Research Article

Formation of Structural Components of Masticatory Muscles in Postnatal Ontogenesis and Their Changes in Experimental Iodine Deficiency

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Abstract

Investigating the blood vessels of masticatory muscles, we have determined a structural age reorganization, which manifests itself in increasing the diameter of all parts of the arterial and venous bed. Also, with age (in mature animals) the number of capillaries per unit of the area and the number of capillaries, which supply with blood one muscular fiber that corresponds to the growth and muscular fibers development demands, increase. Histologically and submicroscopically, muscular fibers acquire a definitive structure. The percentage distribution of different types of fibers is changing: the amount of OGMF decreases with the simultaneous growth of GMF and the amount of OMF is almost unchanged, indicating the decrease of oxidative processes, and it is confirmed by a slight increase of the volumetric fraction of mitochondria in OMF and a moderate decrease of their part in other types of muscular fibers compared with a significant increase of the volumetric fraction of myofibrils.

Under the conditions of iodine deficiency, we have observed the changes in the diameter of the lumen of all parts of the arterial and venous bed: narrowing of the arterial and dilation of venous vessels. One can also note the thickening of the arterial wall due to the edema of all its membranes. These changes are more pronounced in the vessels of the masticatory muscles of the immature animals, which are confirmed by morphometric studies. Also the number of hemocapillaries decreases. Ultramicroscopically, edema is also noted in the endothelial cells. During histological examination of muscular fibers there are changes in the quantitative composition of different types with a tendency to decrease the number of OGMF with simultaneous growth of GMF. Also, there is an increase of the cross-sectional area of all types of muscular fibers. During ultramicroscopic examination, the edematous changes of muscular fibers are also observed, especially in GMF. There is also an increase of the volumetric fraction of mitochondria and the volumetric fraction of myofibrils in muscular fibers, indicating the edematous changes in these structures and the strength of oxidative processes.

Keywords
ontogenesis; hemomicrocirculatory bed; masticatory muscle; iodine deficiency

Problem statement and analysis of the latest research

Due to the deterioration of the ecological situation in Ukraine, the frequency of thyroid gland diseases that can be caused by both pathological changes in the gland itself and thyroid hormones metabolism disorders, increases [1, 3, 4, 5, 10, 16, 17, 19, 20, 25, 27, 32, 36] and regulates all types of metabolism [5, 6, 7, 8, 12, 14, 18, 21, 22, 23]. For normal functioning of the thyroid gland, a person needs to receive 100-200 µg of iodine per day, a decrease of which to 40-80 µg can already be regarded as an indicator of iodine deficiency, which, not leading to clinical manifestations of thyroid disease, reduces the index of mental development of the population [13, 24, 26, 27, 28, 31]. According to the World Health Organization (WHO), one third of the Earth’s population belongs to the so-called “iodine deficiency risk group”. Almost 1 billion of inhabitants of the planet have clinical manifestations of iodine deficiency [4, 10]. For many countries, iodine deficiency has become a major medical and social problem. Therefore, today the interest in research on iodine deficiency states has increased, especially in the age aspect.

It should be noted that in the available biomedical literature we have not found any work for studying the morphology of chewing muscles in iodine deficient conditions, while it is known that the functioning of masticatory muscles determines the state of the tooth-jaw system, organs of digestion and other systems of the body [20, 28, 29, 30, 32, 34, 35]. We did not find any work in which the morphometry could be used and the numerical indices of the analysis of changes in the chewing muscles in the iodine deficient states were obtained. The data on the study of the morphology of chewing muscles in various stages of ontogenesis in iodine deficient states are limited.

Summing up the above-mentioned, it can be argued that this theme is insufficiently studied and contains many con-
tradicions. This indicates the necessity for a comprehensive study during which the morphofunctional peculiarities of the masseter and lateral pterygoid muscles and their structural-metabolic transformations in conditions of the simulated pathology of iodine deficiency could be studied in animals at the stages of postnatal ontogenesis.

The objective of the study is the determination of morphofunctional peculiarities of masticatory muscles of immature and mature rats in norm and in the experimental iodine deficiency.

1. Materials and methods

The material used for the study was the masseter and lateral pterygoid muscles of the 48 white outbred male rats. Animals were divided into groups: group I – intact (n=24) animals including 12 immature and mature ones, which were kept under normal vivarium conditions, in natural food for rodents; group II – under the conditions of iodine deficiency (n=24) including 12 immature and mature animals.

The maintenance, handling, and manipulation of animals was performed in compliance with the ethical and legislative standards and requirements for carrying out of scientific and morphological studies in accordance with the provisions of the “European Convention for the Protection of Vertebrate Animals Used for Experiments and Other Scientific Purposes” (Strasbourg, 1986), Annex 4 to the “Rules for conduction of works using experimental animals”, approved by the Order of the Ministry of Health of Ukraine # 755 of 12.08.1997, the Helsinki Declaration of the World Medical Association (2000), “On Measures for the Further Improvement of the Organization of Forms of Work with the Use of Experimental Animals” and the provisions of the “General Principles of Animal Experiments”, introduced by the First National Congress on Bioethics (Kyiv, 2001); in accordance with the Law of Ukraine # 3447-IV “On the Protection of Animals from Cruel Treatment” of 21.02.2006 (Expert Opinion of the Bioethics Commission of Ivano-Frankivsk National Medical University, protocol # 104/18 dated October 25, 2018).

Euthanasia of animals was performed by intraperitoneal administration of sodium thiopental (2% solution of sodium thiopental in a dose of 25 mg/kg). The material was taken during the 60th day of iodine deficiency development.

The following research methods were used: iodine-deficient state modeling [10, 15]; injection method of the bloodstream bed of the masticatory muscles study; histological examination of blood vessels and tissue elements of the masticatory muscles (coloring with hematoxylin and eosin, histochemical method for the study of masticatory muscles (succinate dehydrogenase (SDG) according to M. Nachlas method) [2]; electron microscopic examination; biochemical composition of blood and urine study [3]; morphometric analysis (mean value of the blood vessels’ lumen and the thickness of their walls; the number of hemocapillaries in 1 µm² of the cross-section of muscular fibers; the number of hemocapillaries in one muscular fiber; percent ratio of OMG, OGMF, GMF, the average size of the muscular fiber); statistical analysis of morphometric parameters in the studied animal groups.

Variationally – statistical processing was performed taking into account individual and group variability.

The analysis of morphometric and biochemical parameters was performed using nonparametric statistics using the Mann-Whitney coefficient and Spearman correlation.

2. Results and Discussion

Investigating the angioarchitectonics of the masseter and lateral pterygoid muscles, in the immature animals, a consistent branching and anastomosing of blood vessels were determined, which form cells in the form of loops that are uniformly, naturally repeated along the course of muscular fiber (Fig. 1). From these arteries, the vessels of smaller diameter (Table 1) that penetrate the muscular bundles and are placed obliquely or along the muscular fibers, deviate at almost at a right angle.

Figure 1. The angioarchitectonics of the muscular fibers of the masseter (a) and lateral pterygoid (b) muscles of immature rats in norm. Staining: injection with a solution of a Parisian blue. Micrphotograph. Magnification: a, b: ×100.

Hemocapillaries in masticatory muscles are long and straight. An analysis of the number of hemocapillaries in 1 µm² of cross-section of muscle fiber showed that there are 1.56±0.26 hemocapillaries in the masseter muscle in 1 µm², in the lateral pterygoid – 1.68±0.36 hemocapillaries. One muscular fiber has in the masseter muscle – 1.42±0.18 hemocapillaries, in the lateral pterygoid – 1.28±0.20.

Ultramicroscopically, in immature animals the nucleus is oval-shaped with evenly spaced heterochromatin in an endothelial cells. Near the nucleus there is a granular endoplasmic reticulum consisting of tubules and cisterns, to which the ribosomes are attached; the Golgi apparatus is represented by vesicles, tubules, cis- and transcisterns. Mitochondria are of small size, with a small number of mitochondrial crests and a matrix of average electron density.

Free ribosomes, lysosomes and pinocytic vesicles are observed (Fig. 2).

During the biochemical study cholesteremia was 1.65±0.08 mmoll/l.

In mature rats the injected vessels form a specific pattern with loops placed along the muscular fibers (Fig. 3). However, during the morphometric study, a greater thickness of the arterial wall is observed in both the masseter and in the lateral pterygoid muscles in comparison with the previous
ontogenetic group. The diameter of the lumen of the vessels also increased significantly (Table 2).

Analysis of the number of hemocapillaries in 1 µm² of cross-section of muscular fiber showed an increase of this index, comparing it with immature animals. Thus, in the masseter muscle in 1 µm² there is 1.68±0.33 hemocapillaries, in the lateral pterygoid one – 1.81±0.33 hemocapillaries. A morphometric study showed that the number of hemocapillaries per muscular fiber increased in the mature rats. In the masseter muscle, the number of hemocapillaries increased to 1.58±0.32, in the lateral pterygoid, respectively, to 1.43±0.18.

Ultramicroscopically, the nuclei of endothelial cells of hemocapillaries of masticatory muscles are of the irregular shape, dense grains of heterochromatin in the nucleus are evenly distributed, seals are encountered along the periphery (Fig. 4).

A rounded nucleus is located in the center or closer to the nuclear shell. Mitochondria are small, of round or elongated shape. Each one is limited by external and internal mitochondrial membranes. The internal mitochondrial membrane forms crests. The granular endoplasmic reticulum is represented by tubules and cisterns, which the ribosomes are attached to. The Golgi complex is closer to plasmolemma and consists of tubules, tiny vesicles and dictyosomes. One can also see a small amount of f pinocytic vesicles. The basal membrane is almost equal, but one can encounter small depressions and invaginations.

In the biochemical study, cholesteremia was 1.70±0.18 mmol/l.

In the mature rats during the injection, some narrowed vessels are observed, resulting in a specific pattern changing its configuration, there is no clear filling of the injectable mass. The arterial net becomes more dense and fine-meshed. The arterial vessels have uneven contours and rectilinear motion (Fig. 7). Morphometrically, a smaller diameter of the lumen with thickening of the arterial wall is observed (Fig. 7). The morphometric study of the vascular bed showed that the arteries have a smaller diameter than the norm, while the thickness of the wall increases. The veins on the contrary increase in diameter with the simultaneous thinning of their walls (Table 3). Analysis of the number of hemocapillaries in 1 µm² of cross-section of muscular fiber showed a decrease of this index. The number of hemocapillaries in one muscular fiber is reduced (Table 4).

Ultramicroscopically, we found that there is a number of changes in endothelial cells of immature animals (Fig. 6). The nuclei of the endothelial cells become irregular, enlarged. There are single vacuoles around the nucleus. Mitochondria are enlarged in size with a rarefied matrix and decomplexing ridges. The dictyosomes of the Golgi apparatus are expanding, and vacuoles with light content are formed in them. The endoplasmic reticulum consists of expanded cisterns and tubules with a smooth surface. In the cytoplasm, multiple pinocytic vesicles appear. The basal membrane in some areas is enlarged and loose with obscure contours.

In the biochemical study, cholesterolemia was 1.37±0.07 mmol/l.

The morphometric study showed that the number of hemocapillaries per unit of area and the number of capillaries per one muscular fiber decreased (see Table 5). Ultramicroscopically in the endothelial cells there are minor changes that occur in the visual increase of the size of the nuclei and the formation of shallow invaginations of the nuclear membrane. Dense grains of chromatin in the nucleus are placed evenly, somewhat marginally. Mito-

### Table 1. Morphometric parameters (in µm) of the circulatory bed of the masticatory muscles of immature animals in norm

| Vessels                        | Immature animals                                                                 |
|-------------------------------|----------------------------------------------------------------------------------|
|                               | Masseter muscle                     | Lateral pterygoid muscle                                                                 |
|                               | Lumen                        | Wall thickness | Lumen                  | Wall thickness |
| 1. Arteries of the I order    | 120.53 ± 15.65 | 38.56 ± 6.38 | 119.13 ± 19.25 | 37.01 ± 5.77 |
| 2. Arteries of the II order   | 80.36 ± 12.19 | 25.16 ± 5.43 | 79.76 ± 19.81 | 24.18 ± 3.04 |
| 3. Arteries of the III order  | 63.16 ± 12.37 | 19.36 ± 2.53 | 62.93 ± 12.34 | 18.52 ± 3.87 |
| 4. Arteries of the IV order   | 52.83 ± 10.00 | 17.01 ± 2.51 | 51.36 ± 6.88 | 16.03 ± 2.88 |
| 5. Arterioles                 | 20.10 ± 3.82 | 8.3 ± 1.40  | 19.01 ± 4.38 | 7.08 ± 1.36  |
| 6. Precapillary vessels       | 17.63 ± 2.83 | 4.05 ± 0.72  | 16.98 ± 2.52 | 3.75 ± 0.61  |
| 7. Hemocapillaries            | 7.05 ± 1.15 | 0.53 ± 0.07  | 6.08 ± 0.90  | 0.33 ± 0.05  |
| 8. Postcapillary vessels      | 18.53 ± 2.77 | 3.53 ± 0.56  | 18.45 ± 3.46 | 3.65 ± 0.49  |
| 9. Venules                    | 36.53 ± 6.78 | 4.05 ± 0.89  | 37.52 ± 5.99 | 3.98 ± 0.79  |
| 10. Veins of endomysium       | 58.17 ± 13.89 | 7.02 ± 1.56  | 60.01 ± 8.93 | 6.15 ± 0.78  |
| 11. Veins of perimysium       | 123.63 ± 15.47 | 10.01 ± 1.83 | 124.57 ± 24.14 | 9.56 ± 1.71 |
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Figure 2. Electron microscopic structure of the wall of the hemocapillary of the masseter (a) and lateral pterygoid (b) muscles of the immature rat in the norm. Electronic Microphotography. Magnification: \( \times 8000 \).

Notes:
1 - the nucleus of the haemocapillary endothelial cell;
2 - pinocytic vesicles in the endothelial cell;
3 - basal membrane of the hemocapillary;
4 - lumen of the hemocapillary;
5 - red blood cells;
6 - the nucleus of the muscular fiber;
7 - the cross section of the muscular fiber.

Table 2. Morphometric parameters (in \( \mu \)m) of the circulatory bed of the masticatory muscles of mature animals in norm

| Vessels          | Mature animals |             |
|------------------|----------------|-------------|
|                  | Masseter muscle | Lateral pterygoid muscle |             |
|                  | Lumen | Wall thickness | Lumen | Wall thickness |
| 1. Arteries of the I order | 122.59 ± 2.87 | 39.15 ± 5.96 | 121.61 ± 23.55 | 38.03 ± 6.80 |
| 2. Arteries of the II order | 82.13 ± 14.11 | 26.45 ± 5.74 | 80.53 ± 19.52 | 25.04 ± 3.34 |
| 3. Arteries of the III order | 64.39 ± 12.01 | 20.65 ± 3.67 | 63.18 ± 14.12 | 19.18 ± 3.02 |
| 4. Arteries of the IV order | 53.36 ± 10.45 | 18.05 ± 3.61 | 51.92 ± 11.44 | 16.57 ± 2.21 |
| 5. Arterioles     | 21.95 ± 5.29 | 9.9 ± 1.23    | 19.97 ± 3.48 | 7.53 ± 1.69   |
| 6. Precapillary vessels | 18.01 ± 4.19 | 4.53 ± 1.09  | 17.13 ± 2.32 | 3.95 ± 0.74  |
| 7. Hemocapillaries | 7.98 ± 1.42 | 0.62 ± 0.09   | 6.93 ± 1.69 | 0.46 ± 0.10  |
| 8. Postcapillary vessels | 19.85 ± 3.93 | 3.61 ± 0.78  | 20.05 ± 2.50 | 3.57 ± 0.78  |
| 9. Venules        | 37.13 ± 8.77 | 4.53 ± 0.98   | 38.01 ± 8.01 | 4.18 ± 0.66  |
| 10. Veins of endomysium | 59.33 ± 10.14 | 7.98 ± 1.17  | 61.18 ± 14.72 | 6.95 ± 1.64  |
| 11. Veins of perimysium | 124.05 ± 1.46 | 10.97 ± 1.67 | 125.15 ± 28.19 | 10.05 ± 2.06 |

Chondria are slightly enlarged, the matrix is sparse. In some mitochondria, the destruction and decomplexation of crests are observed. The endoplasmic reticulum is represented by expanded tubules and cisterns, which the ribosomes are attached to. The Golgi apparatus consists of the expanded tubules, tiny vesicles and dictyosomes. One can also see a small amount of pinocytic vesicles (Fig. 8).

In this group of animals, cholesterolemia was \( 1.44 ± 0.11 \) mmol/l.

In the histological study of muscular fibers in the immature rats, the muscular fibers of the masticatory muscles have a pronounced cross-striation. The regular alternation of dark and light strips is clearly visible. The muscular fibers nuclei are bacilliform, located along the periphery in parallel to the longitudinal axis (Fig. 9a, c). On the cross-section, the muscular fibers have a round or oval shape (Fig. 9b, d).

In the study of SDG-activity, muscular fibers could be divided into three types. Oxidative muscular fibers (OMF) contain a large amount of formazan, so they are colored into a dark blue color, oxidative-glycolytic (OGMF) – with a smaller amount of formazan placed along the periphery of muscular fiber and glycolytic (GMF) contain a small amount of formazan, that’s why they look bright (Fig. 10).

In ultramicroscopic examination of the masseter and lateral pterygoid muscles, we could identify three types of muscular fibers (Fig. 11).

In mature animals, muscular fibers have a pronounced cross-striation with regular alternation of dark and light strips.
Table 3. Morphometric parameters (in µm) of the circulatory bed of the masticatory muscles of immature animals in the iodine deficiency

| Vessels                      | Immature animals                                                                 | Lateral pterygoid muscle                                                                 |
|------------------------------|---------------------------------------------------------------------------------|------------------------------------------------------------------------------------------|
|                              | Masseter muscle                                                                 | Mature                                                                                   |
|                              | Lumen                           | Wall thickness                           | Lumen                           | Wall thickness                           |
| 1. Arteries of the I order   | 119.45 ± 22.42                   | 39.23 ± 7.78                             | 118.87 ± 24.19                   | 37.17 ± 4.75                             |
| 2. Arteries of the II order  | 79.82 ± 18.08                    | 24.98 ± 3.41                             | 79.03 ± 19.86                    | 25.01 ± 4.18                             |
| 3. Arteries of the III order | 62.83 ± 12.22                    | 20.39 ± 3.42                             | 61.98 ± 8.42                    | 19.18 ± 2.86                             |
| 4. Arteries of the IV order  | 51.78 ± 8.91                     | 17.94 ± 2.75                             | 50.85 ± 11.22                   | 17.07 ± 2.34                             |
| 5. Arterioles                | 19.41 ± 2.96                     | 9.19 ± 1.49                              | 18.48 ± 3.17                    | 7.93 ± 1.18 ****                         |
| 6. Precapillary vessels      | 16.96 ± 2.88                     | 4.98 ± 0.84                              | 16.03 ± 3.36 *                  | 4.38 ± 0.90 ***                         |
| 7. Hemocapillaries           | 6.87 ± 0.89                      | 0.78 ± 0.11                              | 5.56 ± 1.01 **                  | 0.54 ± 0.10 ****                         |
| 8. Postcapillary vessels     | 18.97 ± 3.60                     | 3.89 ± 0.67                              | 20.07 ± 2.75 **                 | 4.02 ± 0.61 **                          |
| 9. Venules                   | 36.95 ± 8.07                     | 4.47 ± 0.82                              | 37.84 ± 4.77                    | 4.13 ± 0.66 *                           |
| 10. Veins of endomysium      | 58.71 ± 9.56                     | 7.49 ± 1.12                              | 60.27 ± 10.17                   | 6.38 ± 0.92                             |
| 11. Veins of perimysium      | 124.75 ± 26.31                   | 10.27 ± 1.58                             | 124.91 ± 16.42                  | 9.74 ± 1.61                             |

Notes: * – p<0.05; ** – p<0.01; *** – p<0.001; **** – p<0.0001.

Table 4. Morphometric indices of the number of hemocapillaries in 1 µm² and the number of hemocapillaries in one muscular fiber in the masticatory muscles of various ontogenetic groups with iodine deficiency

|                                | Immature muscle                          | Mature muscle                          |
|--------------------------------|------------------------------------------|----------------------------------------|
| Number of hemocapillaries in 1 µm² | 1.36 ± 0.25 ***                          | 1.57 ± 0.35 *                          |
|                                |                                          | 1.61 ± 0.21 *                          | 1.71 ± 0.31**                      |
| Number of hemocapillaries in one muscular fiber | 1.33 ± 0.24 *                           | 1.56 ± 0.33                            | 1.22 ± 0.17 *                      | 1.42 ± 0.21                           |

Notes: * – p<0.05; ** – p<0.01; *** – p<0.001; **** – p<0.0001.

Table 5. Morphometric parameters (in µm) of the circulatory bed of the masticatory muscles of mature animals with iodine deficiency

| Vessels                      | Immature animals                                                                 | Lateral pterygoid muscle                                                                 |
|------------------------------|---------------------------------------------------------------------------------|------------------------------------------------------------------------------------------|
|                              | Masseter muscle                                                                 | Mature                                                                                   |
|                              | Lumen                           | Wall thickness                           | Lumen                           | Wall thickness                           |
| 1. Arteries of the I order   | 122.02 ± 24.90                   | 39.97 ± 4.95                             | 121.61 ± 9.04                   | 38.98 ± 5.41                             |
| 2. Arteries of the II order  | 81.98 ± 16.44                    | 26.97 ± 6.51                             | 79.97 ± 18.59                   | 25.95 ± 5.01                             |
| 3. Arteries of the III order | 63.93 ± 9.20                     | 20.86 ± 4.37                             | 63.93 ± 14.77                   | 19.75 ± 3.91                             |
| 4. Arteries of the IV order  | 53.06 ± 8.36                     | 18.64 ± 3.71                             | 51.05 ± 10.33                   | 16.97 ± 3.32                             |
| 5. Arterioles                | 21.24 ± 3.05                     | 10.06 ± 1.56                             | 19.86 ± 3.54                    | 7.94 ± 1.33                              |
| 6. Precapillary vessels      | 17.87 ± 2.86                     | 4.88 ± 0.81                              | 16.91 ± 2.26                    | 4.31 ± 0.59                              |
| 7. Hemocapillaries           | 7.47 ± 1.06 *                    | 0.69 ± 0.13 ***                          | 6.25 ± 0.87 *                   | 0.54 ± 0.08 ****                         |
| 8. Postcapillary vessels     | 20.03 ± 4.01                     | 3.87 ± 0.70 **                           | 20.31 ± 3.49                    | 3.91 ± 0.48 **                           |
| 9. Venules                   | 37.31 ± 8.60                     | 4.65 ± 0.95                              | 39.23 ± 9.14                    | 4.21 ± 0.74                              |
| 10. Veins of endomysium      | 59.41 ± 10.35                    | 8.02 ± 1.53                              | 61.23 ± 12.23                   | 7.01 ± 1.03                              |
| 11. Veins of perimysium      | 124.21 ± 5.90                    | 11.04 ± 2.49                             | 125.27 ± 7.29                   | 10.11 ± 1.93                             |

Notes: * – p<0.05; ** – p<0.01; *** – p<0.001.

(Fig. 12). In mature animals, it is also possible to see different types of them both in the masseter (Fig. 13a) and in the lateral pterygoid muscles (Fig. 13b), but the area of their cross-section, as compared with the previous age group, increases.
The percentage difference between different groups of fibers also varies (Table 6).

In submicroscopic studies in mature animals, muscular fibers have a definitive type of structure.

During the examination of muscular fibers in iodine deficiency in immature rats, ioduria increased up to 2.76±0.29 µg/l (p<0.01), compared with normal. Histologically there is an irregular alternation of dark and light strips. The nuclei of muscular fibers are slightly enlarged, they become rounded and lighter (Fig. 14). Endomysium and perimysium are thickened, losing structural organization (Fig. 15).

The cross-sectional area of muscular fibers in the immature animals in experimental iodine deficiency is changing. There is a significant increase of the cross-sectional area of all three types of fibers (Table 7).

In the study of SDG-activity of muscular fibers, we observed a change in the distribution of different types of fibers (Fig. 16).

Ultrastructurally in iodine deficiency a focal disturbance of clear cross-striation of myofibrils and edematous changes, that are especially observed in GMF, were revealed (Fig. 17).

In mature animals with iodine deficiency, ioduria increased up to 3.76±0.36 µg/l (p<0.01) compared to normal.

Muscular fibers have a pronounced cross-striation, but there are the areas with its violation (Fig. 18). There is a slight increase of the cross-sectional area of all types of fibers in comparison with the norm (see Table 7). During the study of SDG-activity, we found a slight decrease of the amount of OGMF and some increase of GMF compared with the norm (Fig. 19, see Table 7).

The sub-microscopic examination confirms the results of the light-optical one (Fig. 20).

In mature animals with experimental iodine deficiency in the GMF, we detected a slight disturbance of the cross-striation of myofibrils, some blurriness and smoothness of the Z-lines, extension and clarification of muscular triads. There are well-defined edematous changes in some muscular fibers.

Thus, in conditions of iodine deficiency, we observed the changes in the diameter of the lumen of all parts of the arterial and venous bed: narrowing of the arterial and venous vessels expansion. It is also possible to note thickening of the arterial wall due to the edema of all its membranes. These changes are more pronounced in the vessels of masticatory muscles of immature animals, which is confirmed by morphometric studies. Ultramicoscopic changes of endothelial cells are also manifested by edematous phenomena. The results of our research could be explained by the influence of thyroid hormones deficiency, which leads to accumulation of glycoproteins in the tissues, which in turn leads to the development of mucosal edema [22]. The inadequacy of thyroid
Table 6. Relative content (%) and cross-sectional area ($\mu m^2$) of OMF, OGMF, GMF in masticatory muscles of different ontogenetic groups in norm

|                     | Amount (%) | Cross-sectional area ($\mu m^2$) |
|---------------------|------------|----------------------------------|
|                     | Immature   | Mature                           |
| Masseter muscle     | Lateral pterygoid muscle | Masseter muscle     | Lateral pterygoid muscle |
| OMF                 | 17.01      | 16.56                            |
|                     | 21.32      | 20.35                            |
|                     | 30.31 ± 3.83 | 32.03 ± 4.54                    |
|                     | 28.72 ± 3.01 | 29.91 ± 3.33                    |
| OGMF                | 72.32      | 66.17                            |
|                     | 69.83      | 64.02                            |
|                     | 36.15 ± 5.06 | 37.18 ± 5.17                    |
|                     | 28.72 ± 3.01 | 36.83 ± 3.97                    |
| GMF                 | 10.67      | 17.27                            |
|                     | 8.85       | 15.63                            |
|                     | 39.17 ± 3.99 | 41.97 ± 4.33                    |
|                     | 38.56 ± 4.50 | 41.71 ± 4.76                    |

Table 7. Relative content (%) and cross-sectional area ($\mu m^2$) of OMF, OGMF, GMF in masticatory muscles of various ontogenetic groups in iodine deficiency

|                     | Amount (%) | Cross-sectional area ($\mu m^2$) |
|---------------------|------------|----------------------------------|
|                     | Immature   | Mature                           |
| Masseter muscle     | Lateral pterygoid muscle | Masseter muscle     | Lateral pterygoid muscle |
| OMF                 | 16.04      | 16.28                            |
|                     | 20.57      | 20.35                            |
|                     | 30.56 ± 3.64 | 32.56 ± 4.74                    |
|                     | 28.93 ± 3.29 | 30.44 ± 4.73                    |
| OGMF                | 62.64      | 66.21                            |
|                     | 65.57      | 61.97                            |
|                     | 36.21 ± 4.21 | 37.19 ± 4.81                    |
|                     | 35.61 ± 3.84 | 36.89 ± 3.86                    |
| GMF                 | 21.32      | 17.51                            |
|                     | 13.86      | 17.79                            |
|                     | 39.21 ± 4.61 | 42.31 ± 6.20                    |
|                     | 38.62 ± 4.76 | 41.86 ± 4.77                    |

Figure 5. Changes in the angioarchitectonics of the endomysium vessels of the masseter (a, b) and lateral pterygoid (c, d) muscles of immature rats under the conditions of an iodine-deficient diet. Staining: a, c – injection with Parisian blue with additional coloring of hematoxylin and eosin; b, d – injection with Parisian blue. Microphotograph. Magnification: a, c – ×200, b, d – ×100.

The performed morpho-functional research is the theoretical basis for the development and pathogenetic substantiation of measures aimed at the correction and prevention of the development of iodine deficiency disorders, which, in turn, could lead to the prevention and reduction of the level of morbidity, its complications, disability and mortality caused by them.
Figure 6. Ultramicroscopic structure of hemocapillary of masseter (a) and lateral pterygoid (b) muscles of immature rat in iodine deficiency. Electronic Microphotography. Magnification: a, b – ×8000.

Notes:
1 - erythrocyte;
2 - lumen of the hemocapillary;
3 - nucleus of the endothelial cell of hemocapillary;
4 - dilated and loose basal membrane;
5 - extended perivascular space;
6 - invagination of the nuclear shell of the muscular fiber of nucleus;
7 - microclasmatosis;
8 - muscular fiber;
9 - nucleus with marginally located heterochromatin in the muscular fiber.

Figure 7. Uneven distribution of endomysium vessels of the masseter (a) and lateral pterygoid (b) muscles of mature rats under conditions of an iodine-deficient diet. Staining: injection with Parisian blue. Photomicrograph. Magnification: a, b – ×100.

4. Conclusions
Under the conditions of iodine deficiency, vascular-stromal-muscular disorders develop in the masticatory muscles of animals of different age groups, with the predominance of pathological changes in immature rats.

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Figure 8. Ultramicroscopic changes of the hemocapillary of the masseter (a) and lateral pterygoid (b) muscles of the mature rat in the iodine deficiency state. Electronic Microphotography. Magnification: a, b – ×4800.

Notes:
1 - erythrocyte;
2 - sludge of erythrocytes;
3 - lumen of the hemocapillary;
4 - lymphocyte;
5 - nucleus of the endothelial cell of haemocapillary;
6 - loose basal membrane;
7 - extended perivasal space;
8 - microclasmatoysis;
9 - muscular fibers;
10 - disorganized mitochondria in muscular fibers.

Figure 9. The bundles of muscle fibers of the masseter (a, b) and lateral pterygoid (c, d) muscles of immature rats in norm. Staining: hematoxylin and eosin. Microphotograph. Magnification: a, c – ×400; b, d – ×200.

Notes:
1 - cross-striation of muscular fiber;
2 - nuclei of muscular fibers;
3 - endomysium;
4 - perimysium;
5 - muscular fibers.

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**Figure 10.** Identification of different types of muscular fibers of the masseter (a) and lateral pterygoid (b) muscles. Staining: succinate dehydrogenase according to M. Nachlas. Microphotograph. Magnification: a, b – ×300.

*Notes:*
1 - OMF;
2 - OGMF;
3 - GMF.

**Figure 11.** Different types of muscular fibers in the masseter (a, b, c) and lateral pterygoid muscles (d, e, f) of immature rats in norm. Electronic Microphotography. Magnification: a – ×1500; b, c, d – ×6400; e – ×4000; f – ×8000.

*Notes:*
1 - OMF;
2 - OGMF;
3 - GMF.

**Figure 12.** The light-optical organization of the masseter (a, b) and lateral pterygoid (c, d) muscles of the mature rats is norm. Staining: hematoxylin and eosin. Microphotograph. Magnification: ×200.

*Notes:*
1 - cross-striation of muscular fiber;
2 - nuclei of muscular fibers;
3 - blood vessel;
4 - endomysium;
5 - perimysium.
Figure 13. Histological structure of different types of muscular fibers of the masseter (a) and lateral pterygoid (b) muscles of the mature rats in norm. Staining: succinate dehydrogenase according to M. Nachlas. Microphotograph. Magnification: a, b – ×400.

Notes:
1 - OMF;
2 - OGMF;
3 - GMF.

Figure 14. Histostructure of muscular fibers of masseter (a) and lateral pterygoid (b) muscles of immature rats under conditions of iodine deficiency diet. Staining: hematoxylin and eosin. Microphotograph. Magnification: ×400.

Notes:
1 - edema of muscular fibers;
2 - enlarged light nuclei of muscular fibers;
3 - full blood vessel;
4 - focal loss of cross-striation.

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Figure 15. Expansion and violation of the structure of the intermuscular layers of the masseter (a) and lateral pterygoid (b) muscles of immature animals in iodine deficiency. Staining: trichrome staining according to Masson. Microphotograph. Magnification: ×200.

Notes:
1 - collagenous fibers and extension of endomysium;
2 - sarcoplasm of muscular fiber.

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Figure 16. Types of muscular fibers in the masseter (a) and lateral pterygoid (b) muscles of immature animals under the conditions of an iodine-deficient diet. Staining: succinate dehydrogenase according to M. Nachlas. Microphotograph. Magnification: a, b – ×200.

Notes:
1 - OMF;
2 - OGMF;
3 - GMF.

Figure 17. Ultramicroscopic changes in the structure of the masseter (a) and lateral pterygoid (b) muscles of immature rat in iodine deficiency. Electronic Microphotography. Magnification: a – ×4800; b – ×8000.

Notes:
1 - focal loss of cross-striation in the muscular fiber;
2 - nucleus with marginally located heterochromatin;
3 - destroyed mitochondria;
4 - enlightened endomysium;
5 - vacuolization of muscular fibers.

Figure 18. Changes in the muscular fibers of the masseter (a) and lateral pterygoid (b) muscles of the mature rats under the conditions of iodine deficiency. Staining: hematoxylin and eosin. Microphotograph. Magnification: ×400.

Notes:
1 - edema of muscular fibers;
2 - enlarged light nuclei of muscular fibers;
3 - focal loss of cross-striation.
Figure 19. Types of muscular fibers in the masseter (a) and lateral pterygoid (b) muscles of the mature rats under the conditions of iodine deficiency. Staining: succinate dehydrogenase according to M. Nachlas. Microphotograph. Magnification: a, b – ×200.

Notes:
1 - OMF;
2 - OGMF;
3 - GMF.
Figure 20. Ultramicroscopic changes in the structure of muscular fibers of the masseter (a) and lateral pterygoid (b) muscles of the mature rat in iodine deficiency. Electronic Microphotography. Magnification: a – ×8000; b – ×6400.

Notes:
1 - enlargement and blurring of the Z-line in the muscular fiber;
2 - invaginations of the nuclear membrane;
3 - expanded and loose basal membrane of muscular fiber;
4 - vacuoles;
5 - expanded and vacuolized endomysium;
6 - hemocapillary;
7 - endothelial cell nucleus.