EVALUATION OF MICROCIRCULATION IN CHILDREN OF 8 AND 10 YEARS OF AGE USING INSPIRATORY BREATH HOLD

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Gas diffusion and transcapillary exchange take place in the microvasculature. Therefore, the evaluation of skin blood flow regulation and functional capacities of the microcirculatory system at various ontogenesis stages is of great importance. Using laser Doppler flowmetry in the group of boys \( (n = 15) \) and girls \( (n = 13) \) of 8 and 10 years of age, skin microcirculation and its regulatory mechanisms were evaluated. The study found an increase in the perfusion index in children between the age of 8 and 10 induced by the shifting roles of mechanisms of the microcirculatory regulation. The comparison of basal microcirculatory parameters did not display statistically significant differences related to sex in 8- and 10-year old participants. However, almost equal perfusion in boys and girls was maintained by different contributions of regulatory mechanisms. The breath holding test showed an increase in the initial microcirculation index and capillary blood flow reserve in the group of 10-year-old boys and girls. Our study revealed differences in various microcirculation parameters, in the intensity of active and passive rhythms of blood flow oscillations and response to inspiratory breath hold, which indicates age-related transformations of microcirculation system.

Keywords: laser Doppler flowmetry, microcirculatory regulation, inspiratory breath hold, capillary flow reserve

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According to present-day concepts, all functions of the organism undergo changes when the organism interacts with the environment. Therefore, the organism’s adaptability at various ages is determined by the morphological maturity of physiological systems and by how adequately the environment matches the organism’s functional capacities [1].

The performance of the cardiovascular system, which is one of the most crucial life-sustaining systems, is often seen as an indicator of the functional status of the whole organism [2]. Still, current physiological studies focus more on the functional interactions of the circulatory and respiratory systems [3].

Such interest is dictated by the fact that the cardiovascular system that serves to deliver oxygen to the organism’s cells and maintain homeostasis is one of the most important physiological systems. It determines both mental and physical performance capacities of the human and his adaptation to different activities [4]. Peripheral circulation provides adequate blood supply to separate organs and tissues in response to the constantly changing metabolism.

One of the high priority issues in the developmental physiology is assessment of separate elements and regulatory mechanisms of tissue perfusion that are ultimately responsible...
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for the normal performance of separate organs and the whole organism. It is also very important to study functional capacities of the microcirculatory system in ontogenesis [5], since it helps to reveal patterns of formation of the microvasculature and the specifics of its functioning, analyze its phenomenology and structure, assess its functional reserve and the conditions which contribute to the effective adaptation of the microcirculatory system at various stages of the child’s development [6, 7].

Due to the extreme importance of the processes that take place in the terminal vessels, microcirculation is of particular interest for researchers. The functional contact of the microvasculature and the tissues plays a key role in maintaining homeostasis through a complex and subtle regulation of microcirculation in accordance with tissue metabolic needs. Because of that, the microvasculature is home for compensatory changes that largely determine the functional state of the organism [8]. A wide range of scientific works have described microvasculature in primary school children [7, 9–11]. Kutryeva et al. showed age-related differences in basal parameters of microcirculation and its regulatory mechanisms in children of 3-4 and 10-12 years of age; age-related changes in the microcirculatory functional reserve were detected.

The most common methods applied to study the microvasculature make use of Doppler ultrasound. Laser Doppler Flowmetry (LDF) combined with functional tests [12] is one of the major methods for studying microcirculation status and its regulatory mechanisms. The advantage of LDF is the ability to measure microcirculation in vivo and noninvasively, which is crucial for evaluating microchemodynamics in children.

The functional respiratory test (inspiratory breath-hold) allows obtaining a large amount of data that characterize microchemodynamics and its reserve capacities and to assess the functional contribution of various elements of microcirculatory regulation. During the vasconstrictive respiratory test is indicative of the impact of both sympathetic innervation and vessel walls on microcirculation.

The inspiratory breath-hold test is highly informative and easy to use. In all healthy individuals, skin areas with high density of sympathetic nerve fibers respond to it positively [13].

The aim of this work was to study microcirculation in children of 8 and 10 years of age using the inspiratory breath-hold test.

METHODS

The study included virtually healthy children of both sexes (a group of boys, n = 15, and a group of girls, n = 13) after written informed consent had been obtained from their legal representatives (parents). The children were examined twice, in 2013 and 2015, when they reached the age of 8 and 10, respectively.

Microcirculation was evaluated by laser Doppler flowmetry using LAKK-02 computerized laser analyzer (LASMA Research and Production Enterprise, Russia).

Skin is a traditional and easily accessible object used to assess microcirculation in clinical practice [14]. For our tests, we chose a distal phalanx of the second finger of the right hand. This area is devoid of hair (glabrous skin). It is rich not only in arteriovenous anastomoses dependent on the sympathetic innervation, but also in autonomic and sensory nerve fibers [13].

The following values were computed: mean perfusion index, mean square deviation (flux, or mean blood flow modulation), coefficient of variation, and amplitude-frequency features of the reflected signal.

Among the elements of microcirculatory regulation, passive and active mechanisms can be distinguished. They form 5 non-overlapping frequency bands in a spectrum of 0.005–3 Hz representative of endothelial activity (0.007–0.017 Hz), neurogenic (sympathetic adrenergic) activity (0.023–0.046 Hz), myogenic (smooth muscle) activity (0.05–0.145 Hz), respiratory rhythm (0.2–0.4 Hz), and cardiac rhythm (0.8–1.6 Hz) [15]. Superimposed oscillations recorded by LDF are induced by active and passive mechanisms of microcirculatory regulation. Active mechanisms (endothelial, neurogenic, and myogenic mechanisms of lumen regulation) generate transverse oscillations of the blood flow by cycles of muscle contractions and relaxations (vasoconstriction and vasodilatation episodes). Passive factors (respiratory and cardiac rhythms) are responsible for longitudinal blood flow oscillations expressed as recurrent changes of pressure and blood volume in a vessel [13].

The amplitude-frequency spectrum of oscillations was computed using wavelet transform; contribution of endothelial, neurogenic and myogenic components of microvascular tone and respiratory and cardiac rhythms was also evaluated [16]. We computed the neurogenic tone (NT) of precapillary resistance vessels and the myogenic tone (MT) of metarterioles and precapillary sphincters, as well as shunt index (SI), using the formulas below.

\[ NT = \left( \sigma \times \frac{P_{max}}{A_{max}} \right) / (A_{init} \times M), \]
\[ MT = \left( \sigma \times \frac{P_{max}}{A_{max}} \right) / (A_{init} \times M), \]
\[ SI = A_{init} / A_{max}, \]

where \( \sigma \) is mean square deviation of perfusion index; \( P_{max} \) represents mean arterial pressure; \( M \) is mean perfusion index; \( A_{max} \) and \( A_{init} \) are maximum averaged oscillation amplitudes of sympathetic adrenergic and myogenic frequency bands, respectively [13].

Due to a large scatter in the results of measurements of oscillation amplitudes, it is difficult to assess performance of a regulatory mechanism using only amplitude values. Therefore, apart from \( A_{max} \) we analyzed the contribution of each component to the modulation of the microcirculatory flow calculated as \( (A_{max} / 3\sigma) \times 100 \% \), and a contribution to the total tissue perfusion calculated as \( (A_{max} / M) \times 100 \% \). Those normalized data were computed automatically after finding \( A_{max} \) in the respective frequency band [16, 17].

To study reserve capacities of the microcirculation, a respiratory vasconstrictive test was used. The subjects were asked to take a deep breath and to hold it for 30 seconds, which led to the short-term reduction of perfusion index followed by the restoration of its initial level (see the picture below).

The following parameters were noted during the respiratory test: \( M_{min} \) — the initial value of perfusion index; \( P_{max} \) — the minimum perfusion value during the test; \( M_{init} \) — perfusion index after normal breathing was restored; \( T_3 \rightarrow T_1 \) — time between the onset of breath-hold and the onset of microcirculatory flow reduction; \( T_4 \rightarrow T_3 \) — time between the onset of microcirculatory flow reduction and the minimal value of perfusion index; \( T_5 \rightarrow T_4 \) — time elapsed from the moment the minimum perfusion index value had been reached till breath recovery.

Using the inspiratory breath-hold test results, perfusion index shift (\( \Delta M \)) was found and capillary flow reserve (CFR, %) was computed using the formulas:

\[ \Delta M = \left( \frac{M_{init} - M_{min}}{M_{init}} \right) \times 100 \% , \]
\[ CFR = \left( \frac{M_{init}}{M_{min}} \right) \times 100 \% , \]

where \( M_{min} \) and \( M_{init} \) are the minimum and the initial values of perfusion index [13].
RESULTS

We observed age-related increase in perfusion index by 36.0 and 42.5 % (p <0.01) in the examined boys and girls, respectively. No statistically significant difference in flux (ρ) was found in both groups. Blood flow variability (Kf) in the group of boys increased by 30.7 % (p <0.01), while girls displayed no statistically significant changes in Kf.

Parameters M, σ and Kf give a general idea of the microcirculatory system performance. A more detailed analysis was carried out at the second stage of the study when processing the amplitude-frequency spectrum of blood flow oscillations.

Boys showed age-related decrease in the normalized amplitude of endothelial rhythms by 19.7 % (p <0.05) the amplitude of myogenic oscillations increased by 24.4 % (p <0.01). In the group of boys aged 10 the values of maximum and normalized amplitudes in all frequency bands of passive factors of microcirculation regulation (respiratory and cardiac rhythms) were statistically higher compared to the data obtained from the same participants at the age of 8. The myogenic tone and shunt index decreased with age by 12.2 % (p <0.05) and 22.5 % (p <0.01), respectively (see table 1).

The functional contribution of endothelial rhythm and neurogenic oscillations to microcirculatory flow modulation decreased by 23.6 and 20.9 % (p <0.05) respectively in girls between the age of 8 and 10 years. The contribution of myogenic rhythms to total tissue perfusion decreased by 29.3 % (p <0.05). Maximum amplitudes of respiratory and cardiac rhythms increased by 84.2 (p <0.001) and 38.9 % (p <0.05). The neurogenic tone value increased by 31.4 % (p <0.05), shunt index decreased by 15.2 % (p <0.05) (see table 1).

DISCUSSION

It is known that the organism develops unevenly: long stable periods of development are followed by short unstable “critical” periods. It is those critical periods of development that are a basis for intensive formation of new properties and physiological systems, which is associated with the activation of energy metabolism [18].

The literature reports that mechanisms of microcirculatory regulation are formed at the age of 6, while specific patterns of the microcirculatory system performance are finally shaped in puberty followed by the formation of mature microcirculation [19].

In his work Litvin notes that tissue perfusion has a tendency to increase with age [1].

Our study demonstrated a conspicuous perfusion growth between the age of 8 and 10, which is probably associated with age-related changes in the microcirculatory system resulting from a more intensive metabolism. The increased amplitude of the pulse wave coupled with the increased perfusion index is indicative of a stronger arterial blood flow to the microvascular bed [16].

Decreased shunt index in the subjects indicates reduced muscle tone of precapillaries responsible for the regulation of nutritive blood flow. The reasons for it are different: reduced myogenic tone in boys and increased neurogenic tone in girls, which suggests a larger volume of blood coming into the nutritive capillaries.

When comparing basal parameters of microcirculation, we observed no significant differences in perfusion values in 10- and 8-year old boys and girls; however, in 10-year-old children an almost equal level of perfusion was maintained against various ratios of regulatory factors.

Respiratory oscillations originating from venular components penetrate into skin microvasculature; they are...
registered mainly in venules. Formation of those oscillations in human skin microvasculature is affected by at least two mechanisms: a mechanistic transmission of respiratory variations of intrathoracic pressure mediated by the venous system (suction effect of the thorax during inspiration as veins get filled with blood) and an autonomic interaction of cardiovascular and respiratory centers. A passive hydrostatic nature of the former mechanism means that respiration-related oscillations originate from pressure wave propagation, while active mechanisms of vascular tone regulation are not involved. In the latter case, blood flow oscillations form active vasocostrictive mechanisms of neurogenic nature, one of which is a well-known vasomotor reflex that is expressed as short-term reduction in tissue perfusion in response to the inspiratory gasp. The inspiratory vasomotor reflex is implemented through sympathetic peripheral innervation [20].

The vasomotor reflex triggered by the inspiratory gasp induces constriction of arterioles and short-term reduction in skin blood flow. Reduced perfusion during the respiratory test is a result of sympathetic regulation limited mainly by the neurovascular synapse [13].

Levin’s work [21] showed that between the age of

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**Table 1.** Perfusion index and mechanisms of perfusion regulation in children of 8 to 10 years of age

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Boys (n = 15)</th>
<th>Girls (n = 13)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>8.13 ± 0.34 years</td>
<td>10.00 ± 0.33 years</td>
</tr>
<tr>
<td>Perfusion index</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M, PU</td>
<td>19.65 ± 5.34</td>
<td>26.77 ± 6.00**</td>
</tr>
<tr>
<td>M, PU</td>
<td>2.29 ± 0.85</td>
<td>2.22 ± 0.66</td>
</tr>
<tr>
<td>KV, %</td>
<td>13.09 ± 4.91</td>
<td>9.07 ± 3.11**</td>
</tr>
<tr>
<td>Endothelial rhythms</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A_200, PU</td>
<td>1.58 ± 0.83</td>
<td>1.28 ± 0.67</td>
</tr>
<tr>
<td>(A_200 / 300) × 100 %</td>
<td>20.84 ± 5.17</td>
<td>16.72 ± 5.23*</td>
</tr>
<tr>
<td>(A_200 / M) × 100 %</td>
<td>8.23 ± 3.67</td>
<td>4.82 ± 2.88*</td>
</tr>
<tr>
<td>Neurogenic rhythms</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A_200, PU</td>
<td>1.36 ± 0.49</td>
<td>1.35 ± 0.67</td>
</tr>
<tr>
<td>(A_200 / 300) × 100 %</td>
<td>18.02 ± 3.19</td>
<td>18.33 ± 3.90</td>
</tr>
<tr>
<td>(A_200 / M) × 100 %</td>
<td>7.16 ± 2.67</td>
<td>5.13 ± 2.44*</td>
</tr>
<tr>
<td>Myogenic rhythms</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A_200, PU</td>
<td>1.00 ± 0.35</td>
<td>1.12 ± 0.41</td>
</tr>
<tr>
<td>(A_200 / 300) × 100 %</td>
<td>13.49 ± 2.92</td>
<td>16.84 ± 3.82**</td>
</tr>
<tr>
<td>(A_200 / M) × 100 %</td>
<td>5.19 ± 1.36</td>
<td>4.25 ± 1.47</td>
</tr>
<tr>
<td>Respiratory rhythms</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A_200, PU</td>
<td>0.31 ± 0.10</td>
<td>0.58 ± 0.19***</td>
</tr>
<tr>
<td>(A_200 / 300) × 100 %</td>
<td>4.34 ± 1.08</td>
<td>8.41 ± 2.49***</td>
</tr>
<tr>
<td>(A_200 / M) × 100 %</td>
<td>1.52 ± 0.36</td>
<td>2.16 ± 0.54**</td>
</tr>
<tr>
<td>Cardiac rhythms</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A_200, PU</td>
<td>0.18 ± 0.04</td>
<td>0.35 ± 0.11***</td>
</tr>
<tr>
<td>(A_200 / 300) × 100 %</td>
<td>2.67 ± 1.02</td>
<td>5.28 ± 2.11**</td>
</tr>
<tr>
<td>(A_200 / M) × 100 %</td>
<td>0.99 ± 0.24</td>
<td>1.32 ± 0.34**</td>
</tr>
<tr>
<td>NT, AU</td>
<td>1.84 ± 0.38</td>
<td>1.82 ± 0.32</td>
</tr>
<tr>
<td>MT, AU</td>
<td>2.37 ± 0.48</td>
<td>2.08 ± 0.51*</td>
</tr>
<tr>
<td>SI, AU</td>
<td>1.38 ± 0.36</td>
<td>1.07 ± 0.15*</td>
</tr>
</tbody>
</table>

**Note:** here and in table 2 data are presented as arithmetic mean ± standard deviation. M represents mean perfusion index; e represents mean square deviation (mean blood flow modulation); K_e represents the coefficient of variation of perfusion index (blood flow variability); NT — neurogenic tone; MT — myogenic tone; SI — shunt index; A_200 (A_200 / 300) × 100 % and (A_200 / M) × 100 % — maximum and normalized amplitudes.

* — p < 0.5; ** — p < 0.01; *** — p < 0.001 in comparison with the same parameters in children of an earlier age; PU — perfusion units; AU — arbitrary units.

**Table 2.** Perfusion parameters in 8- and 10-year-old children during the inspiratory breath hold

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Boys (n = 15)</th>
<th>Girls (n = 13)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>8.13 ± 0.34 years</td>
<td>10.00 ± 0.33 years</td>
</tr>
<tr>
<td>M_200, PU</td>
<td>20.26 ± 5.49</td>
<td>26.91 ± 6.49**</td>
</tr>
<tr>
<td>P_200, PU</td>
<td>10.12 ± 4.06</td>
<td>16.17 ± 7.90*</td>
</tr>
<tr>
<td>M_200, PU</td>
<td>18.87 ± 4.40</td>
<td>26.90 ± 6.65**</td>
</tr>
<tr>
<td>T3–T1, s</td>
<td>11.28 ± 3.95</td>
<td>9.23 ± 2.26*</td>
</tr>
<tr>
<td>T4–T3, s</td>
<td>8.01 ± 4.10</td>
<td>4.74 ± 1.88*</td>
</tr>
<tr>
<td>T5–T4, s</td>
<td>10.64 ± 4.64</td>
<td>17.81 ± 6.75**</td>
</tr>
<tr>
<td>CFR, %</td>
<td>54.09 ± 17.81</td>
<td>59.57 ± 24.35</td>
</tr>
<tr>
<td>ΔM, %</td>
<td>50.20 ± 26.10</td>
<td>39.70 ± 23.10*</td>
</tr>
</tbody>
</table>

**Note:** M_200 is the initial value of perfusion index before the inspiratory breath hold; P_200 is minimal perfusion during the respiratory test; M_200 is the value of perfusion index after normal breathing was restored; T3–T1 is a time interval between the onset of breath hold and the onset of microcirculatory flow reduction; T4–T3 is a time interval between the onset of microcirculatory flow reduction and the minimum value of perfusion index; T5–T4 is a time interval between the minimum value of perfusion index and breath recovery; CFR is capillary flow reserve; ΔM is perfusion index shift.

* — p < 0.05; ** — p < 0.01; *** — p < 0.001 when compared to the corresponding values in children of an earlier age.
7 and 20, the functional reserve of the microcirculatory system grows. The respiratory test we used demonstrated the increase in the microcirculatory reserve capacity accompanying short-term hypoxia in girls; boys showed a conspicuous tendency to increased CFR.

The vascular response to the activation of adrenergic fibers is affected by sympathetic innervation and vessel wall reactivity. Therefore, the degree of blood flow reduction during the respiratory test reflects a combination of both factors that cannot be assessed separately. Because of that, for adequate sympathetic perivascular innervation assessment, LDF data should be interpreted carefully taking into account the initial neurogenic tone at rest and a relative value of perfusion index reduction during the respiratory test [13]. Changes in NT and ΔM in 10-year-old girls lead us to conclude that sympathetic activity increases with vasoconstrictive stimulation, while in 10-year-old boys changes in the perfusion index shift are induced by a weaker reactivity of preganglionic neurons against the functional load [16].

The perfusion index shift measured during the respiratory test is a result of sympathetic regulation limited mainly by the neurovascular synapse. Therefore, the age-related decrease of ΔM in boys and girls indicates a less conspicuous response of the vascular wall to the inspiratory breath hold. With age, perfusion index reduction becomes weaker in response to the respiratory test.

Physiologically, the time interval between 8 and 10 years of age is crucial because it lies between two critical periods: a growth spurt (at the age of 5–6) and puberty. The former is associated with significant morphological and functional changes in the nervous system [22]. Puberty is characterized by hormonal and muscle changes.

In 8–10-year-old children the intensity of oxidative processes remains pretty high, though metabolism remains quite stable. However, at this age the majority of physiological systems, including the cardiorespiratory system, enhance their capacities. Tissues and organs demand more oxygen, which leads to a specific performance pattern of the cardiovascular and respiratory systems. Though the circulatory and respiratory systems are not that resource conserving in children as in adults, they are very co-operative [22]. Close functional interconnection of the respiratory and cardiovascular systems implies their interdependence. Changes in the respiratory system performance lead to adaptive changes in the circulation and oxygen delivery to blood tissues.

In the ontogenesis, the cardiorespiratory system develops heterochronically in close interaction with the physical development of the organism, morphological changes in the lungs, heart, thorax, age-related metabolism dynamics and the development of regulatory mechanisms. Because of that, the cardiorespiratory system of a primary school child at various ages has different qualitative and quantitative characteristics based on the continuous development of morphological structures and functional changes [23].

CONCLUSIONS

Our study has revealed changes in the peripheral blood flow characterized by the age-related increase in tissue perfusion in the group of boys and girls. Those changes are most likely to be associated with the shifting roles of various regulatory mechanisms; oscillation amplitudes of active modulation components contribute less to the microcirculatory regulation, while passive elements of microhemodynamics modulation contribute more. A considerable growth of the pulse wave indicates a bigger arterial blood flow to the microvasculature. The increased amplitude of the respiratory wave resulting from venous pressure indicates decreased microcirculatory pressure.

The study demonstrated that basal microcirculatory parameters in boys of girls of 8 and 10 years of age did not differ significantly. However, active and passive factors of microcirculatory regulation made different contribution to maintaining equal levels of perfusion in boys and girls.

While assessing reserve capacities of microcirculation using the inspiratory breath hold, we found that improvement of autonomic regulation of the microvasculature between the age of 8 and 10 was expressed as decrease in the vessel wall reactivity in response to sympathetic adrenergic stimuli.

Further research is necessary to study the microvasculature and its regulatory mechanisms at various ontogenesis stages, including puberty which gives birth to sex-related differences in hemomicrocirculation performance. Such studies will help to understand human microcirculation dynamics better and to explore age-related specifics of peripheral circulation.

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