Histochemical and Morphometric Characteristics of the Vastus Lateralis Muscle in Children

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Abstract

Autopsy samples of vastus lateralis muscles of 50 healthy children were pursued at several age intervals, between 14 days and 15 years. During postnatal development percentage of type I and type II fibres changes with ages. Percentage of type I fibres increases during the first 3 years and decreases after 10 years of life. High percentage of IIA fibres in the first year decreases during second postnatal year, at the same time the percentage of type I and IIB fibres increases.

The mean diameters of fibre types increase linearly with age. In the age group of 10 to 15 years they reach the lower limit of fibre diameters characteristic of the adult vastus lateralis muscle. The arrangement of fibre types within different layers of fascicles is specific in all age groups and after the first postnatal year is similar to the typical arrangement in normal adult muscles. On the periphery beneath the perimysium there is a preponderance of type IIB and in the internal regions of type I fibres. Transformations of fibre types occurring during growth and development do not influence the mosaic pattern of fibre type distribution expressed on the cross plane of normal adult muscles.

Key words: skeletal muscle, histochemistry, fibre types, growth and differentiation, spatial arrangement.

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The growth and development of human skeletal muscle is a long-lasting and complex process. Knowledge about its normal pattern is still not complete. During maturation of the nervous system and increasing muscle activity, several age-related adaptive mechanisms influence the growth, development and metabolic turnover of muscles [18]. Skeletal muscles are composed of heterogenous muscle fibre populations reflecting a high degree of functional specialization. This heterogenity is due to the ability of muscle fibres to adapt to specific functional demands within genetically determined borderlines. The response to exogenous factors relates to the fact that most myofibrillar proteins exist as various isoforms, encoded by multigene families, which provides the genetic basis for the variability of contractile tissues [11]. The rapid turnover of macromolecular constituent cell proteins, especially myosin heavy chains, constitute the basis for gross changes in muscle morphology [8]. The continuous dynamic transformations of muscle fibres, which occur throughout a lifetime, are even more pronounced during development and reflect the plasticity of a muscle as a whole.

A close correlation exists between the morphology of a muscle and its function. It was shown that type I muscle fibres contract slowly and are capable of sustained activity while type II fibres contract quickly and are mainly adapted for intermittent or rapid activity. The first mentioned characteristic is due to the presence of the isoforms of myosin heavy chains, manifesting different myofibrillar adenosinetriphosphatase activity [2, 7, 38], and the second is due to the presence of different metabolic enzymes [31]. Both the intrinsic programming of myogenic precursor cells and extrinsic factors have been shown to modulate the appearance and accumulation of myosin heavy chains [1].

The continuous phenotypic modulations of muscle fibres could be demonstrated by histochemical methods as transformations of fibre types.

With a histochemical reaction, demonstrating myofibrillar adenosinetriphosphatase in alkaline and acidic media, muscle fibres differentiate into type I and type II fibres; the

latter can be subdivided further into IIA, IIB and IIC fibres [4].

In this study, the morphometric characteristics of muscle fibre types in a cross-section were determined. The main purpose of the study was to gain a better insight into different stages of maturation in normally developing human muscles. Morphological data at several successive age intervals would be useful in comparative studies of normal human muscles and those changed in diseases.

Materials and Methods

Materials

Vastus lateralis muscles of 50 healthy children, aged 14 days to 15 years, who had suffered sudden death, were examined. The muscle samples were excised from the right leg on the border between the middle and the distal third of the femur, 8 to 30 hours after death. None of the children had a history of neuromuscular disease. No evidence of pathological abnormalities was detected at postmortem examinations of muscle specimens. Muscle samples were gathered partly at the Institute for Forensic Medicine in Ljubljana and partly at the Institute of Pathology, Psychiatric Hospital in Leipzig, Germany.

Histochemistry

The muscle specimens were immersed in liquid nitrogen and cut into 10 μm transverse sections. In muscle samples of 38 subjects, fibres of type I and II were demonstrated after combined staining for myofibrillar adenosinetriphosphatase and diaphorase I at pH 7.2 [41], or calcium method staining for myofibrillar ATPase at pH 9.4 [26]. In 12 muscle samples, the histochemical reaction demonstrating myofibrillar ATPase activity was demonstrated with the calcium method at pH 9.4 and after preincubation at pH 4.5 and 4.3 [10]. By combining observations from 3 serial cross sections, the muscle fibres were classified as types I, IIA, IIB and IIC [4].

Sampling

Randomly chosen areas or fascicles, at least three from one muscle sample, each containing 100 fibres on average were photographed with an Opton photomicroscope with a final magnification of 90 to 640 x. Photographs of sections were used for further analysis.

The muscle samples were sorted into age groups. Within every age group, there were no differences between muscle samples of boys and girls. Therefore they were not treated separately in the statistical analysis. The muscle samples of first postnatal years of life, in which greater changes were expected, were sorted into more frequent age intervals than the muscle samples of older children. Concerning the material gathered, morphometric characteristics were calculated for all or only for some muscle samples. Details of the analysis were limited by material (short fibres, already stained cross-sections).The statistical significance of the results was tested by Student t-test.

Data acquisition and preprocessing

The contours of the fibres were digitised with the aid of a Cherry graphic tablet coupled to an IBM PC/AT compatible computer, which also served for further calculations. A computer-assisted method [28, 30] was applied to determine the fibre type, numerical density, mean diameter, the distribution of fibre types on the cross section of a muscle and the arrangement of fibre types within fascicle layers.

Spatial distribution analysis

On the cross section of a muscle, the spatial distribution of muscle fibre types was determined by the index of normalised dispersion. The index value was zero when the muscle fibres of a particular type were arranged randomly. It was -1 when the fibre types were maximally disjoined and it reached a positive value when the muscle fibres of a particular type were grouped [30].

Analysis of fascicles

Muscle fibres within fascicles, well-defined by their perimysium and not comprising smaller fascicles, were decomposed into different layers. The fibres on the border of the fascicles lying under the perimysium formed the peripheral layer (layer 1) and the internal nodes constituted the internal region. Layer 2 was composed of fibres adjacent to the edge of the fibres in the first layer.

The proportion of types I, IIA IIB and IIC was calculated in layer 1, layer 2 and internal region. Normalised fibre type proportion was used to permit comparison of fascicles with a different fibre type frequency distribution [30]. A value greater than 1 indicated a higher fibre type proportion in a layer and a value lower than 1 indicated a lower fibre type proportion in a particular layer of the fascicle than in the whole fascicle.

Results

Percentage of muscle fibres

The data on numerical density (N_N) of fibre types are presented in Table 1 and Table 2. In the age range from 14 days to 1 year, a low numerical density of type I fibres (32%) was observed. In the second year, it increased to 52.12% ($p < 0.05$). It remained constant till the age of 10 years and during the period from 10 to 15 years it decreased to 44.19% ($p < 0.01$). Type II fibres changed reciprocally. In type II fibre group, IIA fibres predominated (46.7% of the whole fibre type population) in the first year of life; 14.90% were type IIB fibres and few undifferentiated IIC fibres were observed (2.7%). In the second year of life, the percentage of type IIA fibres dropped to 12.30% ($p<0.05$). In the age range from 6 to 8 years, the numerical density of IIA and IIB fibres was equal (18.20% of IIA and 18.40% of IIB fibres). IIC fibres, however, were rare (2.9%). The highest proportion of IIC fibres was found in the age period between the first and second year of life.

Table 1. Percentage (N_N), mean diameter ($\bar{D}$) [μm] and spatial distribution analysis ($DISP_{NORM}$) of fibre types I and II at different age intervals ($\bar{x} \pm SE$).

Age (years)	Percentage		Mean diameter		$DISP_{norm}$	
	I	II	I	II	I	II
0-1	32.30 ± 4.64	67.70 ± 4.64	9.46 ± 1.86	10.08 ± 1.44	0.03 ± 0.09	0.06 ± 0.10
	n=5		n=5		n=5	
1-2	52.12 ± 3.48	47.88 ± 3.48	13.32 ± 2.50	16.37 ± 2.51	-0.12 ± 0.05	-0.18 ± 0.06
	n=6		n=5		n=6	
2-3	59.15 ± 2.59	40.85 ± 2.73	19.70 ± 4.75	22.28 ± 6.23	0.05 ± 0.05	0.12 ± 0.10
	n=6		n=6		n=6	
3-5	57.89 ± 3.24	42.11 ± 3.24	34.83 ± 5.46	31.83 ± 5.15	-0.16 ± 0.04	-0.07 ± 0.09
	n=4		n=3		n=4	
5-10	58.76 ± 1.86	41.24 ± 1.86	40.06 ± 5.68	38.27 ± 4.84	0.00 ± 0.08	-0.14 ± 0.09
	n=11		n=8		n=11	
10-15	44.19 ± 2.56	55.81 ± 2.56	41.54 ± 2.56	40.67 ± 2.38	-0.02 ± 0.05	-0.18 ± 0.05
	n=18		n=17		n=18	

Muscle fibre size

The data on the mean diameters of type I and type II muscle fibres are collected in Table 1. In children up to the first year of life the mean diameter of type I fibres was 9.46 μm and of type II fibres 10.08 μm. At the age range 10 to 15 years, the mean diameter increased up to 41.54 μm in type I fibres and up to 40.67 μm in type II fibres. There was no difference in muscle fibre diameters between boys and girls.

The comparison of the mean diameters in successive age periods did not display statistically significant changes. Up to 3 years, the mean diameters of type II fibres were slightly larger. After that period, the mean diameters of type I fibres were slightly larger.

Arrangement of fibre types within fascicles

The proportions of fibre types differ significantly across the layers of a fascicle. Fig. 1 presents normalised fibre type proportions in layer 1, layer 2 and inside the fascicle in 4 different age groups. In layer 1, there is a preponderance of IIB fibre type in all age groups. On the average, it was 25% higher than in the whole fascicle in the first year of life, 43% higher in the second year and 57% higher in the age group from 6 to 8 years. In the second layer, the proportion of type I fibres was 28 to 30% higher than in the whole fascicle. IIA fibres were nearly equally dispersed in all fascicle layers. In the age group from 10 to 15 years, only normalised proportions of type I and II fibres were analysed. In the first layer, the proportion of type II fibres was 21% higher and of type I fibres 15% lower than in the whole fascicle. In the second layer and in the internal region, the situation was just the reverse. IIC fibres were rare in all layers of the fascicle in all age groups.

Spatial distribution of fibre types on the skeletal muscle cross-section plane

Table 1 shows data on the spatial distribution of type I and type II muscle fibres in randomly chosen areas or in fascicles from six successive age intervals. Both fibre types

Table 2. Percentage (N_N) of muscle fibre types and subtypes at three age intervals ($\bar{x} \pm SE$).

Age (years)	n	Fibre types I	IIA	IIB	IIC
0-1	4	35.70 ± 7.87	46.70 ± 12.30	14.90 ± 6.85	2.70 ± 0.70
1-2	4	57.00 ± 3.74	12.30 ± 3.14	24.60 ± 2.46	6.00 ± 1.11
6-8	4	60.50 ± 2.43	18.20 ± 2.42	18.40 ± 1.38	2.90 ± 1.57

n = number of muscles analysed

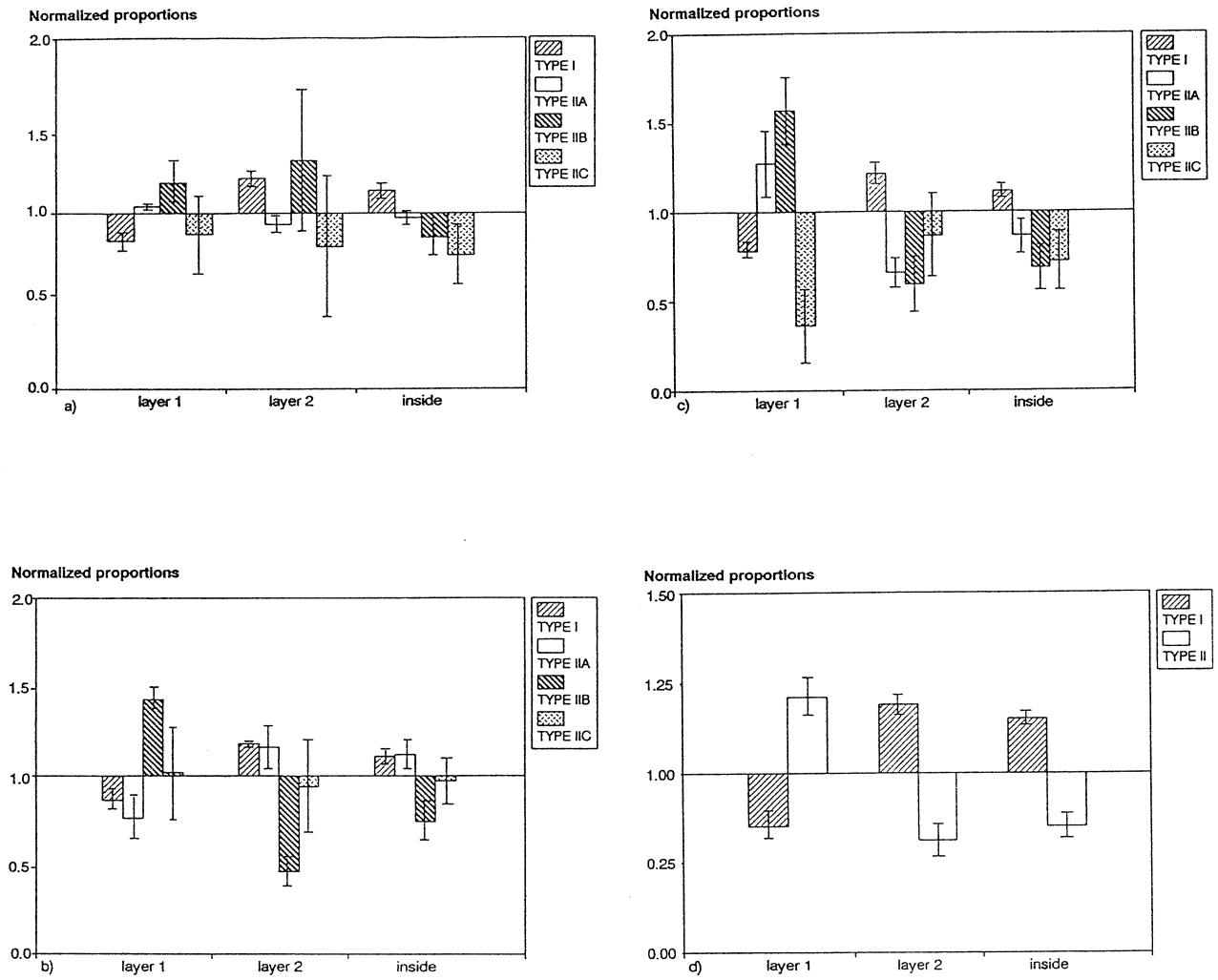

Fig. 1. Arrangement of muscle fibre types and subtypes within fascicles. Normalised proportions in first layer, in second layer and inside fascicles at different age intervals ($\bar{x} \pm SE$). a) 0-1 year, b) 1-2 years, c) 6-8 years, d) 10-15 years.

are arranged randomly or slightly disjoined in the whole age period.

Discussion

Differentiating muscle fibre types by histochemical procedures

It is easy to distinguish between type I and type II muscle fibres - histochemical procedures where the capturing agents are calcium ions [26] and those where phosphate is precipitated by lead ions [20] can be applied with equal certainty (our own observations). Though different staining procedures were used in our study [26, 41], we do not think that any bias can be claimed against differentiating type I and type II fibres. Subtyping IIA, IIB and IIC fibres is more tricky. In the muscles of younger children (0-1 year) we found it better to differentiate only type I and type II fibres, because subtypes of group II were practically impossible to determine with certainty. In certain cases differentiation caused problems even in the muscles of older children but the computer-aided method [28] does include some precautions regarding fibre typing. So-called "undetermined fibres", which are absent or very few (less than 5 percent) in the successful determination of fibre categories become numerous in critical cases - this is a good indicator that the histochemical reaction was not successful. Such samples were not included in the analysis. Histochemical reactions were repeated with some modifications of preincubation and incubation times on serial sections. If even further attempts did not succeed in providing clearer differentiation of fibre subtypes, only type I

and type II categories were determined. The main reason for impossible differentiation into IIA and IIB fibres is most probably coexistence of myosin heavy chains IIA and IIB in the same fibre.

New findings about isomyosins and about the possible presence of IIX heavy chains will probably lead to new classifications of fibre types and give a new insight into the processes going on during postnatal development [36].

Percentage of muscle fibre types

The fibre type composition of vastus lateralis muscle changes with age. The percentage of type I fibres increases from 32.2% in the first year of life to 59.15% during the age period from 2 to 3 years. Since the total number of muscle fibres in a muscle remains constant after birth [23], the number of type II fibres changes reciprocally. A similar increase in the percentage of type I fibres was observed by Oertel [25] within the first 2 postnatal years. This finding is in contrast with the previous widely accepted view that the histochemical differentiation of human skeletal muscles is completed at birth [6] and concurs with studies on experimental rats which state that under normal growth conditions, continous transitions of fibre types occur for many weeks to reach an adult profile [15]. In the age interval from 3 to 10 years, the percentage of both fibre types remains constant. Unchanged proportions of both fibre types are probably due to the matured neuromuscular system and balanced muscle fibre type conversions induced by locomotion. In that age period, histochemically matured muscle fibres just grow. In the age period from 10 to 15 years the percentage of type I fibres decreases to 44.19%. A similar decrease in the percentage of type I fibres was observed by Vogler and Bove [40] after 7 years and by Oertel [25] after 12 years of life. According to our knowledge, data about the percentage of muscle fibre subtypes are not available in literature. The results of our study show that type IIA fibres predominate (46,7% of whole fibre population) in the first postnatal year; the numerical density of IIB fibres is low (14.9%). Nearly all fibres are histochemically differentiated, while fibres of IIC type, whose presence indicates that transformational processes occur, are rare.

During the second postnatal year, the percentage of IIA fibres decreases and of IIB fibres increases. It is evident that IIA fibres partly convert into type I fibres and partly into type IIB fibres. This leads to the conclusion that during the growth and development of the neuromuscular system, the number of fibre types with the most specialised function increases.

At the age of 6 to 8 years, the percentage of muscle fibre types remains constant; only the percentage of muscle fibre subtypes changes. Since type IIA and IIB proportions are equal, and IIC fibres are rare, it has been concluded that in this period muscle fibre differentiation is in the final stage. It is evident that all transformations between slow type I and fast type II occur through IIA fibres. The same conclusions regarding transformations of fibre types can be drawn from immunohistochemical studies of myosin heavy chains. Some authors [34, 37] state preferential combinations of myosin heavy chains and thus assign probable directions of transformations between fibre types. Gorza [9] suggests that, in the view of newly discovered myosin heavy chain IIX in some animal species, transformations of fibre types occur according to the following scheme:

I ↔ I/IIA(IIC) ↔ IIA ↔ IIA/IIX ↔ IIX ↔ IIX/IIB ↔ IIB

The data indicate that type II to type I transformation during development is not completed at birth, but persists postnatally as a normal pattern of the maturational process. At the passage from intrauterine to postnatal development, fibre type differentiation is qualitatively and quantitatively unchanged.

Muscle fibre size

The average diameters of the both fibre types increase linearly with age. Only successive age groups can be compared, though no statistically significant differences were observed. Transversal growth of muscle fibre size does not differ between boys and girls. The mean diameters of muscle fibre subtypes are nearly equal. The diameter values are in close correlation with Oertel's data [25]. The diameters of muscle fibres in the age group of 10 to 15 years reach those values, which are the lower ones of the diameters for healthy adult muscles [3].

Arrangement of fibre types within fascicles

Most studies dealing with the arrangement of fibre types within fascicles are qualitative [13, 24]. Lexell at al. [17] were the first investigators to show the difference in the proportions of type I and type II fibres between the periphery and the internal regions of human muscle fascicles. Pernuš and Erzen [29] were the first who showed the difference in normalised proportions of fibre types I, IIA and IIB in layer 1, layer 2, layer 3 and internal region in vastus lateralis muscle of young healthy men. In this study, normalised proportions of fibre types I, IIA, IIB and IIC were determined in layer 1, layer 2 and in the internal region of the fascicles in different age groups. A higher proportion of type II fibres was detected in the first layer of fascicle and type I fibres in layer 2 and in the internal region in all age groups. In the type II fibre group, IIB fibres predominate in the first layer in all age groups. Two possible explanations for the specific topographic distributions of fibre types have been proposed. According to the first hypothesis local factors such as connective tissue and vascularization and different functional demands on different skeletal muscles are the principal cause for the specific fibre type distribution [27, 35]. The second hypothesis supposes that a specific fibre type arrangement results from the developmental processes in fibre type differentiation [19, 32]; a combination of both explanations is not excluded [29]. Primary myotubes containing slow myosin are germs of type I fibres, which constitute a primary generation of fibre types. Under the basement membrane of primary generation the second generation of myotubes containing fast myosin differentiates and they

are destined to be fast twitch fibres. The next generations, tertiary myotubes, are formed within the basement membrane of secondary myotubes [5]. Containing fast myosin, they could be predestined IIA fibres, which may further develop into type I or type IIB fibres. Because each new generation of myotubes originates around the previous, such topographical distribution is reasonable (with a preponderance of earlier formed fibres inside and the latest formed fibres on the periphery of a fascicle).

Spatial distribution of fibre types on the muscle cross plane

In healthy adult skeletal muscles, both fibre types are distributed at random and give a mosaic pattern on a muscle cross plane. In some circumstances (denervation, followed by collateral innervation, cross-innervation) the fibre types become arranged in groups or clusters [12]. For spatial distribution estimation the following measures were proposed: z [39], number of enclosed fibres (NEF) [14], co-dispersion index (CDI) [16]. No one measure has been accepted widely. In the present study an attempt was made to set standards of spatial distribution at several successive periods of healthy developing muscles, applying the newly developed measure index of normalised dispersion (DISPnorm) [30]. Since fibre types were distributed randomly to slightly disjoined at all age intervals, the mosaic distribution pattern was proved, which is characteristic of healthy adult muscles. Data on the comparison of other measures for normal autopsy material of vastus lateralis muscles of different age will be given elsewhere (in preparation).

Though alterations of the normal mosaic distribution pattern due to conversions of fibre types throughout the developmental period were expected, the results lead to conclusions about specific genetically determined distribution, governed by developmental processes. It is well known that multiple types of intrinsically different myoblasts exist in particular stages of myogenesis and are predestined to form different muscle fibre types [22]. Their specific location determines the distribution pattern of developing muscle fibre types [21, 33] and most probably limits their extensive translocations.

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