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Muscle fiber size in healthy children and adults in relation to sex and fiber types

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Abstract

Background: In adult males, cross-sectional area (CSA) for type II muscle fibers is generally larger than for type I fibers. In this cross-sectional study the aim was to compare sex-related CSAs of various muscle fiber types during childhood-to-adulthood transition.

Methods: Percutaneous biopsy samples were obtained from vastus lateralis in 10-y-old children (10 males and 5 females) and in young adults (9 males and 7 females). Fiber types were classified by myofibrillar ATPase and CSAs from NADH-dehydrogenase staining.

Results: Type IIA were larger than type I fibers in adult males, but not in adult females or children (age x sex x fiber type, $P < .002$). When including all participants, body weight and sex explained 78% of the variation in type IIA CSA but only body weight contributed for type I.

Conclusions: Sex-specific patterns in CSA of the muscle fiber types appears to develop during the transition from childhood to adulthood.

KEYWORDS
age, cross-sectional area, human, muscle biopsy, vastus lateralis

1 | INTRODUCTION

The cross-sectional areas (CSAs) of various skeletal muscle fiber types differ between sexes in adults. Generally, the CSAs of the various muscle fiber types are larger in males compared to females and the sex difference in CSA is larger in type II than in type I fibers. Moreover, type IIA fibers are typically the largest in males, whereas in females type I fibers are either larger or of similar size as type IIA fibers.[1-3] While there are some indications in the literature that such sex-related patterns develop during the transition from childhood to

adulthood,[4-8] the number of male and female children in relevant age spans did not allow for a sex-related statistical evaluation. One concern is that these data were based on autopsy or clinical material and no information on anthropometrics or physical training status was presented. We are aware of only two earlier studies examining biopsy material collected from leg muscles of healthy children of both sexes, although only fiber type composition and not CSA was reported.[9,10]

Additional studies in healthy children, therefore, are warranted to confirm earlier indications from autopsies or clinical material about the development of these sex-related differences in CSA of the various muscle fiber types typically apparent in adults. Extending the existing reference material in children by including biopsy material from

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Abbreviations: ANOVA, analysis of variance; BMI, body mass index; CSA, cross-sectional area; CV, coefficient of variation; MHC, myosin heavy chain; NS, not significant.

healthy children of both sexes could also be important for the diagnosis of skeletal muscle diseases.[4]

Accordingly, the aim of the present cross-sectional study was to examine sex-related differences in CSA of different muscle fiber types during the transition from childhood to adulthood. It was hypothesized that the typical male pattern with larger type IIA than type I fibers would not be observed in children.

2 | METHODS

2.1 | Participants

The children were recruited from individuals undergoing minor surgery, such as for inguinal hernia. They were otherwise healthy. The young adults were recruited from healthy university students. Data on muscle fiber type composition have been published earlier from these subjects,[11] but not on CSA of the muscle fibers. Body weight and height were measured and body mass index (BMI) (kg × cm^{-2}) was calculated.

All children participated in various types of physical activities such as swimming, horseback riding, soccer, basketball, or ice hockey for approximately 2 h/wk in addition to the regular physical education in school. All of the young adults participated in physical activities, such as jogging, aerobics, or tennis, for approximately 45 min/wk following the regular physical education during their time in school.

All the female children were pre-menarche, and all the female adults reported normal menstruation. All subjects and the parents of the children were fully informed verbally and in writing before consenting to participate in the study. The study was approved by the Ethics Committee of the Karolinska Hospital.

2.2 | Experimental protocol and analyses

Percutaneous needle biopsies[12] were obtained from vastus lateralis muscles of the children under general anesthesia and from the adults under local anesthesia. The biopsies were mounted in an embedding medium, frozen in isopentane precooled with liquid nitrogen, and stored at -80C° until analysis. The biopsies were freeze-cross-sectioned with a thickness of 10 μm after careful orientation to obtain cross-cut and analyzed histochemically using a myofibrillar adenosine triphosphatase (ATPase) stain at pH 9.4 after preincubations at pH 10.3, 4.6 and 4.3 for the identification of the four fiber types I, IIA, IIB, and IIC.[13] The CSA of the various fiber types was measured by planimetry by one observer from a NADH-dehydrogenase[14] stain as described earlier.[15] ATPase fiber classification combined with the NADH-dehydrogenase stain for area measurements was performed as previously published.[16] Biopsies were carefully oriented to obtain cross-cut sections of the muscle fibers. The values of CSA from each biopsy represent a mean value of 20 fibers for type I and of 15 fibers for each of type IIA and type IIB. There were too few IIC fibers to enable CSA measurement of this type. The given value of CSA for each individual is based on two biopsy samples in the adults but only one sample in the children due to their thin muscle bellies.

The variability in CSA is considerably less within a biopsy than between biopsies on the assumption that 15–20 fibers were measured per fiber type and sample.[17] In order to decrease the methodologic error, it is therefore recommended that measurements be based on two biopsies as done for the adults in the present study instead of increasing the number of measured fibers within a biopsy sample. The coefficient of variation was reported to be 10–15% when 15–20 fibers of each fiber type were measured in one single biopsy and 7–11% based on two biopsies.[17]

2.3 | Calculations

Fiber type composition was expressed as the relative number of the different fiber types I%, IIA%, IIB%, and type IIC%. Fiber type composition was also expressed as relative fiber type area by the formula (type I% + IIC%) x type I CSA x 100 ((type I% + IIC%) × type I CSA + type IIA% × type IIA CSA + type IIB% × type IIB CSA))$^{-1}$. The relative type IIA and type IIB was calculated applying corresponding equations. The intraindividual variation in CSA of the various fiber types was presented by the coefficient of variation (CV), calculated by dividing the intraindividual SD of CSA for each of the fiber types by the mean of the CSA for each individual multiplied by 100.

2.4 | Statistics

A two-way analysis of variance (ANOVA) was applied to analyze the effect of age (children and adults) and sex (females and males) on background variables and fiber type variables. In case of interaction, Student's t-test for group was performed. A three-way ANOVA was applied to analyze the effect of age, sex, and fiber type (I, IIA, and IIB; I and IIA; I and IIB or IIA and IIB) on CSA. (Figure 1). In the case of an
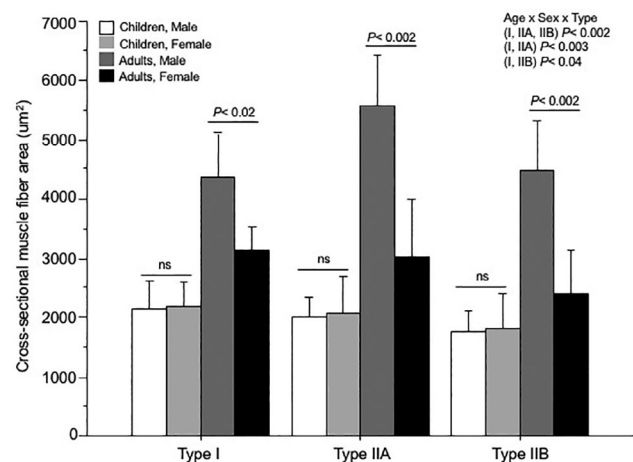

FIGURE 1 Muscle fiber CSA of type I, IIA, and IIB in 10 male and 5 female children and 9 male and 7 female adults. The statistical outcome of the three-way ANOVAs (age × sex × fiber type) is indicated by P-values. Statistical significance between the sexes for each age group is indicated by P-values between the bars

interaction between these three factors, a two-way ANOVA was applied to analyze the effects of age and fiber type or of sex and fiber type on CSA. In case of interaction, Student's t-test for group or for paired analysis was performed. Multiple regression analysis was applied to analyze the impact of body weight and sex on CSAs, with the dependent variables CSA of type I, IIA, or IIB and the independent variables sex, body weight, and interaction sex × body weight. Differences were accepted as statistically significant at the level of $P < .05$.

2.5 | Sex or gender

Sex and gender are often used terms and interchangeable in scientific papers, but sex strictly refers to biological and gender to social and cultural differences. In reality, however, the body is influenced by both sex and gender and both contribute to form the phenotype.[18] Regarding skeletal muscle, for instance, CSA is influenced by sex (chromosomes or hormones), but also by behavioral factors such as physical activity. The nomenclature males and females corresponds to the term sex. Women (girls) and men (boys) or other non-binary identities corresponds to gender. We have chosen the nomenclature sex, females, and males in the current study, even though we are aware that phenotype is influenced by both sex and gender.

3 | RESULTS

3.1 | Participants

In total, 31 individuals of both sexes took part in the study, 15 of whom were children and 16 were young adults. The children were, on average, 10 (range 9–11) y old and the adults 21 (range 19–25) y old with no significant difference in age between males and females. In the children, no sex differences were found for height and body weight, while the adult males were taller and heavier than the adult females (Table 1).

3.2 | Fiber CSA

A sex-related pattern with larger type IIA than type I fibers in adult males compared with adult females or children was indicated by the three-way ANOVA (Figure 1). For further statistical evaluation, the study population was dived by age (sex and fiber type included in a two-way ANOVA). In children, there were no significant differences in CSA between sexes for any of the fiber types (no interaction between sex and fiber type or main effect of sex, t-tests presented in Figure 1). However, both type I (t-test $P < .004$) and type IIA (t-test $P < .001$)

TABLE 1 Muscle fiber characteristics and anthropometrics in children and adults of both sexes

Group of age	Children		Adults		P<		
Gender (n)	Male (10)	Female (5)	Male (9)	Female (7)	Age	Sex	Age x sex
Age (y)	10 ± 1	11 ± 1	21 ± 2	21 ± 1	0.001	NS	NS
Body weight (kg)	37 ± 8	35 ± 7	77 ± 8***	57 ± 5	0.001	0.001	0.002
Height (cm)	144 ± 9	148 ± 7	184 ± 6***	166 ± 5	0.001	0.03	0.001
Type I CSA (μm²)	2133 ± 441	2173 ± 411	4243 ± 812**	3140 ± 409	0.001	0.02	0.01
Type IIA CSA (μm²)	2048 ± 363	2076 ± 628	5449 ± 910***	3036 ± 977	0.001	0.001	0.001
Type IIB CSA (μm²)[a]	1732 ± 368	1805 ± 592	4479 ± 847***	2420 ± 723	0.001	0.001	0.001
CV of type I CSA (%)	28 ± 6	30 ± 8	28 ± 6	32 ± 5	NS	NS	NS
CV of type IIA CSA (%)	21 ± 5	23 ± 7	22 ± 5	18 ± 6	NS	NS	NS
CV of type IIB CSA (%)[a]	24 ± 6	24 ± 6	20 ± 5	20 ± 8	NS	NS	NS
Type IIA/I CSA	0.98 ± 0.2	0.95 ± 0.2	1.30 ± 0.2***	0.95 ± 0.2	0.03	0.01	0.03
Type IIB/I CSA[a]	0.82 ± 0.2	0.81 ± 0.2	1.03 ± 0.2**	0.76 ± 0.1	NS	0.04	0.05
Type IIB/IIA CSA[a]	0.86 ± 0.1	0.87 ± 01	0.80 ± 0.1	0.82 ± 0.2	NS	NS	NS
Type I (%)	63 ± 6	57 ± 10	56 ± 10	53 ± 5	0.07	NS	NS
Type IIA (%)	28 ± 5	29 ± 6	32 ± 10	33 ± 7	NS	NS	NS
Type IIB (%)	9 ± 5	13 ± 6	11 ± 7	13 ± 4	NS	NS	NS
Type IIC (%)	1 ± 2	0 ± 0	1 ± 2	1 ± 1	NS	NS	NS
Type I CSA (%)	65 ± 7	60 ± 10	53 ± 10	57 ± 6	0.02	NS	NS
Type IIA CSA (%)	28 ± 7	28 ± 5	37 ± 10	33 ± 7	0.04	NS	NS
Type IIB CSA (%)	7 ± 5	12 ± 6	10 ± 7	10 ± 3	NS	NS	NS

Note: Values are mean and SD.
*** Denotes $P < .001$ and ** denotes $P < .01$ and indicates statistical level of sex difference.
Abbreviations: CV, coefficient of variation of the intraindividual variation in CSA (see methods).
[a]Children: male, n = 9; adults: male, n = 8.

fibers were larger than type IIB in children, independent of sex (main effect of fiber type, $P < .001$). In adults, the CSA of all fiber types were larger in males than females (t-tests presented in Figure 1) with a larger sex differences for both type IIA (t-test, $P < .001$) and type IIB (t-test, $P < .02$) than for type I (sex × fiber type, $P < .001$). The study population was alternatively divided by sex (age and fiber type included in the two-way ANOVA). In adult males, the type IIA were larger than type I fibers ($P < .0005$), but not in male children (NS); (age × fiber type, $P < .001$). In adult females and female children, however, the type IIA and type I fibers were of similar size (age x fiber type, $P > .05$).

3.3 | Ratio of CSA

Interactions were found between age and sex for ratio of CSA IIA/I and IIB/I (Table 1) and t-tests were performed to identify the differences between the groups. In the children, there were no significant differences in ratio of CSA IIA/I, IIB/I, or IIB/IIA between sexes ($P < .76$, $P < .92$ and $P < .98$). In adult males, the ratio of type IIA/I and IIB/I were both higher than in adult females ($P < .001$ and $P < .004$). The ratio of IIA/I and IIB/I was higher in the adult males compared to the male children ($P < .002$ and $P < .03$), while there was no significant difference in any of the ratios between adult females and female children.

3.4 | Intraindividual variation in CSA of various fiber types

The intraindividual variation in CSA for each of the different fiber types (CV of CSA) did not differ between sex or between age (no main effects of sex or age or no interactions sex x age, Table 1). When comparing the intraindividual variation of the CSA between the fiber types by a three-way ANOVA (age, sex, and fiber type), the intraindividual variation in CSA of type I fibers (CV of CSA) was larger than for type IIA and type IIB fibers independent of age and sex (main effect of fiber type, $P < .001$). CV of type I CSA was larger than both CV of type IIA and IIB CSA (t-test, both $P < .001$).

3.5 | Fiber type composition and relative fiber type area

The percentage of type I fibers (type I %) tended to be higher (see main effect of age, Table 1) in children than in adults (61 ± 8% vs 55 ± 8%, pooled values for each age, not given in Table 1). The relative type I fiber area was significantly higher (63 ± 8% vs 54 ± 8%, pooled values for each age not given in Table 1) and the relative type IIA area was significant lower (28 ± 6% vs 35 ± 9%) in children than in adults (see main effects of age, Table 1). The proportion of the intermediate fiber type IIC (type IIC%) was generally low in all groups and the relative type IIC area was not calculated.

3.6 | Relationship between CSA and body weight

A direct relationship was demonstrated between CSA and body weight for all three fiber types, when including all participants (Figure 2, correlation for type IIB not presented). When analyzing the impact of body weight and sex on CSA (dependent variable) for the different fiber types a multiple linear regression was applied. An interaction was found for CSA of type IIA (Figure 2) and a tendency for type IIB (sex × weight; $P = .05$), but not for type I, in the multiple regression analysis. This means that, for one and the same body weight, the CSA of fiber type IIA was significantly larger in males than in females. Body weight and sex explained 78% of the individual variation in type IIA fiber CSA. For type I fibers, only body weight
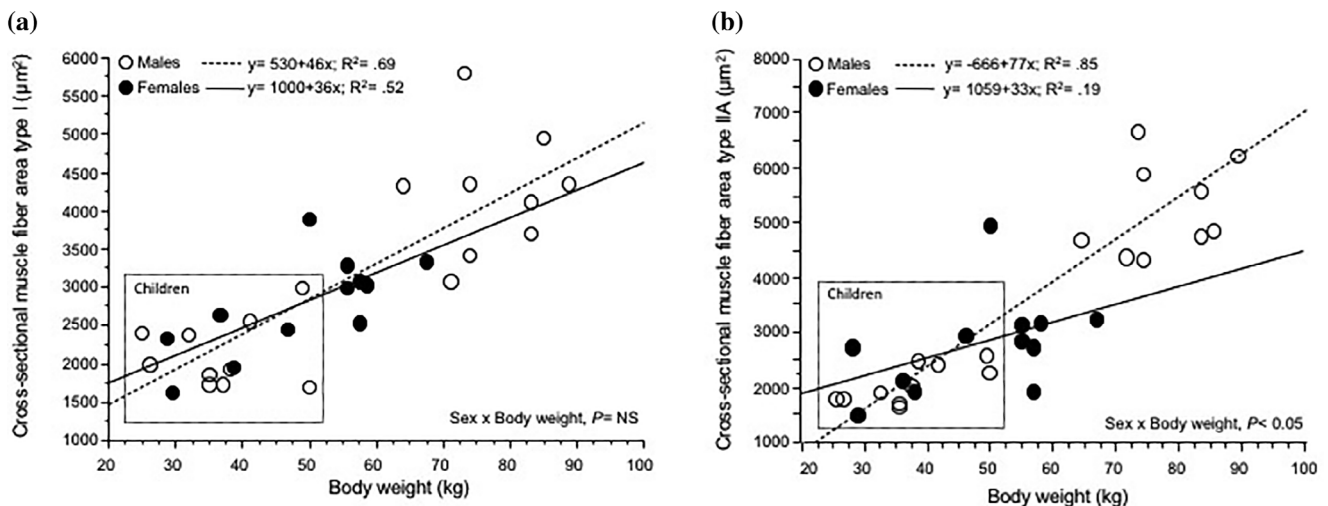

FIGURE 2 Relationship between muscle fiber CSA of type I and body weight (A) and type IIA and body weight (B). All 31 subjects were included in the multiple regression analysis. The level of significance of the interaction of sex and body weight is indicated by P-value and the degree to which the regression model explains the data is denoted by R^2

contributed significantly and explained 67% of the variation in CSA, (P < .02). Age might be a confounding variable at least for the children, as age tends to correlate with body weight until adulthood is achieved. This was tested in multiple linear regression analyses, including all subjects, with CSA as dependent and age, body weight, and sex as independent variables. Only body weight contributed significantly to explain the variation in CSA for each of the fiber types and may have captured the variation in CSA explained by age and sex.

4 | DISCUSSION

4.1 | Main findings

The typical male pattern regarding the size of the various muscle fiber types develops during the transition from child-to-adult: A shift in CSA from type I = IIA > IIB to type IIA > IIB = I was seen in the males but not in the females. Moreover, both body weight and sex contributed to explain the variation in type IIA CSA, but for type I, only body weight contributed as demonstrated by multiple regression analyses including all subjects.

4.2 | Comparisons with earlier studies in children

The findings of equal size of type I and type IIA fibers and slightly smaller type IIB in children of both sexes in our study confirm earlier indications by Oerthel[6] and Brooke and Engel.[4] Oertel et al.[6] examined autopsy samples from children that had suddenly died in accidents. However, the number of children of each sex in relevant age span was too low to address the current sex-related study question. Brooke and Engel[4] examined biopsy samples from children with neuromuscular diagnoses with varying degrees of symptoms, but no data were available from healthy children. Oertel et al.[6] examined autopsy samples from children that had suddenly died in accidents. However, the number of children of each sex in relevant age spans was too low to address the current sex-related study question. The biopsy samples in the present study were derived from children that underwent a minor surgery but were all physically active and otherwise healthy.

4.3 | Comparisons with earlier studies in adults

The sex difference in fiber area ratio type IIA/I is generally very consistent among young and middle age adults[1,2,19] and old adults,[19,20] even though the IIA/I ratio is generally lower in the old than in young adults.

The present study reports data from the lower limb muscle, vastus lateralis, as most studies on sex-related differences on morphological characteristics of adult human skeletal muscle.[1,2,6] There are, however, some reports of data from upper limb muscles, such as biceps brachii and deltoid in adults.[5,6,21,22] Principally, the same sex-related pattern is found in the upper limb muscles with a larger IIA/I ratio in males than females in both young and old adults.[5,21,22]

4.4 | Sex-related pattern in muscle CSA of fiber types

The reason for the sex-related pattern in muscle CSA of the various fiber types in adults but not in children with a larger type IIA/I ratio in males than in females is unknown. In the present study the influence of body weight was analyzed. The interaction between body weight and sex for type IIA CSA (Figure 2) indicates that the larger body weight in adult males compared with females is not the only factor explaining the larger type IIA CSA in adult males. Conversely, the lack of interaction between body weight and sex for type I CSA indicates that body weight is a major factor explaining the interindividual variations in type I CSA.

As sex-related hormonal profiles develop during puberty, it is natural to seek explanations of the sex-related fiber area pattern among changes within hormonal profiles.[23-27] In humans, however, there is no support for a testosterone-induced selective type II fiber hypertrophy.[28] On the contrary, in a study of resistance training in healthy men, a selective increase in myonuclear number was recorded in type II fibers that was blocked by suppression of testosterone. Such an increase in myonuclear number may increase the hypertrophy potential in type II fibers.[29]

Another possible explanation is related to sex-related differences in physical activity pattern, such as type and intensity of exercise. For instance, studies in 16 y-old teenagers demonstrated that boys performed more exercise at vigorous intensity than did girls.[30] Possibly, a sex-related physical activity pattern develops from childhood to young adulthood as indicated in a recent cross-sectional study on boys and girls from the age of 5–18, where the amount of vigorous physical activity decreased more in girls than boys over this period.[31] This may contribute to the sex-related phenotype that develops from child-to-adulthood. Moreover, studies on resistance or sprint exercise training in young adults found that all fiber types can increase in size and especially type II fibers in women, thereby achieving a more adult male fiber area pattern.[32,33] However, after 20 wk of resistance training, the type IIB fibers in women were still smaller than type I, that is within the range of the female pattern. In female elite bodybuilders, a more male fiber area pattern was evident, with a IIA/I fiber area ratio > 1, but this ratio was even larger in male elite bodybuilders.[9] In line with this, Brooke and Engel[5] reported that mothers of severely disabled children had a fiber area profile more like adult males in biceps brachii, but still a sex difference was demonstrated.

It is concluded from the literature that the sex-related pattern in muscle CSA of the various fiber types in adults, but not children, could be due to sex differences in physical activity pattern, systemic hormonal differences, or possible interactions between such factors.

4.5 | Relative type II fiber, anaerobic potential and muscle power

The finding of a larger relative type II fiber area in the adult males than in the male children in our study suggest that the adult males have a

greater local anaerobic potential. This agrees with earlier findings[34] reporting that adult males as compared to male children had a more anaerobic profile, with both higher basal muscle phosphofructokinase activity and greater maximal muscle lactate accumulation in response to exercise. Moreover, a well-known sex-specific increase in muscle power from child-to-adult with larger increase in males[35] could partly be related to the sex-specific increase in relative type II fiber area. This fiber type is known to develop higher peak power than type I even after adjustment for size.[36]

4.6 | Child-to-adult difference in muscle size

In the present study, there was a highly significant correlation between body weight and the CSA of the muscle fibers. This together with the finding that the fold-increase from child-to-adult of the CSA of both the muscle fibers and the body weight were similar, supports the earlier finding that the increase in muscle size from child-to-adult results mainly from hypertrophy, that is an increase in size of the existing fibers, rather than from hyperplasia, that is an increase in the number of fibers.[37]

4.7 | Limitations

One limitation is the low number of female children included in the study. The reason for this was that they were recruited from children who underwent a minor surgery for at first hand inguinal hernia, which is much more common in male children than in female children. However, the findings of a similar size of type I and type IIA fibers and somewhat smaller type IIB fibers in the female children were supported by data of seven 10 y-old female children that a few weeks before the biopsy had started professional dance training (type I, $1987\mu m^2$; type IIA, $1920\mu m^2$; and type IIB, $1556\mu m^2$; our unpublished data). Moreover, the specific hypothesis of the study that the typical adult male pattern with larger type IIA than type I fibers develops during the transition from child-to-adulthood was independent of the number of female children included in the study, which reduces the significance of this limitation.

In the present study the classical ATPase stain after alkaline or acid preincubation was applied to identify type I, IIA, IIB, and IIC fibers.[13] This might be regarded as a limitation because new antibody techniques have been developed to better distinguish between various fiber types with a broader range and a higher specificity of myosin or myosin heavy chain (MHC) isoform. Strong correlations have been demonstrated between mATPase staining patterns and myosin or MHC isoforms in human limb muscle with some limitations for hybrid fibers.[38-42] Therefore, in the present study of vastus lateralis biopsies in children and young adults it is not very likely that the results and conclusions would be different if fiber types had been identified by antibody techniques, especially as very low numbers of the intermediate fibers (IIC) were identified in both children and adults. The advantages with the ATPase stain is that it is inexpensive, reproducible, stable, and is not dependent on antibody specificity and availability.

4.8 | Conclusions

The typical adult male pattern with larger type IIA than type I fibers was apparent in young male adults but not in male children. The sex-related pattern in CSA of the various fiber types in young adults thus likely develops during the transition from childhood to adulthood. A major portion of the interindividual variation in CSA of type IIA and of IIB fibers, in the combined group of children and adults, could be explained by body weight and sex. However, for type I fibers, only body weight contributed to the variation in CSA. From the literature there are indications that the sex-related pattern in muscle CSA of the various fiber types in adults, but not children, could be due to differences in physical activity or systemic hormonal levels between the sexes that develops during transition from childhood to adulthood.

CONFLICTS OF INTEREST

None of the authors has any conflict of interest to disclose.

ETHICAL PUBLICATION STATEMENT

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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