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Original Article

Energy properties of skeletal muscles in human fetuses as a function of gender, age and constitutional type

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Abstract:
Constitutional approach has significant practical meaning for human health assessment, developing individual recommendations for optimal adaptation to environment, prognosis and treatment of pathological conditions.The aim of the research is to determine qualitative and quantitative indicators of glycogen, creatinine, total lipids and proteins; to establish interconnection of energy features in skeletal muscles depending on age, gender and morphological criteria of constitutional type in human fetuses.To establish types of constitutional structure in fetuses and to clarify energetic features of striated skeletal muscles, we have used complex of anthropometric, biochemical and micro-macroscopic methods.It has been found that at the beginning of the 5th month of prenatal development in females, amount of glycogen is significantly higher than in males, with a reverse trend with lipids. There is an increase in levels of creatinine and proteins; critical periods occur on the 8th and 9th month. Indicators of lipids in skeletal tissue are critical during 7th month, after which there is a significant decline until birth. On the contrary, glycogen begins to grow with each passing month in both sexes. There exists an interdependence of energy properties with body constitution. Objects with normostenic type of body structure are characterized by the lowest content of creatinine and the average values of other energy substrates. Hyperstenics are characterized by the lowest levels of glycogen and the largest indexes of lipids. The reverse trend is observed in asthenics: level of glycogen is the highest, and lipids levels are the smallest. The age dynamics of protein content is proportional to age; in particular - increases with each passing month. In general, protein content in males is higher than that one in females in all constitutional types. Obtained data can serve for health assessment, can be used in clinical medicine, in particular in obstetrics during childbirth.
Key words: prenatal human development, skeletal muscles, humanfetuses, constitutional type.

Introduction

Constitutional approach has significant practical meaning for human health assessment, developing individual recommendations for optimal adaptation to environment, prognosis and treatment of pathological conditions (13,15). Despite the fact that constitutional type is a combination of morphological, physiological and psychological characteristics of an individual that are inherited and remain constant throughout life, most clinical studies are based specifically on morphological constitution criteria in postnatal period of human ontogenesis (9, 14). However, theories about particular qualities of energy supply during prenatal human development are still fairly limited.

A special role in formation of skeletal muscles' energy peculiarities plays a constitutional type, which is incorporated into genetic apparatus before birth. It was found that after 20 weeks of prenatal development all muscle fibers are homogeneous and don't take part in any energy activity. First differences happen appear at the end of 22nd week of prenatal development and relate only to activity of myosin ATPase. Differentiation processes in muscle fibers begin at 28th week of prenatal development (most intense in muscles of upper extremities and diaphragm), later – in quadriceps and soleus muscles (8, 10). Classification byShevkunenko and Heselevych (1926) is wide-spread and identifies types of constitution depending on anatomical features, and precisely, correlations of different body parts dimensions (1, 6, 7). In dolihomorph type predominate longitudinal body sizes, narrow shoulders, long limbs, short trunk and above-average height (according to asthenic type by Chernorudskyi). Brachiomorph type is characterized by predominance of transverse body sizes, short neck, long

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body, broad chest (corresponding to hypersthenic type); mesomorph type is an intermediate type between dolihomorph and brahiomorph types.

Classical morphology usually cannot serve as a basis for valuation determinant areas of science development on typological features in human. Therefore, for predicting individual paths of ontogenetic development, morphology knowledge should be combined with knowledge of skeletal muscles' energy supply processes (5, 9, 11).

Materials and methods

The aim of the research is to determine qualitative and quantitative indicators of glycogen, creatinine, total lipids and proteins; to establish interconnection of energy features in skeletal muscles depending on age, gender and morphological criteria of constitutional type in human fetuses.

Research has been conducted on muscle homogenate of 91 humans'fetuses' corpses. We have determined quantity and quality indexes of glycogen, creatinine, total lipids and proteins. Material for study was obtained from Chernivtsi Regional Pathologists Office. Research was performed in compliance with fundamental bioethical regulations by Council of Europe Convention on Human Rights and Biomedicine (from 04.04.1997), of the World Medical Association Declaration of Helsinki Ethical Principles for Medical Research Involving Human Subjects (1964-2008) And MOH Ukraine № 690 from 23.09.2009. Commission on Biomedical Ethics of Bukovynian State Medical University (protocol 4 from 01.19.2014) hasn't found any violations of ethical standards at carrying out this research work.Periods of fetal development were systematized by G.A. Schmidt (1955) classification, considering "Instruction on criteria definition of prenatal period, neonate and still born", approved by the Ministry of Health of Ukraine №179 from March 29, 2006.

To establish types of constitutional structure in fetuses and to clarify energetic features of striated skeletal muscles, we have used complex of anthropometric, biochemical and micro-macroscopic methods.

As shown in Figure 1, age structure of studied objects was determined by B. M. Petten tables (12) A.G. Knorre, B.P. Hvatova and Yu.N. Shapovalov (10, 13). This tables use tape to determine parietal-coccygeal length (PCL), parietal-heel length (PHL) and transverse dimensions (transverse breast size; the distance between anterior superior iliac spines) by using calipers.Quantitative and age structure of research objects is presented in Table 1.

According to received measurements, coefficients of constitutional type K have been calculated (V.N. Shevkunenko et al., 1935; T.F. Lavrov, 1979) (9).

K1 = intercostal distance / interstitial distance x 100; *(1)*

Table 1. Age and quantitative compound of research objects

Age of fetuse				Research methods		
weeks	months	PCL, mm	PHL, mm	Anthropometric	Biochemical	Total objects
13-16	4	85,0-130,0	160,0-200,0	12	12	12
17-20	5	135,0-185,0	210,0-250,0	14	14	14
21-24	6	190,0-230,0	255,0-300,0	18	18	18
25-28	7	240,0-270,0	310,0-350,0	12	12	12
29-32	8	280,0-310,0	360,0-400,0	11	11	11
33-36	9	320,0-340,0	410,0-450,0	12	12	12
37-40	10	350,0-370,0	460,0-500,0	12	12	12
Total:						91

Under control of magnifying optics (2x-3x), the front part of hip was drawn to muscles. Part of quadriceps was removed for further biochemical investigation. A homogenate was prepared from this part of muscle for further quantitative and qualitative determination of creatinine (3, 4), glycogen (5), total lipids (10) and proteins (20, 21). The research has been conducted on the basis of Department of Bioorganic and Biological Chemistry and Clinical Biochemistry of Bukovinian State Medical University (Chernivtsy, Ukraine).To determine concentration of glycogen in studied material we used method by E.O. Danchenko and A.A. Chirkin (5). This technique is conducted by staining glycogen molecules in tissue by iodine without changing its structure.

Calculation of glycogen concentration was carried out by formula [2]:

C=(E)/F*10*k (mg/gr), [2]

where C – glycogen concentration; E – optical density of obtained material; F – factor by which the tangent's angle of inclination of gradation is calculated; 10 – coefficient for conversion in milligrams per 1,0 g of homogenate; k – coefficient of solution sample.

Normally the amount of glycogen in skeletal muscles in adults' ranges from 3 to 20 mg/g (300-2000 mg%, 0,45-0,70 g/kg) (5).

Concentration of creatinine was determined by using a set of "Filisit-diagnostics" reagents. The principle of this method is that the picric acid interacts in alkaline medium with creatinine and forms a red product. The intensity of color in resulting homogenate solution is proportional to creatinine level in the sample.

Calculation of creatinine concentration in homogenate was carried out using the following formula [3]:

$$C = C(cal) \times \frac{E\,(exp)}{E\,(cal)}$$ μmol/l (mg%), [3]

where: C – creatinine concentration in a homogenate; C (cal) – calibrating concentration of creatinine; E (exp) – optical density of experimental test; E (cal) – optical density of calibrating test.

Determination of common lipids was carried out by color reaction with sulfophosphovanillin reagent (4). The principle of this method is that products of decomposition of unsaturated lipids give a color reaction with reagent, which consists of sulfuric, orthophosphoric acids and vanillin. Content of total lipids was calculated by formula [4]:

$$C = E\,(\exp) \times \frac{C(st)}{E(st)},$$ [4]

where: E(exp) – extinction of the experimental sample; E (st) – extinction of the standard sample; C – concentration of a standard solution, mg/100 ml.

Qualitative and quantitative protein content was calculated by the Lowry method (11). The method is based on receiving colored products of aromatic aminoacids with a Folin-Chekalteu reagent in conjunction with a biuret reaction on peptide bonds.

Statistical processing of results was carried out using computer with mathematical apparatus software with spreadsheets "StatPlus 2005 Professional 3.5.3" (AnalystSoft). To analyze obtained data, commonly accepted methods of descriptive statistics and correlation analysis have been used.

When processing results that fell under normal distribution, methods of the statistical variation have been used, with deduction of average value and average square error of arithmetic mean. The probability of obtained results were determined by using a Student's reliability criteria (t). For assertion the likelihood of discharges, have been taken generally accepted in medical biology the magnitude of probability level – $p < 0,05$.

Results

Gender and age dynamics of creatinine changes. Starting from the 5th month of prenatal ontogenesis is observed the first increase in creatinine level in skeletal muscle of both male and female fetuses, due to first fetal movements. In female fetuses creatinine values in muscle tissue are 4,15 μmol/l, which is 2% less than that in male fetuses (4,16 μmol/l). In 6-7-month-old fetuses content of creatinine increases only in males – up to 4,29 μmol/l, which is approximately an increase of 3%. At the end of third trimester (8-9th month of prenatal development), creatinine values are the highest (4,368 μmol/l), therefore this developmental period can be considered as the critical one. It can be assumed that quantitative and qualitative content of creatinine depends on endogenous factors, as well as on structural and functional rearrangement of energy supply mechanisms, which somewhat vary (depending on the gender of a fetus) which is shown in Figure 1.

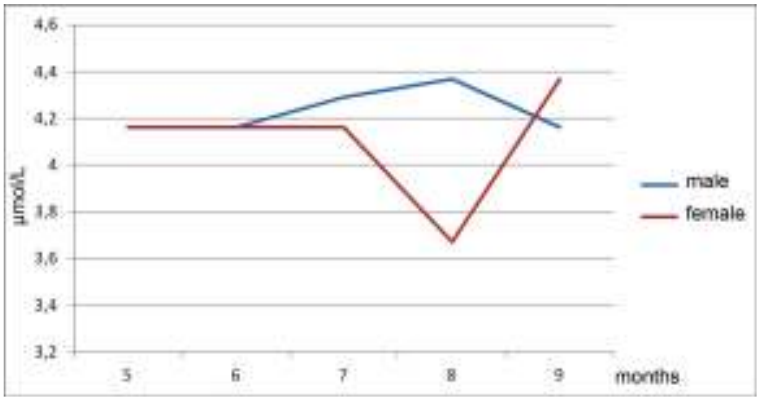


Fig. 1. Gender and age dynamics of creatinine levels.

Gender and age dynamics of glycogen changes. Studies have been conducted on fetuses of 6th – 9th months of prenatal development. It should be noted that glycogen levels in female fetuses were1,35 μmol/l, which is almost as twice high as in males - 0,75 μmol/l. During 7th month of development is observed a

significant increase in content of glycogen in female fetuses – 2,73 μmol/l (51%), and an increase in male fetuses up to 0,97 μmol/l (31%). Fetuses of 8th month of prenatal development are characterized by a slight increase of glycogen level in muscles, in particularly in females – up to 2,96 μmol, and in males up to 1,18 μmol. As shown in Figure 2, in 8-9-month-old fetuses the amount of glycogen in skeletal muscle increases and liver begins to function fully. A distinctive feature of this period is a significant increase of glycogen level in males – up to 2,88 μmol, which is almost three times higher than at the beginning of third trimester. In females this increase is negligible (up to 3,04 μmol).

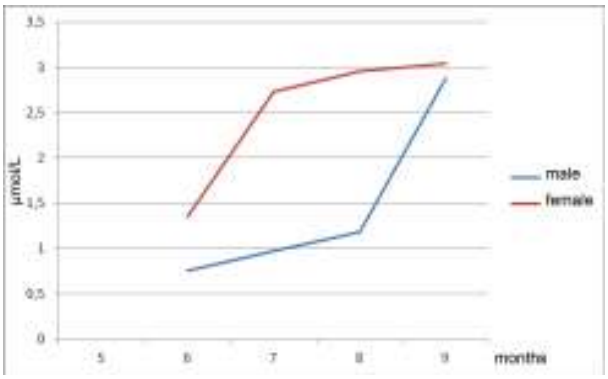


Fig. 2. Gender and age dynamics of glycogen changes.

Gender and age dynamics of proteins changes. During the study of protein content in skeletal tissue in fetuses during beginning of the 5th month of prenatal development, its average rate in male fetuses was 13,6 μmol/l, and in females less – 12,5 μmol/l. Subsequently, at the 6th month, males' rate has increased by 84% - up to 25 μmol/l, and in female fetuses this figure has increased by 120% and reaches 27,5 μmol/l. At the 7th month of development, synthesis of protein in males increases in contrast to females, which may be due to uneven development of synthetic apparatus in muscle fibers - ribosomes and Golgi complex in particular. In male fetuses was found that the amount of protein has increased by almost 100% and (38,5 μmol/l), while in female fetuses a gain of 52% is determined (34 μmol/l). According to our data and as is shown in Figure 3, 8th month of prenatal development is critical in both sexes, as the index of protein in skeletal tissue during this period sharply decreases until the beginning of 9th month of prenatal development. During the 8th month, total protein is increased by 42% (44,3 μmol/l) in male fetuses. Closer to the 9th month, the index is reduced by 27% (40,5 μmol/l). The 8th month is characterized by a significant increase in protein levels (by 142%) compared to the previous month (52,13 μmol/l).

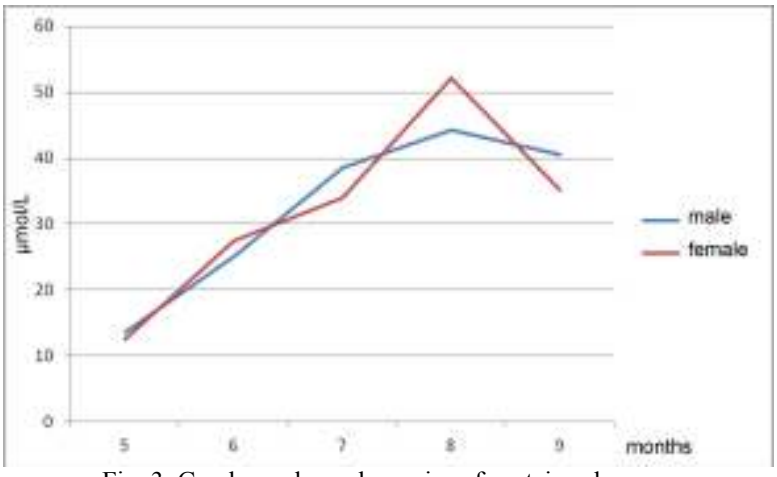


Fig. 3. Gender and age dynamics of proteins changes.

Gender and age dynamics of lipid changes.Results of our research show, that in female fetuses the amount of lipids at the beginning of the 6th month of prenatal ontogenesis reaches 83 μmol/l. By the middle of the 7th month, lipid content in skeletal muscle has increased by 7,5 times, compared to the previous month (602 μmol/l), which can be considered a critical period of development.At the 8th month of prenatal development, lipid content is the lowest – 48 μmol/l. Later, by the end of the third trimester, lipid index becomes stabilized and is 217 μmol/l. In male fetuses this indexes are somewhat different - 155 μmol/l at the beginning of the 6th month. Further, its content is almost tripled and by the middle of the 7th month it reaches 442 μmol/l, which is a critical

period for male fetuses. The last trimester is characterized by a decrease: at the 8th month - up to 362 μmol/l, on the 9th month – up to 130 μmol/l.

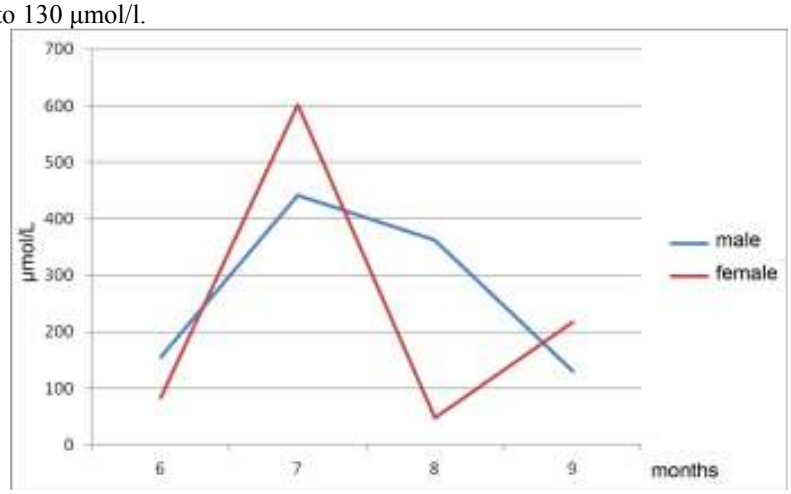
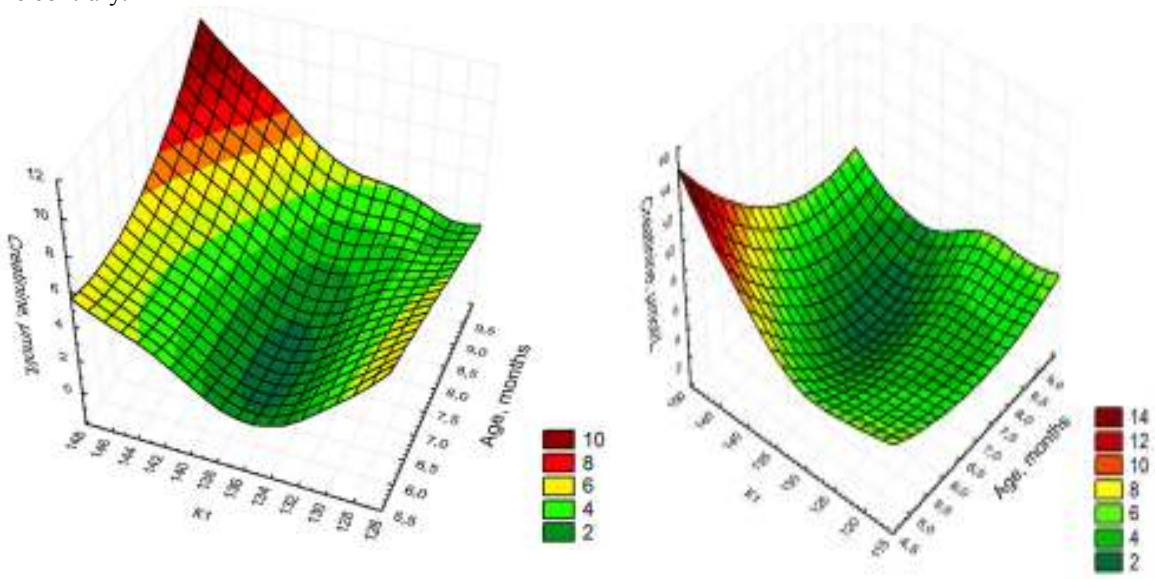


Fig. 4. Gender and age dynamics of lipid changes.

Discussion

Analysis of interdependence dynamics of energy properties, depending on age and constitutional type coefficient.It has been established that in both sexes, the lowest creatinine values were found in objects with average values of constitutional type coefficient (K) (normostenic type of body structure). We have detected the dependence of creatinine level on gender of a fetus at the edges of variability range of constitutional type coefficient, as shown in Figure5. Thus, in male fetuses, the level of creatinine is lower on edges of anatomical variability (the highest and the lowest K values). In females with maximum K indexes, level of creatinine is higher than in males. Moreover, in female fetuses with a decrease of K level, the level of creatinine is practically not reduced with age. Whereas in men with a decrease in K, the level of creatinine increases, but with age almost doesn't change. So, if males with the highest K index creatinine increases with age, but in females' decreases, on the contrary.



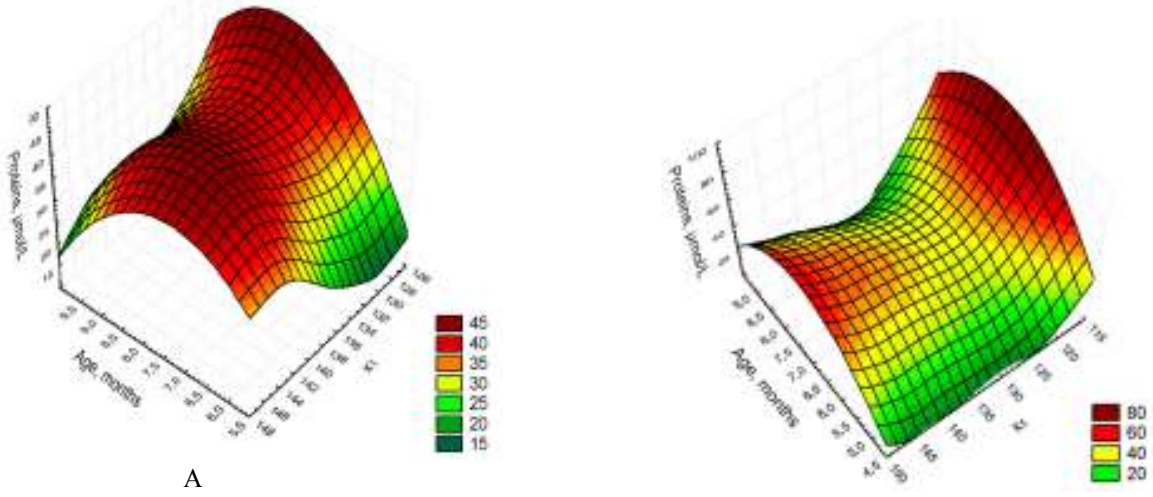
A

B

Fig. 5. Level of creatinine depending on age and constitutional type. A – in male, B – in female.

The analysis of dynamics of protein content, age and constitutional type interconnection in fetuses of both sexes, has shown that with age protein concentration increases, but starting from the 9th months of development the growth rate slows down and tends to decrease before birth, as shown in Figure 6. It should be noted that the highest rates of protein were found in individuals with the lowest constitutional-type ratios in both sexes. The lowest rates of protein concentration growth in muscles are observed in fetuses with an average type of constitution. At the beginning of fetal period, there is practically no dependence of proteins level on the constitutional type in female fetuses. In the middle of fetal period, the greatest correlation was found between

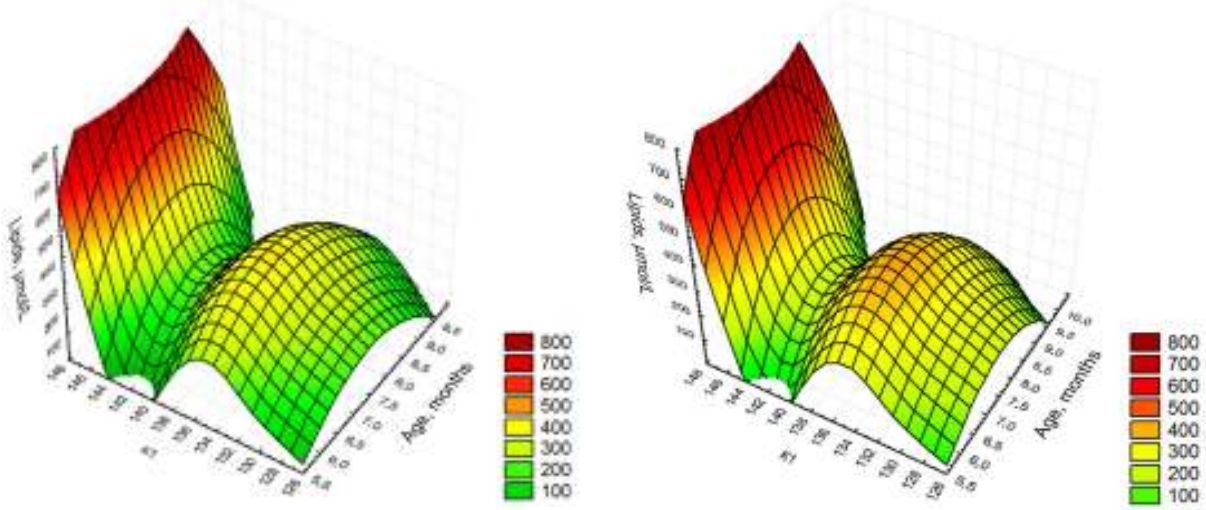
age, protein level and constitutional type, which we can explain by intensive growth processes and structure-functional alterations of the organism during this period.



A B

Fig. 6. Level of protein depending on age and constitutional type. A – in males, B – in females.

It has been established that in both sexes the highest lipid content was observed in objects with the highest constitutional type – as seen in Figure 7; vice versa, the level of lipids has decreased with decreasing K index. In females, the content of lipids is slightly lower than that of men. In female fetuses with the lowest K index, level of lipids almost does not change during the prenatal period of ontogenesis. In men with normosthenic type of body structure (average values of K), content of lipids is higher than that in women with the same coefficients of constitutional type; its level increases in dynamics during fetal period. A similar trend has been observed in objects with the highest rates for both genders. In fetuses with high K index, lipid values are significantly higher than those with smaller K.



A B

Fig. 7. Level of lipids depending on age and constitutional type. A – in males, B – in females.

Probable differences in ratio of creatinine content to peculiarities of constitutional structure and age in male and female fetuses were not detected. It has been established that with age, creatinine values increase in fetuses of both sexes, regardless of type of constitutional structure, but its growth rate varies depending on K index, that is seen in Figure 8. The slowest growth rates are observed in objects with average values of K, and these changes are particularly noticeable at the end of fetal period. The greatest dependence is found in fetuses with the lowest values of K, and in comparison with other types of body structure, the content of glycogen is much higher, especially at the end of the prenatal period. There is a critical period in this constitutional type and

the level of glycogen continues increasing until birth. In the upper K, glycogen levels are highest in the second trimester of pregnancy, and growth rates are negligible compared with normostenics.

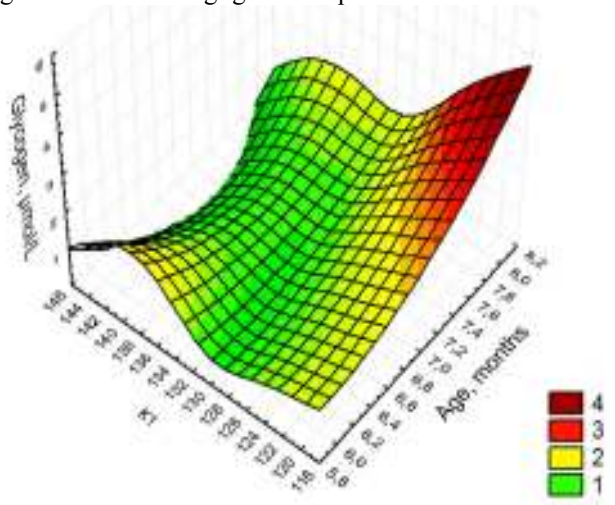


Fig. 8. Level of glycogen depending on age, gender and constitutional type.

Biochemical parameters of skeletal muscle tissue in normosthenics, asthenics and hypersthenics. It has been established that fetuses of both sexes with normostenic constitutional type have the lowest level of creatinine, in comparison with other constitutional types. The age dynamics of protein content is relatively proportional to age - it increases with each month of maturation. In normosthenics (in comparison with asthenics) the level of glycogen is slightly lower, but there remains a tendency to a slight increase. It can be assumed that in objects with this constitutional type, the ratio of muscle fibers changes, in particular in type I and type II (as well as in those with asthenic type). The analysis of age-related changes in quantitative indices of lipids in skeletal muscle tissue has shown that normostenics have mean values of triacylglycerols in comparison with other constitutional types, and slightly increase during fetal period. According to indices of energy substrates in fetuses of normostenic type, a structural reorganization of fiber composition in skeletal muscles takes place.

Fetuses of asthenic constitution type are characterized by high levels of glycogen, which gradually increases throughout the fetal period, which may be due to reduction of red oxidizing fibers (type I) and increase of white glycolytic fibers (types IIa and IIb). Indicators of proteins increase by the end of prenatal development, which shows structural and functional changes in muscles. The level of creatinine in asthenics is higher than that of normostenics, but lower than in hyperstenics. In males, creatinine is slightly higher than that of females, which is usually considered as a norm.

From results of our studies, it follows that hyperstenics have the highest energy substrate rates among all types, except for glycogen parameters. In objects with this constitutional type there are reversible age trends, compared with asthenics. It is assumed that in asthenic constitutional type muscle fibers are differentiating from slow-type I into the fast type II. In fetuses with hyperstenic type, lipid and glycogen content have to inverse changes and type II fibers are transformed into type I fibers. The activity of protein synthesis is somewhat different, especially in males of 7th month of prenatal development; their content is much higher than in other types, which may which may be explained by intensive rearrangement processes in skeletal muscles.

Analyzing age dynamics of skeletal muscle development in prenatal ontogenesis, it should be noted that during fetal period, all muscles are characterized by the same tendency: advantage of undifferentiated fibers and their replacement during second and third trimester of pregnancy. The data obtained from the research indicates that energy properties of skeletal muscles differ significantly in different constitutional types. Our results partially coincide with results of R.V. Tambovtseva(14, 15). By studying the flabby muscle, we have become convinced of the non-standard development of this structure. This muscle in adults consists mainly of fibers of the same type. It is commonly believed to be slow-type fiber type I. Studying peculiarities of age development of this muscle's fibers makes it doubtful whether these allegations are correct. In analysis of biochemical results, a wave of energy changes has been observed. In particular, the ratio of glycogen level, proteins, lipids and creatinine. Internal mechanism of these rearrangements requires further integrated study.

Conclusions

1. It has been found that at the beginning of the 5th month of prenatal development in females, amount of glycogen is significantly higher than in males, with a reverse trend with lipids. During development, there is an increase in levels of creatinine and proteins; critical periods occur on the 8th and 9th month. Indicators of lipids in skeletal tissue are critical during 7th month, after which there is a significant decline until birth. On the contrary, glycogen begins to grow with each passing month in both sexes. 2. According to the results of our

studies, there exists an interdependence of energy properties with body constitution. In particular, objects with normostenic type of body structure are characterized by the lowest content of creatinine and the average values of other energy substrates. Hyperstenics are characterized by the lowest levels of glycogen and the largest indexes of lipids. The reverse trend is observed in asthenics: level of glycogen is the highest, and lipids levels are the smallest. The age dynamics of protein content is proportional to age; in particular - increases with each passing month. In general, protein content in males is higher than that one in females in all constitutional types.
3. Obtained data can serve for health assessment, can be used in clinical medicine, in particular in obstetrics during childbirth. Forecasting and treating pathological conditions has a great practical importance and relevance, but this direction needs further profound research.

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