach has two glandular and non-glandular regions, and the thickness of both areas was measured in µm in the experiment (Table 2). The average thickness of the mucus in the sample of gastric mucosa tissues shows that the lack of dietary protein caused the thickness of the gastric mucosa to significantly increase from 615.29 to 1298.03 µm in the glandular region (P=0.003). The average gastric mucosa thickness in the non-glandular low protein group also increased significantly from 427.16 to 859.65 µm (P=0.009) (Table 2).

### The thickness of the submucous layer of the stomach wall

The morphometrical analysis of the thickness of the submucous layer in the glandular and non-glandular part of the stomach indicated that deficiency of dietary protein significantly increased the average submucosa thickness from 93.42 µm in the control group to 114.47 µm in the experimental group (P=0.029). In the non-glandular area, the average thickness of the submucosa increased significantly from 100.64 µm in the control group to 126.72 µm in the experimental group (P=0.01). So, according to the results, the thickness of the stomach submucosa in the glandular and non-glandular area was significantly increased in the group of rats that received a low-protein diet (Table 2).

### The thickness of the muscular layer of the stomach wall

The thickness of the muscle layer of both glandular and non-glandular regions of the stomach tissue is presented in Table 2. Due to the lack of dietary protein, the average thickness of the muscle layer of the non-glandular area of the stomach has increased from 155.74 µm in the control group to 201.55 µm in the experimental group (P=0.11), which is far from the significant level. In the glandular region of the stomach, the average thickness of the muscle layer increased significantly from 104.05 µm in the control group to 246.38 µm in the experimental group (P=0.002).

**Table 3.** The thickness of the muscular layer of the glandular and non-glandular parts of the stomach, stomach pit depth and the parietal cells count in the stomach pit in the control and experimental groups

| Variables | Mean±SD | | | |
|---|---|---|---|---|
| | Muscular Layer Thickness (µm) | | Stomach Pit Depth (µm) | The Parietal Cells Count in the Stomach Pit |
| | Glandular | Non-glandular | | |
| Control | 104.05±10.70[a] | 155.74±25.77[a] | 94.80±11.81[a] | 9.83±1.34[a] |
| Experimental | 246.38±76.54[b] | 201.55±53.38[a] | 185.30±31.17[b] | 25.67±1.97[b] |
| P | 0.002 | 0.11 | 0.0001 | <0.0001 |

Note: Dissimilar letters in each parameter indicate significant differences between the groups.

**Table 4.** Calcium, phosphorous and glaucous levels of mice blood serum in the control and experimental groups (n=6)

| Variables | Mean±SD | | |
|---|---|---|---|
| | Calcium (mg/dL) | Phosphorus (mg/dL) | Glaucous (mg/dL) |
| Control | 5.57±0.96[a] | 11.42±3.37[a] | 78.00±32.61[a] |
| Experimental | 6.17±0.93[a] | 8.82±1.85[b] | 38±12.86[b] |
| P | <0.05 | <0.05 | <0.05 |

Note: Dissimilar letters in each parameter indicate significant differences between the groups.

Therefore, a low protein diet caused a significant increase in the thickness of the stomach muscles in the glandular area and a non-significant increase in the thickness of the non-glandular area muscles (Table 3).

### The stomach pit depth

According to Table 3, due to lack of dietary protein, the stomach pit depth was significantly increased from 94.80 µm in the control group to 185.30 µm in the experimental group (P=0.0001) (Table 3).

### The parietal cells count in the stomach pit

The result of counting the number of parietal cells in the stomach pit in both control and experimental groups showed that the average number of cells in the control group increased significantly from about 9.83 to about 25.67 in the field of vision in the experimental group (P<0.0001) (Table 3).

### Biochemical parameters of blood serum

The assessment of the phosphorus, glucose, cholesterol, triglycerides, urea, and creatinine levels in the blood serum of experimental and control groups indicated that the reduction of dietary protein caused the mentioned blood chemical parameters to decrease in the experimental group compared to the control group. However, the calcium parameter was non-significantly increased in the experimental group (P>0.05) (Tables 4 and 5).

### Liver enzymes

The evaluation of aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) levels in the blood serum of the experimental and control groups showed that the ALT level decreased significantly in the experimental group compared to the control group. The AST and ALP levels significantly increased in the experimental group (P<0.01) (Table 6).

## Discussion

Previous studies have shown that various diets or medications can have long-term effects on the histological and histomorphometric structure and, consequently, on the function of different layers of the stomach walls. These studies have also indicated that the growth and survival of organisms are significantly dependent on essential nutrients such as protein (Fazelipour et al., 2016a; Limbach et al., 2021).

The present study investigated the effects of adequate protein intake or protein deficiency on the tissue layers of the stomach walls, which are fundamental components of the gastrointestinal tract. The results indicated that a protein-deficient diet led to a significant increase in the layers

**Table 5.** Cholesterol, triglyceride, urea and creatinine levels of mice blood serum in the control and experimental groups (n=6)

| Variables | Mean±SD | | | |
|---|---|---|---|---|
| | Cholesterol (mg/dL) | Triglyceride (mg/dL) | Urea (mg/dL) | Creatinine (mg/dL) |
| Control | 157.50±14.22[a] | 155.66±59.46[a] | 67.25±7.38[a] | 0.53±0.13[a] |
| Experimental | 123.83±9.98[b] | 118.66±5.27[b] | 48.78±3.27[b] | 0.28±0.7[b] |
| P | <0.05 | <0.05 | <0.05 | <0.05 |

Note: Dissimilar letters in each parameter indicate significant differences between the groups.

**Table 6.** The ALT, AST and ALP levels of mice blood serum in the control and experimental groups (n=6)

| Variables | Mean±SD | | |
|---|---|---|---|
| | ALT (IU/L) | AST (IU/L) | ALP (IU/L) |
| Control | 6.17±0.93[a] | 8.82±1.85[a] | 38±12.86[a] |
| Experimental | 5.57±0.96[b] | 11.42±3.37[b] | 78.00±32.61[b] |
| P | <0.01 | <0.01 | <0.01 |

Abbreviations: AST: Aspartate transaminase; ALT: Alanine transaminase; ALP: Alkaline phosphatase.

Note: Dissimilar letters in each parameter indicate significant differences between the groups.

of the mucosa, submucosa, and muscles of the stomach wall, as well as an increase in the number of parietal cells and the depth of the gastric pits. In this study, the thickness of the mucosal layer in both the non-glandular and glandular parts of the stomach showed a significant increase in the protein-deficient group compared to the control group.

In a study, it was observed that a diet containing soy, which replaced animal protein, also resulted in a significant increase in the thickness of the mucosal layer in both the glandular and non-glandular parts of the stomach (Fazelipour et al., 2016a). In line with the present study, the number of parietal cells in the protein-deficient group was higher than in the control group. The increase in these cells probably leads to an increase in stomach acid, and the increased thickness of the mucosa may be due to the increased stomach acid. Thus, the mucosal layer of the stomach wall increases in thickness to protect the wall and prevent damage from excessive acid. This protection is achieved through mucus secretion by all epithelial cells of the glandular part of the stomach, providing a greater protective role. The present study also showed that the increase in the depth of the pits could be another protective mechanism against acid, allowing for longer-term protection by preventing acid penetration into the stomach.

In a study by Karam, it was observed that ranitidine, which was administered to mice, reduced the secretion of parietal cells responsible for stomach acid production. This study shows that the longer the treatment duration with ranitidine, the less acid is secreted, as the drug reduces acid secretion and destroys some parietal cells, thereby decreasing acid secretion (Karam & Alexander, 2001). The examination of parietal cells in this study indicates that the vacuolization and dilation of the canaliculi, and possibly their destruction, effectively reduces acid levels, aligning with the present study. Therefore, the increase in the mucosal layer observed in this study is a compensatory response of the stomach to protect the wall against increased acid.

In another study, it was shown that long-term consumption of aspartame, an artificial sweetener in beverages, led to the destruction and disorganization of the epithelial mucosa of the stomach wall and atrophy of some glandular cells in the glandular part of the stomach lining. This study demonstrates that high doses of aspartame could cause an increase in the thickness of the glandular part of the stomach wall in mice, which could be attributed to the long-term effect of aspartame on the stomach's epithelial cells. Another result of this study was an increase in the thickness of the muscular layer of the stomach wall in the glandular part of the mouse stomach, which is similar to the human stomach. The increase in the muscular layer may increase the mechanical activity of the animal's stomach, possibly due to a digestive disorder and the compensatory response of the stomach wall by increasing the muscular layer and enhancing mechanical activity in response to digestive disturbances. In this context, the presence of an extensive vascular system in the stomach wall should also be considered related to these reactions, as some drugs with high doses can influence blood flow in the stomach wall, affecting the thickness of the layers and the activity of the muscular layer. Additionally, in a study on the muscular layer and stomach wall of diabetic mice, the observed increase in the muscular layer thickness supports the present study's findings (Tootian et al., 2022).

Masuoka et al. found that mice on a protein-deficient diet experienced weight loss and decreased urea levels (Masuoka et al., 2020). The present study also found that a protein-deficient diet significantly reduced serum urea levels compared to the control group. This outcome can be attributed to the fact that urea is formed from the deamination of amino acids in the liver and excreted by the kidneys; thus, protein deficiency leads to fewer amino acids and, consequently, lower urea levels. Fazelipour et al. (2016b) also observed that dietary changes could reduce serum urea levels.

Additionally, our study indicates that a protein-deficient diet could affect liver enzymes, with serum ALT levels decreasing and AST and ALP levels increasing in the experimental group. Morovvati et al. (2018) found that administration of dianabol in rats increased serum AST, ALT and ALP levels, aligning with this study's findings.

The present study also shows significant reductions in serum glucose, triglycerides, cholesterol, and creatinine in the experimental group compared to the control group. Fazelipour et al. (2020) found that nanoparticles affected blood factors, increasing glucose, triglycerides, cholesterol and creatinine levels compared to the control group.

Thus, dietary changes and medications can rapidly alter serum blood factor levels. Mousaie et al. (2011) also reported a significant decrease in triglycerides in the protein-deficient diet group compared to the control group.

The experimental protein deficiency reduces the activities of a constellation of hepatic enzymes and enzymatic complexes. The hepatic enzymes are generally more severely affected by protein deprivation than the same enzymes in other tissues. Since most hepatic enzymes diminish in proportion to the reduction in total hepatic protein or even to a greater extent, certain hepatic proteins are more affected than others. However, the unique ability of hepatic enzymes is not a characteristic of the enzymes but instead of hepatic protein metabolism. The quality of the dietary protein appears to be important in the maintenance of the enzymatic profiles of the liver, as indicated by the fact that a dietary deficiency of methionine, tryptophan, or histidine results in the reduction of xanthine oxidase and betaine transmethylase. Rats deprived of protein show a substantial increase in the activity of hepatic amino acid-activating enzymes, and the same occurs in malnourished children. Controversial results have been reported in the case of hepatic cytochrome oxidase activity in protein-deficient rats, which has been found to increase in some studies and decrease or be unaltered in others. Alkaline phosphatase activity was reported to increase in livers of rats fed protein-free diets (Porta & Hartroft, 1970; Lenox, 2021). In this study, the level of ALP decreased significantly in the experimental group, which is aligned with Porta and Hartroft (1970).

It is difficult to determine from the available data if protein deficiency affects the enzymes of a particular intracellular location more extensively than other parts of the cell. Reduced enzymes have been found in practically all subcellular fractions, but most results are derived from protein-free animals rather than those fed with low-protein diets. On the other hand, the reported increases in amino acid activating enzymes, alkaline phosphatase, and cytochrome oxidase in rats and the increased levels of catalase, malic dehydrogenase, transaminase, and alkaline phosphatase found in livers of patients with protein malnutrition would suggest that some adaptive changes may have taken place under nutritional stress to spare the more vital enzymes. It is logical to assume that a low protein intake is less catastrophic than a complete lack of dietary protein concerning enzymatic lability (Porta & Hartroft, 1970; Lenox, 2021). In the present study, the experimental group's liver enzyme levels were significantly altered. The AST and ALP levels increased dramatically in the protein deficiency group, which can be indicated as the primary fatty liver due to protein metabolism disorders in the liver.

## Conclusion

As the results of the present research and other research have shown, regarding the change in the amount of protein in the diet, the lack of protein consumption in the long term causes changes, and sometimes unfavorable ones, in the histology and histomorphology of the digestive system tissues. Also, blood parameters and liver enzymes change as a result, which can have an adverse effect on the growth and health of living beings in the long run. Therefore, getting enough protein is essential for good health.

## Ethical Considerations

### Compliance with ethical guidelines

This study was approved by the Ethics Committee of Tehran Medical Sciences Branch, Islamic Azad University, Tehran, Iran (Code: IR.IAU.TMU.REC.1402.166).

### Funding

The paper was extracted from the general medical doctorate thesis of Pooya Mahjoub, Simin Fazelipour, Parivash Davoudi, and Mohammad Babaei, approved by the Department of Anatomical Sciences and Cognitive Neurosciences, Faculty of Medicine, Tehran Medical Sciences Branch, Islamic Azad University, Tehran, Iran.

### Authors' contributions

Conceptualization and supervision: Simin Fazelipour, Parivash Davoudi, and Mohammad Babaei; Methodology: Pooya Mahjoub, and Mohammad Babaei; Investigation and writing: Mohammad Babaei and Simin

Fazelipour; Data collection: Pooya Mahjoub; Data analysis: Pooya Mahjoub and Mohammad Babaei; Funding acquisition and resources: Simin Fazelipour and Pooya Mahjoub.

## Conflict of interest

The authors declared no conflict of interest.

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