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Dynamic of the Changes in the Thickness of the Wall of the Main Leg Vessels during the Prenatal Ontogenesis. Quantitative Analysis

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The structure and development of the lower limb vessels are subject to elevated scientific interest due to the varied heavy pathology affecting these. Histotopographic slices from 16 lower limbs of eight 135 mm to 388 mm long fetuses and longitudinal and transverse slices of isolated main vessels from the lower limbs of six mature still-born human fetuses were studied. To characterize quantitatively the vessel wall development two indexes are suggested: average week increment for the investigated period (shows the average speed of the vessel wall growth in μm/week) and growth index of the increment (shows the average increase of the vessel wall per mm increase of the crown-heel fetal length in μm/mm). Both indexes show common dynamics and render the investigated cases comparable. The comparative morphometric analysis allows substantiating the divergence in the prenatal histogenesis of the wall of the arteries and of the deep and the superficial veins of the lower limb.

Key words: prenatal development, vessel wall, chronic arterial insufficiency, chronic venous insufficiency.

Introduction

The vascular systems of the upper and the lower limb of the human show principally the same structure. They are affected, however, by considerably different in character, occurrence, duration and severity pathological processes. When compared to the vessels of the upper limb, the analogical arterial, venous and lymphatic vessels of the lower limb suffer more often and much earlier from systemic vascular diseases, as well as from specific local disorders. The Chronic Arterial Insufficiency of the Lower Limb (CAILL) and the Chronic Venous Insufficiency of the Lower Limb (CVILL) are leading amongst the latter.

The pathological processes affecting the lower limb develop progressively and cause irreversible changes in the arterial, venous and lymphatic vessel wall. The treatment, in the best case, slows down the process, but does not stop it [2, 10, 12, 13, 14, 20, 28]. The reasons for these differences are probably complex. The opinion is formed, however, that the leading cause is the insufficient adaptation of the lower limb vascular system in the process of evolution towards upright posture and bipedal locomotion [5, 19, 24]. The

lower limb vessels are in unfavorable position even during the prenatal ontogenesis, because before birth they are supplied by blood with lower oxygen contents. The anticipating in size and mass growth of the lower limb during postnatal life sets higher demands in front of its arterial system. On the other hand, the haemodynamic conditions in its venous system worsen through the high hydrostatic pressure. These circumstances burden the adaptation mechanisms of the lower limb vasculature. Sometimes these mechanisms turn out inadequate and the respective parts of the lower limb vasculature — arterial, venous or lymphatic, decompensate. The proof of this hypothesis needs considerable widening of the insufficient literature data about the mechanisms and dynamics of the discrete changes in wall of the main lower limb vessels during the phylo- and ontogenesis [6, 7, 8, 25]. Clarification of these changes could give valuable criteria for discrimination between normal mechanisms of adaptation and the initial pathological changes [9, 26, 27]. Consequently, the objective of this investigation is to clarify and compare the dynamic of the changes in the thickness of the wall of the main arterial and venous leg vessels during the prenatal ontogenesis.

Material and Methods

The study was carried out on cadaver material from the fund of the department of anatomy, histology and embryology at the University of Medicine "Prof. Dr Paraskev Stoyanov", Varna, following all the ethical rules for work on cadaver material. Subject of the examination were:

— histotopographical slices from proximal, middle and distal third of the legs of 16 lower limbs taken from 8 human foetuses with crown-heel lengths of 135 mm, 140 mm, 198 mm, 200 mm, 256 mm, 257 mm, 306 mm and 388 mm;

— longitudinal and transverse slices mostly from the middle third of the legs from 6 lower limbs from 6 full-term human still-borns.

The average age was calculated after the rule of Haase. Slides were stained with hematoxilin-eosin, orcein, after van Gieson and with the combined method of Weigert-Mallori. The following vessels were examined:

I group — main arteries of the leg: *a. tibialis anterior* (Ata), *a. tibialis posterior* (Atp);

II group — deep main veins of the leg: *v. tibialis anterior* (Vta), *v. tibialis posterior* (Vtp);

III group — superficial main veins of the leg: *v. saphena magna* (Vsm), *v. saphena parva* (Vsp).

The observation was carried out with Olympus BX50 microscope. Representative areas of the vessel wall were photographed with Ikegami ICD-840P and Olympus Camedia 5.1 digital cameras. With the help of the programs ImageTool 3.0 [17] and ImageJ 1.35d [1, 15] the digital images were processed and the common media-intima thickness of every captured vessel was measured. On the examined histotopographical slides the border between adventitia and surrounding connective tissue was quite unclear, therefore the latter wasn't measured.

Results were analized statistically by means of Student test and factorial analysis of variance (ANOVA) and parameters of the dynamic changes of the vessel wall thickness were calculated.

Results and Discussion

The examined vessels were divided in 6 age groups (Table 1).

Table 1. Length and age of the examined fetuses

Lunar Month (LM)	Number of fetuses, crown-heel length (mm)
III 3/4	Two fetuses, 135 mm and 140 mm
IV 1/2	Two fetuses, 198 mm and 200 mm
V	Two fetuses, 256 mm and 257 mm
VI	One fetus, 306 mm
VII 3/4	One fetus, 388 mm
X	Six full-term still-borns

The measurement results are displayed on an extended scatter diagram (Fig. 1). According to the factorial ANOVA the greatest influence upon the dispersion belongs to the factor "crown-heel length", which is linked to the fetal age, F=115.26 (F_{crit} = 2.76 for p=0.01); second in importance is the factor "vessel type" (artery, deep vein or superficial vein) — F=99.20 (F_{crit} = 4.75 for p=0.01). Lack of data renders impossible to estimate statistically adequate the influence of the factor "level of measurement" (proximal, middle or distal third of leg) on the dispersion. We eliminate the influence of this factor by averaging of the results from different measurement levels for every investigated vessel of every fetus (Table 2, Fig. 2).

Table 2. Average results according to the age and investigated vessel

Fetal crown-heel length (mm)	Average age of the fetuses in months (weeks) after Haase	Mean common intima-media thickness in um					
		arteries		deep main veins		main superficial veins	
		Ata	Atp	Vta	Vtp	Vsp	Vsm
135	III ¾ (15 weeks)	6.53	7.06	2.70	3.46	-	4.10
140	III ¾ (15 weeks)	8.02	6.43	2.87	3.99	7.08	6.19
198	IV ½ (18 weeks)	13.00	11.39	9.84	10.49	11.62	13.44
200	IV ½ (18 weeks)	9.13	9.49	11.05	11.26	12.92	14.80
256	V (20 weeks)	11.12	12.67	12.66	12.61	15.37	19.16
257	V (20 weeks)	12.00	17.52	13.30	16.64	-	16.29
306	VI(24 weeks)	13.51	17.47	15.63	17.56	21.11	38.96
388	VII ¾ (31 weeks)	22.14	25.04	15.17	16.52	67.90	57.63
500	X (40 weeks)	47.52	46.47	-	17.72	87.08	63.48

The Student test does not show any statistically meaningful differences in the variance between the vessels from the same group — t_{art}=0.4228, $t_{deep\ veins}$ =0.2433, $t_{superf.\ veins}$=0.3352 (t_{crit}= 2.92, for p=0.01). The effect of the internal casual and individual aspects was eliminated by means of averaging of the results from the vessels of the respective groups for every age (Fig. 3). Two periods can be distinguished on this diagram. Between III and V LM (Crown-heel length 135-257 mm) the three vessel groups follow common hardly different linear trend in the increment of the common intima-media thickness. In VI LM (306 mm) the wall thickness of the superficial veins increases harshly followed in VII lm (388 mm) by a shift in the development of the arterial wall. Between VI lm and X lm the common intima-media thickness of the wall of the deep veins remains almost unchanged.

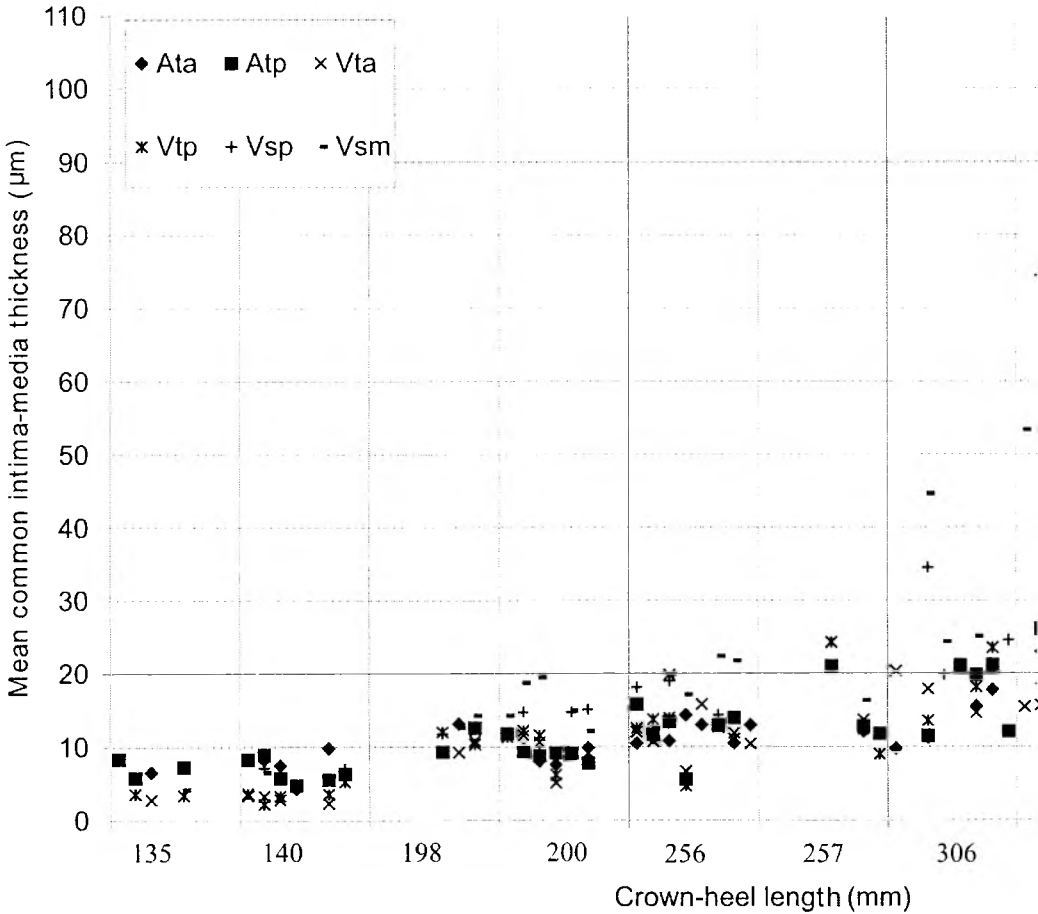

Fig. 1. Scatter diagram of the initial results

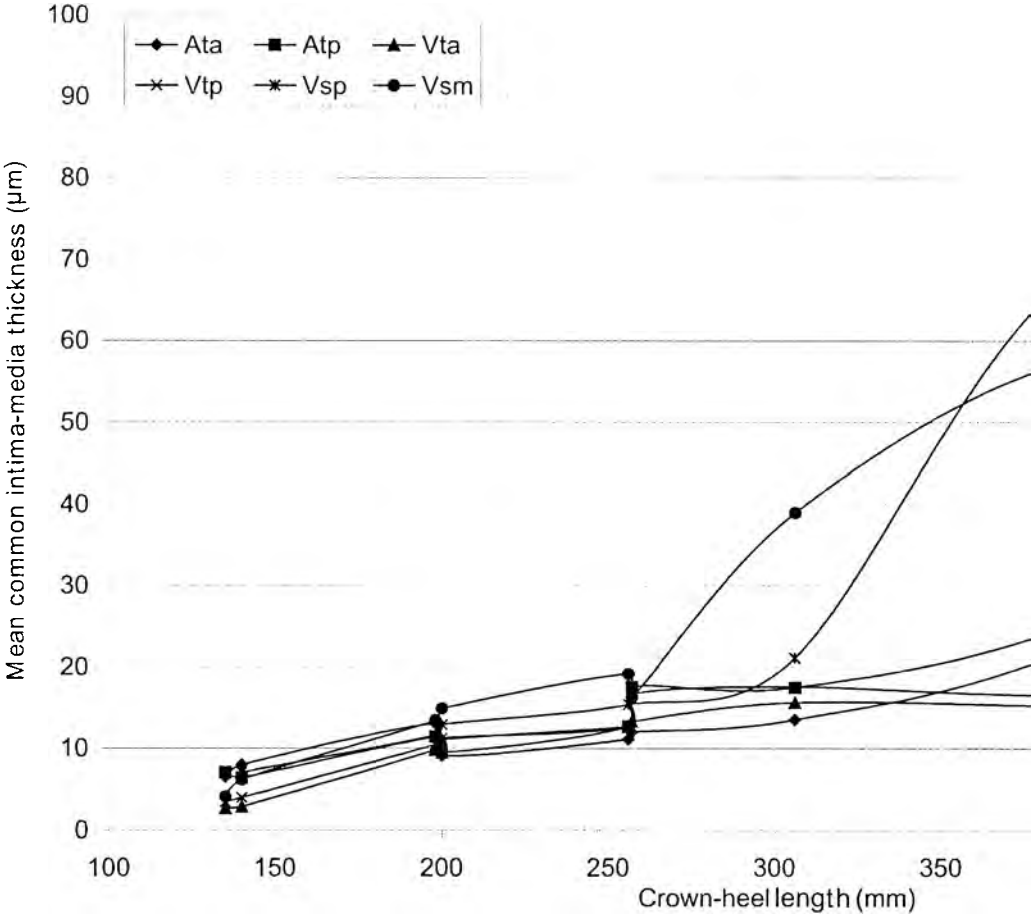

Fig. 2. Dynamic in the growth of the mean common intima-media thickness (µm) relative to the crown-heel

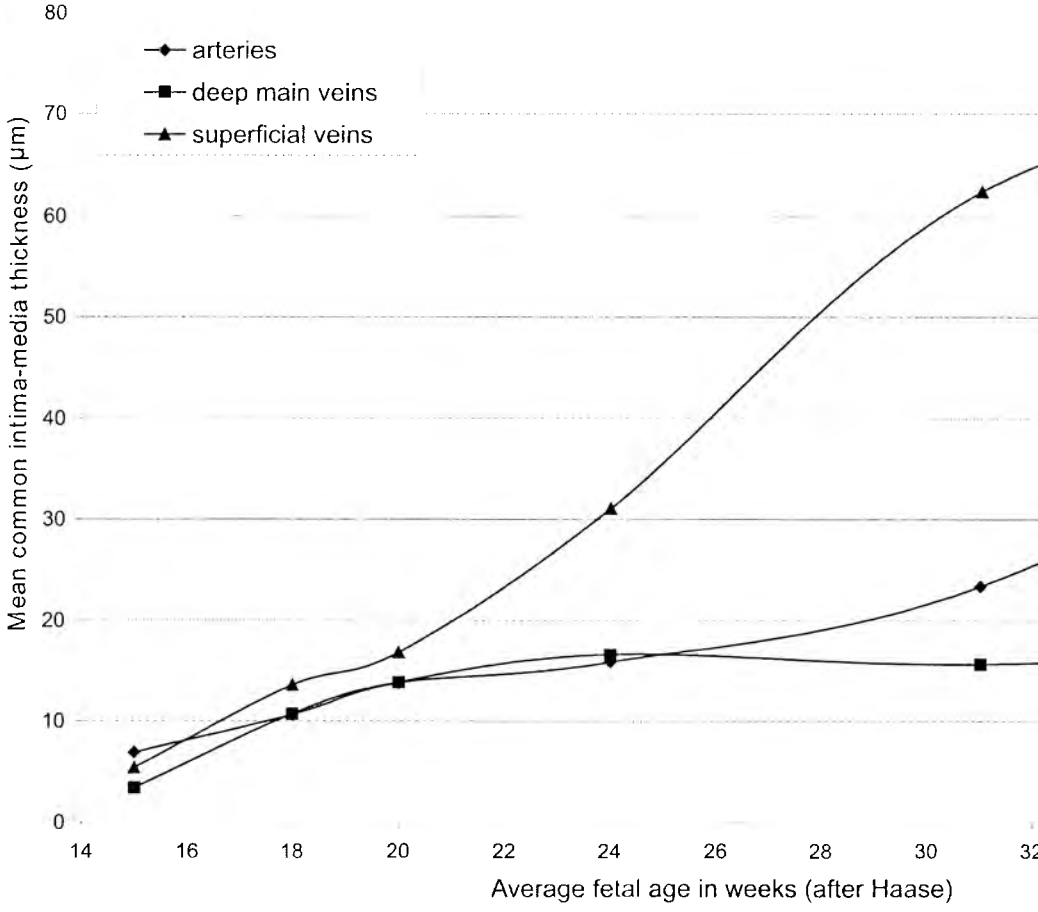

Fig. 3. Age related dynamic of the growth of mean common intima-media thickness (μm) in the different vessel groups

The found minimal variance is probably due to casual and individual peculiarities.

We calculated two indexes, to characterize the dynamic of the common intima-media thickness during the prenatal development:

— average week increment for the investigated period — the absolute growth of the mean intima-media thickness of the vessels between two neighboring age groups, divided by the difference in the average fetal age in weeks; the resulting numbers express the mean week growth of the investigated parameter (common intima-media thickness) for the respective period (μm/week) (Fig. 4);

— index of the increment — the absolute growth of the mean intima-media thickness of the vessels between two neighboring age groups, divided by absolute growth in the crown-heel length. This index shows the change in the investigated parameter for 1 mm change in the fetal crown-heel length (μm/mm) (Fig. 5).

Both indexes express common dynamic throughout the investigated period — 15 - 40 weeks from gestation. In the beginning (15-18 weeks) the growth speed of the intima-media thickness is relatively high for all of the three vessel groups and eventually decreases. A second peek in the growth speed can be observed during 20-31 week for the superficial veins and during 24-40 week for the arteries. In both cases this growth acceleration is connected to increase in the quantity of smooth muscle cells in the media [11, 22].

In our opinion, one of the most plausible causes for the harsh increment of the wall thickness of arteries and superficial veins is the increased burden of the vessel wall. In arteries this is connected to the increment of the arterial transmural blood pressure [3, 4]. In superficial veins the increased load of the vessel wall is probably due to deteriorated through the posture venous drainage — in the last trimester the lower limbs of the fetus are flexed in the hip and sometimes also in the knee joint. The altered rheological properties of the fetal blood — physiological erythrocytois and macrocytosis, elevated hematocrit and decreased erythrocyte sedimentation rate by the pregnancy term, also contribute to the increase of the vessel wall load [3, 4, 16, 21]. These factors, however, do not exert their influence upon the wall of the deep main veins of the leg. This could be explained through the effect of two factors, which are absent in the superficial veins:

1) in this period of the pregnancy the muscle pump is already functional;

2) through the arterial wall pulsations the transmural arterial blood pressure is acting upon the deep veins from the outside in the area where they touch the arteries and renders the drainage easier. A proof for this is the decrease of the wall thickness of the deep veins of the leg in the same area [23, 22].

The established fluctuation in the developmental dynamic of the main arterial and venous leg vessels in the prenatal ontogenesis represent undoubtedly an interesting fact. Especially interesting are the "accelerated growth periods". The discovered in our previous investigations qualitative changes in the vessel wall structure in these periods show, that they are based upon increase in the active contractile elements (smooth muscle cells) and in the passively elongated elastic fibers [11, 22]. This shows that even in these early developmental stages the vascular system of the lower limb is evolving and adapting to the changing hemodynamics. This adaptation must be observed in the light of different specific peculiarities of position and interaction of the arterial and venous vessels with other surrounding structures in the leg region. What is the potential possibility in time for adequate adaptation of the vessel wall to the constantly changing with age hemodynamic conditions, is a question that still awaits its answer.

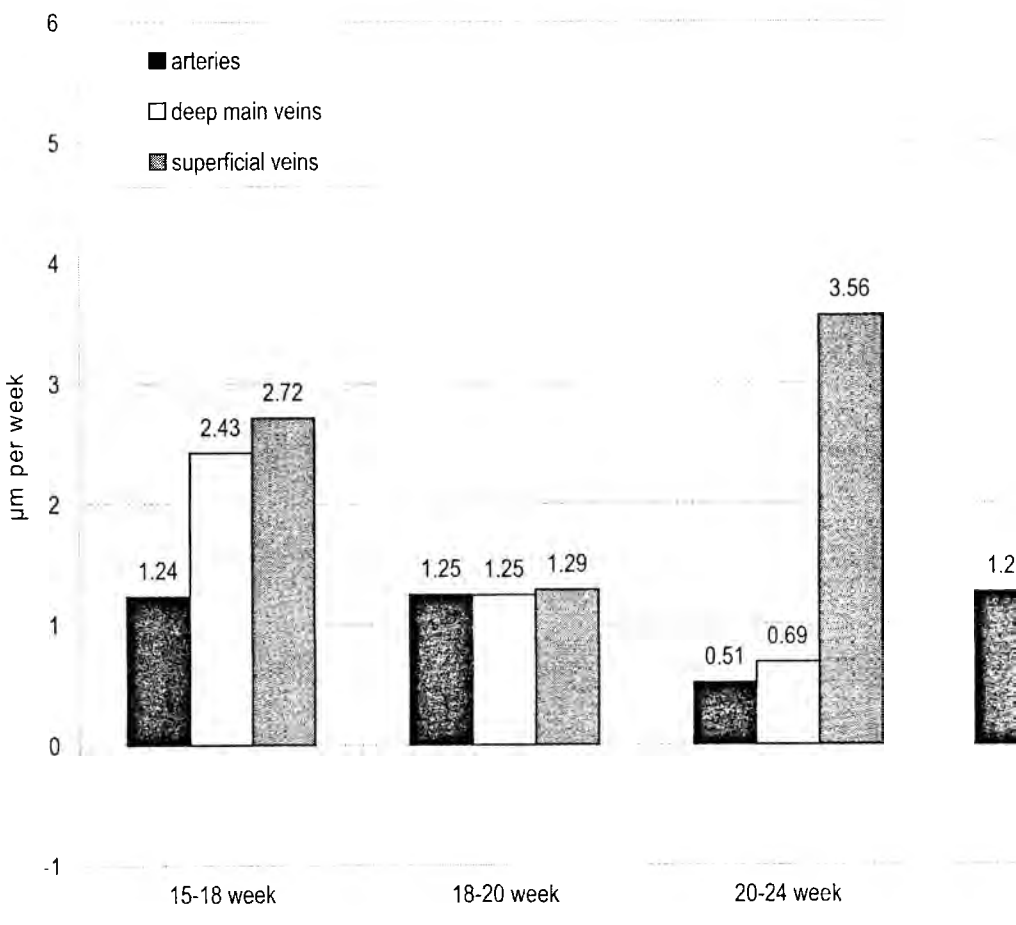

Fig. 4. Average week increment of the mean common intima-media thickness (µm) for the investigated per

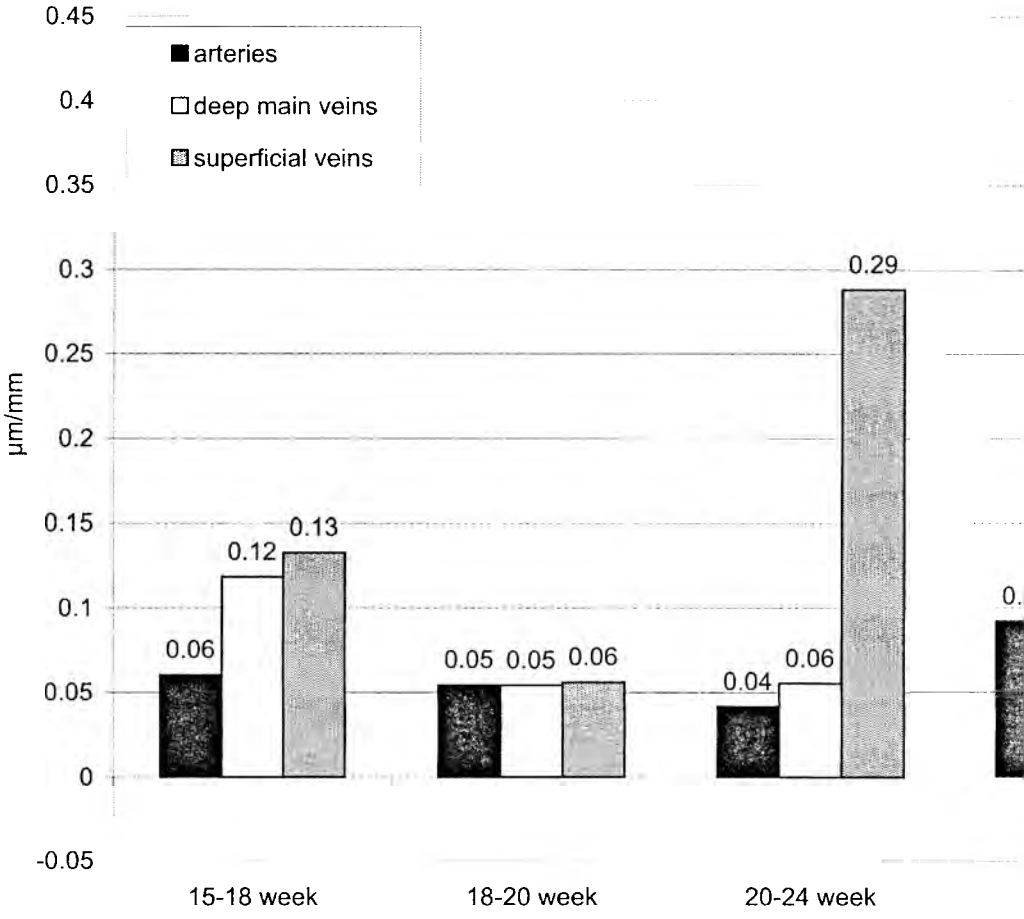

Fig. 5. Index of the increment of the mean common intima-media thickness (µm)

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