Comprehensive morphometric assessment of deltoid muscle development in children: A cross-sectional study

Teresinha Evangelista,[a,b,c,i,*] Malick Kandji,[d,i] Emmanuelle Lacene,[a] Anaïs Chanut,[a] Mai Thao Bui,[a] Rudy Marty,[d] Laurent Buffat,[e] Kenneth Knoblauch,[f,g] Brian B. Rudkin,[d,f,h,j,**] and Norma Beatriz Romero[a,b,c,j]

[a]AP-HP, H. Pitié-Salpêtrière, Institut de Myologie, Unité de Morphologie Neuromusculaire, France
[b]AP-HP, H. Pitié-Salpêtrière, Centre de référence des maladies neuromusculaires Nord/Est/Ile de France, France
[c]Sorbonne Université, INSERM, Institut de Myologie, Centre de Recherche en Myologie, France
[d]CARPACCIO.cloud, Lyon, France
[e]AltraBio, Lyon, France
[f]Univ Lyon, Université Claude Bernard Lyon 1, Inserm, Stem-cell & Brain Research Institute, Bron, France
[g]National Centre for Optics, Vision and Eye Care, Faculty of Health and Social Sciences, University of South-Eastern Norway, Kongsberg, Norway
[h]Shanghai Jiao Tong University Institute of Medical Genetics, Shanghai Children's Hospital, Shanghai, PR China

Summary

Background Normative values for different morphometric parameters of muscle fibres during paediatric development, *i.e.* from 0 to 18 years, are currently unavailable. They would be of major importance to accurately evaluate pathological changes and could be used as reference biomarkers for evaluating treatment response in clinical trials, or physiological adjustments in sports or ageing.

Methods Data were derived from 482 images with a total of 33 094 fibres from 10 μm cross-sections of snap-frozen muscle from 83 deltoid muscle biopsies from patients, 0–18 years, without neuromuscular pathology stained with ATPase 9.4. Data was acquired and analysed with patented image analysis algorithms from "CARPACCIO.cloud". Several parameters were extracted or calculated, including cross-sectional area (CSA), fibre type, circularity, as well as the Minimum diameter of Feret (MinFeret).

Findings This study illustrates changes in quantitative parameters for muscle morphology over the course of paediatric development and the pivotal changes occurring around puberty. Only fibre size parameters (MinFeret, CSA) are dependent on gender, and only after puberty. All other parameters vary in a similar manner for females and males. The proportion of type 1 fibres is essentially constant from birth to age 10, decreasing to ≈40% by age 18. Circularity decreases with age, to plateau after age 10 for both fibre types.

Interpretation Normative values and reference charts for muscle fibre types in this age range have been generated to allow comparison of data from patients in pathology laboratories working on neuromuscular diseases.

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Keywords: Normal skeletal muscle; Human; Morphometry; Paediatric age; Automated analysis

Introduction

Data on skeletal muscle fibre size and proportion, in histologically normal subjects in the paediatric age, is scarce. In 1969, Brooke et al. published a series of papers on the histographic analysis of human muscle biopsies. In the first publication[1] the authors analysed the

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Research in context

Evidence before this study
Data on skeletal muscle fibre size and proportion, in histologically-normal subjects in the paediatric age, is scarce. In 2000, Staron et al. published what became one of the reference papers for the normative values of the *vastus lateralis* muscle fibre type distribution and histographic composition in young adults.

Added value of this study
With this work, we have defined normative values for muscle fibre diameter and cross-sectional area, as well as the fibre type distribution in morphologically normal skeletal muscles biopsies obtained from the deltoid muscle in children aged 0–18 years old.

Implications of all the available evidence
This study allows us to offer graphs that can be used as a reference for pathology laboratories analysing biopsies of deltoid muscle. These graphs are of major importance to accurately evaluate pathological changes, and can be used as reference for determining treatment response in clinical trials, or to evaluate physiological adjustments in sports or ageing.

variation in fibre size and fibre type distribution in adult muscle biopsies and compared male and female values. The muscle biopsies included three heterogeneous groups: asymptomatic relatives, individuals with neuromuscular complaints without evidence of organic disease and those with organic disorders who had no evidence of neuromuscular involvement at the time of the biopsy. The last paper in this series of 4 is dedicated to biopsies of children under the ages of 15 years old.[2] The authors analysed different disease categories, and the results were presented according to the histographic appearance of the biopsies. Moore et al.[3] published a study in which they showed the normal mean cross-sectional diameters of human striated muscle fibres in post-mortem material ranging in age from five months gestation through senescence. Measurements represented the mean narrow fibre diameter of celloidin-embedded material. One paper[4] studied 28 healthy controls with an age range 18–34 years old. In this paper, the authors used the nicotinamide-adenine dinucleotide-tetrazolium reductase (NADH-TR) reaction to determine the fibre type distribution. There is a mention of the diameter of the fibres without a proper explanation on how those values were achieved. The authors report mainly on incidental findings in normal individuals such as central nuclei, necrosis, or type grouping. An interesting study published in 1991[5] that reports a cohort of 69 boys and 47 girls age 16 years old, establishes some guidance regarding the inter-individual and inter-sex variation in the proportions of muscle fibre types, the muscle fibre area and muscle enzyme activities. The authors conclude that the percentage of type I fibres in the *vastus lateralis* was 54% for the boys and 52% for the girls and that, according to the literature and their work, there is a tendency for physically trained individuals to have a higher percentage of type I fibres. This study showed that boys had larger type IIA than type I fibres, unlike girls, where the size of IIA and type 1 fibre was similar. Moreover, the boys had equal size type IIB and type I fibres, whereas the girls had smaller type IIB than type I fibres. However, no normative values were given in this study. In 2000, Staron et al.[6] published what became one of the reference papers for the normative values of the *vastus lateralis* muscle fibre type distribution and histographic composition in young adults.

A systematic evaluation of the cross-sectional area, fibre diameter and fibre type distribution in frozen sections of morphologically normal muscle biopsies in the age range 0–18 years old is not available. The existence of normative values for fibre size and fibre type distribution helps to evaluate the pathological changes, such as fibre type predominance, or atrophy/hypertrophy of fibres, but can also be used as a biomarker for treatment response in clinical trials, or to express physiological adjustments occurring in sports or ageing.

This work aimed to define normative values for muscle fibre diameter and cross-sectional area, as well as the fibre type distribution in morphologically normal skeletal muscles biopsies obtained from the deltoid muscle in children aged 0–18 years old. Since accurate measurement of muscle fibre size and evaluation of fibre type distribution in cross-sectional areas is a time-consuming subjective process, when done manually, we have used the image analysis software from the innovative start-up in digital pathology, CARPACCIO.cloud to generate standardized results.

Methods
Study design and participants
We analysed 83 deltoid muscle biopsies referred to our laboratory for diagnostic purposes and found to be morphologically normal. The biopsies were open muscle biopsies. Two-experienced pathologists (TE and NBR) reanalysed the muscle biopsies to confirm the absence of histological abnormalities. The subjects were between the ages of 0 and 18 years old, 33 females and 50 males. Age at biopsy was expressed in years and calculated as the difference between the date of biopsy and date of birth.

Ethics

The individuals or their legal guardians gave written informed consent for the biopsy and for reuse of data for research.

The study was not submitted to an ethics board as the biopsies were done previously to the study in a diagnostic setting. However, at the time of the biopsy, all patients give informed consent for the use of the biopsy/biopsy material for research.

Sample preparation and imaging

We have used 10 μm cross-sections of snap-frozen muscle stained with adenosine triphosphatase pre-incubated at pH 9.4 (ATPase 9.4) (Fig. 1A).[7] Digital photographs of the biopsies were obtained with a Zeiss AxioCam HRC linked to a Zeiss Axioplan Bright Field Microscope and processed with Axio Vision 4.4 software (Zeiss, Germany).

We used between one and fifteen non-overlapping images (average of 6) per biopsy at the magnifications of ×40, ×20 or ×10 depending on the age of the child, with a mean of 69 fibres per image. The aim was to include at least 100 muscle fibres per individual - the average was 390 (range 81–2146) per individual. Data from a total of 482 images with a total of 33 094 fibres were acquired and analysed.

Image analysis

We have used the image analysis software from the innovative start-up in digital pathology, "CARPACCIO.cloud" for data capture. The software determines the position of the perimeter of each fibre and qualifies the cytoplasmic labelling. For this study, we extracted or calculated the following parameters for each muscle fibre: perimeter, cross-sectional area (CSA); x, y co-ordinates of the centre, indicators of circularity, solidity and intensity and the fibre type: type 1 or type 2, as well as the overall surface occupied by the fibres (Fibre surface density). The fibres' smallest diameter i.e. Minimum diameter of Feret (MinFeret) was also determined to avoid processing artefacts such as kinking of the muscle or obliquity of the fibres. Fibres at the periphery of the sections that were not entirely captured in the photographs were excluded from the analysis (Fig. 1).

Statistical analysis

Our objective was to find a reasonable description of the data in an agnostic fashion since we did not have any a priori knowledge of a mechanism for the dependence on age, nor the distribution of the response. We proceeded in an exploratory fashion using Local Polynomial Regression modelling i.e. LOcally Estimated Scatterplot Smoothing (LOESS) and Box–Cox transformation to obtain a good account of the data. Before model optimization, the data for each parameter was fitted using LOESS to find the local trends in the data and to ascertain which types of models could best reflect the evolution of the parameters within the observational age window. This was performed according to gender and fibre type. Initially, a linear initial model was evaluated: if the hypotheses of a linear regression model were not satisfied, data was transformed using Box–Cox transformation, otherwise the work was done on non-transformed data. Dependency on gender, age or fibre type was systematically determined via backward and forward model selection; then nested likelihood ratio tests and subsequent analyses were implemented taking into account the corresponding results.

Parameters were plotted as a function of age, gender and fibre type. Best-fit curves and models were generated from the untransformed or transformed data using R software[8] via the RStudio interface.[9] For each parameter, a global model was created using a linear, polynomial, bi-linear or Gompertz regression. The choice of explanatory variables (age, sex, fibre type) was achieved
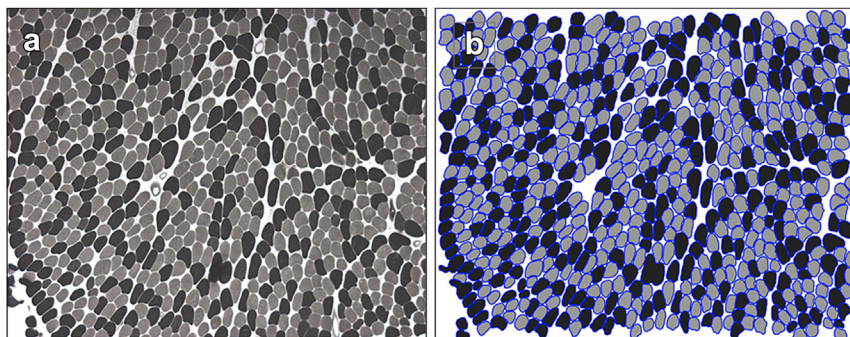

Fig. 1: Image of a Muscle cross-section and corresponding rendering of CARPACCIO.cloud. **a.** Muscle cross-sections stained with ATPase at pH 9.40 are photographed, then analysed using the CARPACCIO.cloud algorithms. **b.** The software identifies the fibre perimeter and the fibre type (Light: Type 1; Dark: Type 2), generating a rendering representing the individual fibres and their type, excluding the peripheral ones that are not intact. The quantitative data subsequently extracted is based solely on the intact fibres.
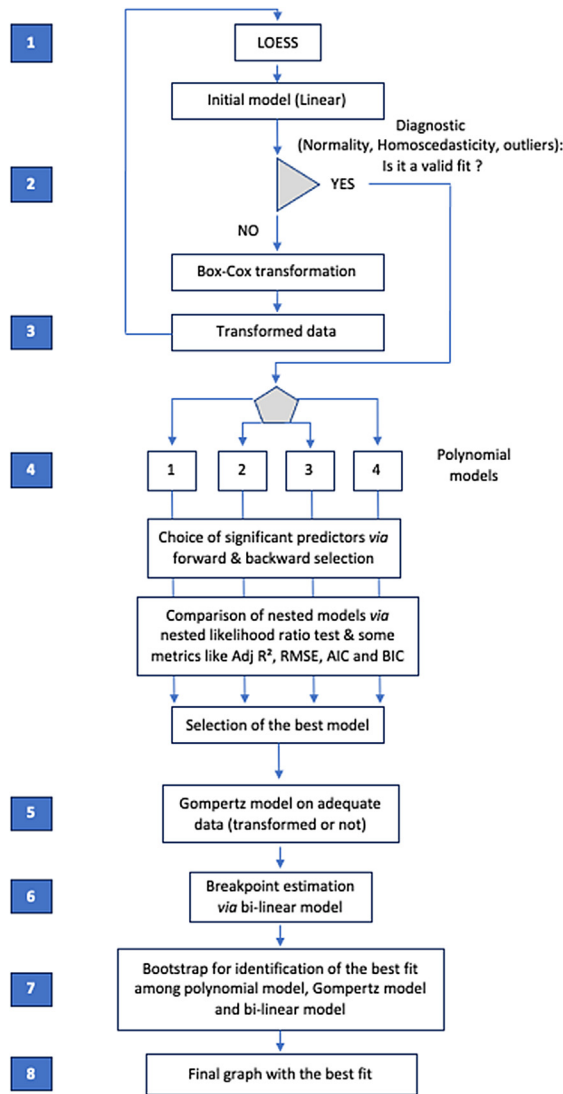
Fig. 2: Flow diagram of statistical analysis and optimal model determination. Key steps in the analysis of data derived from this cohort of normal individuals are indicated with succinct description offered in the text.

using forward and backward model selection and the level of significance between models was determined using nested likelihood ratio tests as detailed in Supplemental Material.

A summary of the process is offered below in the form of a flow chart (Fig. 2) and a brief description.

Best model selection

1. LOESS on the original data
2. Initial model (Linear): Evaluate the pertinence of the hypothesis for application of multiple linear regression models

- Residuals *vs* Fitted (verify if a linear model can be adapted to the data)
- Normal Q–Q (verify normality of residuals)
- Scale-Location (verify variance of residuals)
- Residuals *vs* Leverage (verify if there are outliers which may impact fitting results)

Additional statistical tests were implemented for evaluating quality of fit:

- Breusch-Pagan & Non-constant Variance Score tests to verify heteroscedasticity
- Shapiro–Wilk normality test to verify normality of residuals
  ⇒ Validity of hypothesis
  ⇒ If hypothesis is not validated, Decision to identify optimal transformation *via* Box–Cox transformation
  ⇒ 95% Confidence interval of parameter λ informs as to what might be the best transformation.
  ⇒ Test transformation depending on λ
3. After the transformation (generally log), the same procedure is applied as above
4. Apply a Polynomial degree 1 to "n" on the original data depending on all predictors.
   Choice of significant predictors *via* backward & forward selection. Model selection was performed with the stepAIC function from the MASS package of R[10] specifying both forward and backward selection. Selection at each step was based on the criterion of the AIC. The procedure was not reiterative.
   Comparison of nested models *via* nested likelihood ratio test & some metrics like Adjusted R^2 (Adj R^2), Root Mean Squared Error (RMSE), AIC, BIC (more penalizing than AIC)
5. Gompertz model was applied as a potential solution due to the non-monotonicity of the polynomial fit.
6. Breakpoint estimation using bi-linear regression models
   Age breakpoints for given parameter and inherent dependencies (*i.e.* fibre type, gender) were ascertained by fitting a bi-linear regression function with a least squares criterion in R[8]. This bi-linear regression function was also compared to a linear one *via* nested likelihood ratio test. The corresponding mean values are indicated by a vertical line in the optimal models presented in the manuscript, with the standard error indicated numerically above it.
7. Identification of best fit model using a "Bootstrap" approach
8. Final graph in main article.

Role of funders

Funders did not have any role in study design, data collection, data analyses, interpretation, or writing of report.

Group	Age range	Number of individuals	Gender (male/female)
Group 1	0–24 month	21	13/8
Group 2	25 months–10 years	30	16/14
Group 3	11 years–18 years	32	21/11
Number of individuals per age group.			

Table 1: Cohort age distribution.

Results

Demographic data

We have analysed biopsies from 83 individuals; 50 males and 33 females. The age range is from 28 days up to 18.8 years (mean age 8 years). The distribution regarding the different age groups and gender can be seen in Table 1.

Fibre type distribution

Comparison of the percentage of type 1 fibres was performed for each gender as a function of age. This percentage is relatively constant, around 50% from birth up to age 11 ± 3 years, in both females and males, after which it decreases to approximately 40%, without reaching a minimum in the experimental window (up to 18.8 years) (Fig. 3).

Fibre type diameter (Minimum diameter of Feret (MinFeret))

The mean MinFeret of type 1 and type 2 fibres increases at a similar rate in males and females (≈3 μm/year) until approximately age 5, at which point there is an apparent

slowing of growth in females (≈2 μm/year) *vs* males (≈3 μm/year), essentially plateauing after age 11 and ≈ 16 respectively. Estimation of the breakpoint was achieved using a bi-linear model with non-linear least squares regression encompassing both male and female data points (*cf.* Supplemental Materialsl). For females the breakpoint was estimated to be 11.0 ± 0.7 years and for males 15.8 ± 1.1 (Fig. 4).

Fibre cross-sectional area (CSA)

As with MinFeret, the CSA is essentially identical for type 1 & type 2 fibres in a gender-dependent manner. The mean CSA of type 1 and type 2 fibres increases up to age 10, at which point there is an apparent slowing of growth in females *vs* males (≈160 μm²/year vs 255 μm²/
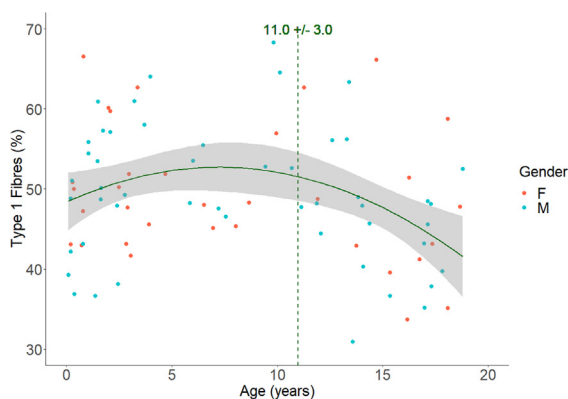

Fig. 3: **Percentage of type I fibres as a function of age**. The ratio of type 1 fibres per total fibres in each image derived from an individual biopsy, was determined and the mean ± 2 × standard error calculated using a polynomial model fit in R[8] (*cf.* Supplemental Material). Estimation of the breakpoint (11 ± 3 years; Mean ± Standard error) (Dotted vertical line) was achieved by fitting a bi-linear regression function with a least squares criterion in R[8] (*cf.* Supplemental Material). The optimal model curve was generated from data derived from both female and male subjects since it was independent of gender. The grey zone represents the 95% confidence interval. Female (Ochre dots); Male (Turquoise dots).
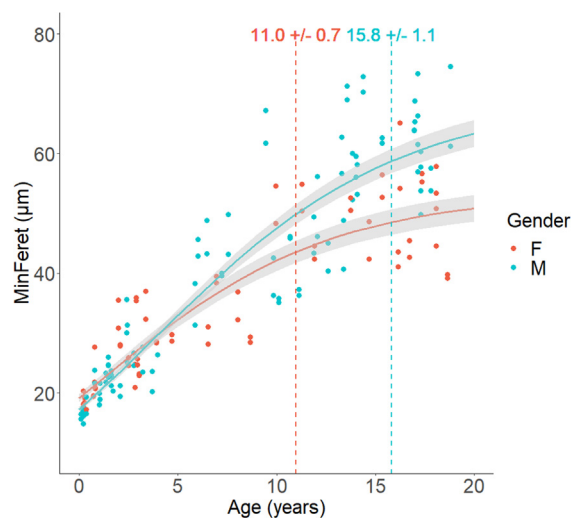

Fig. 4: **Evolution of the Minimum diameter of Feret (MinFeret) in all fibres (type 1 & type 2 together) *vs* age and gender**. Mean values for the MinFeret of both type 1 and type 2 fibres combined for each gender, Females (Ochre) and Males (Turquoise), calculated for each individual were used to determine the optimal model curve representing the evolution of this parameter using a Gompertz model fit in R[8] (*cf.* Supplemental Material). No significant difference was observed for this parameter between type 1 and type 2 fibres for a given individual of either gender, allowing grouping in a single curve representing the mean for each gender. The grey zone represents the 95% confidence interval for each curve. The vertical dotted lines indicate the breakpoint derived from a bi-linear regression model of the data from each gender (*cf.* Supplemental Material).

year respectively). Estimation of the breakpoints was achieved using a bi-linear model with non-linear least squares regression encompassing both male and female data points (*cf.* Supplemental Materials). For females the breakpoint was estimated to be 11.0 ± 0.6 years and for males 14.4 ± 0.7 (Fig. 5).

Fibre surface density

The relative surface of a muscle image occupied by type 1 or type 2 fibres was determined to be independent of gender. It appears that the density for both fibre types remains essentially constant and equivalent prior to age 10. Subsequently, the surface occupied by type 1 fibres decreases, while that of type 2 fibres increases (Fig. 6). This correlates with the evolution of the number of each fibre type per mm^2 over this age range, as shown below.

Fibre number per mm^2

From birth until around 10 years there is a 5-fold decrease in the mean number of fibres per unit of surface (*i.e.* per mm^2) from approximately 1000 to 200 fibres per mm^2. Subsequently, in males there are fewer fibres overall compared to females, thus correlating with
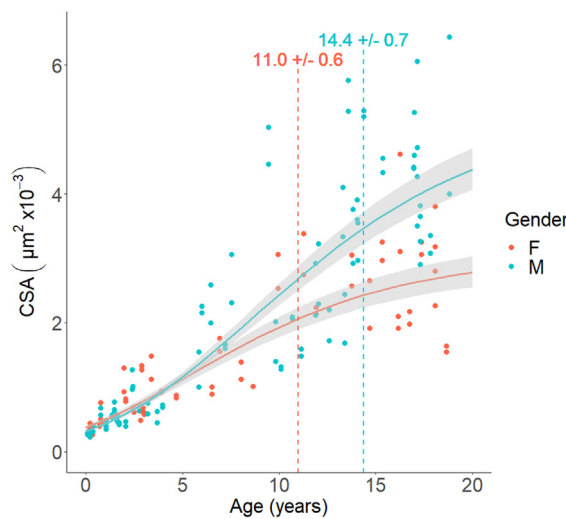

Fig. 5: **Evolution of fibre Cross-Sectional Area (CSA) *vs* age and gender.** Mean values for the CSA of type 1 and type 2 fibres, calculated for each individual were used to determine the optimal model curve representing the evolution of this parameter using a Gompertz model fit in R^8 (*cf.* Supplemental Material). No significant difference was observed between type 1 and type 2 fibres for a given individual of either gender allowing grouping in a single curve representing the mean for each gender (Female: Ochre; Male: Turquoise). The grey zone represents the 95% confidence interval for each curve. The vertical dotted lines indicate the breakpoints derived from a bi-linear regression model of the data from each gender (*cf.* Supplemental Material).
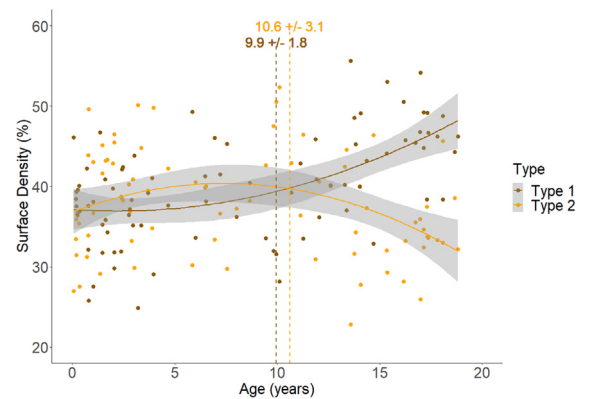

Fig. 6: **Evolution of fibre surface density *vs* age and gender.** Mean values for the surface occupied by type 1 (Yellow) and type 2 (Brown) fibres, calculated for each individual were used to determine the optimal model curves representing the evolution of the mean for this parameter using a polynomial model fit in R^8 (*cf.* Supplemental Material). No significant difference was observed for a given fibre type between females and males, thus allowing grouping in a curve representing the mean for each type. The grey envelope represents the 95% confidence interval for each curve. The vertical dotted lines indicate the breakpoints derived from a bi-linear regression model of the data from each type calculated individually (*cf.* Supplemental Material).

the increased CSA in males. Though not statistically significant, after age 14 in both males and females, there appears to be a decrease in the number of type 1 fibres per mm^2, whereas the number of type 2 per mm^2 increases (Fig. 7). This observation is supported in the measurement of the percentage of type 1 fibres as a function of age (*cf.* Fig. 2).

Fibre circularity

Circularity is essentially identical for type 1 & type 2 fibres in a gender-independent manner. It decreases until around age 10 then remains constant until the end of this observation window (Fig. 8). The degree of circularity of the muscle fibres is important to ascertain certain patterns of diseased muscle and is one of the parameters consistently observed by the pathologist. In normal adult muscle, fibres have a polygonal shape, but in pathological situations they may become rounded, as in muscular dystrophies or congenital myopathies or very angulated, as may be seen in denervation.[11]

Discussion
Study limitations

One of the limitations of this study is the relatively small number of cases and the fact that, in certain age ranges, the number of females was lower than the number of males. However, compared with the literature, ours is one of the largest and most comprehensive series. The
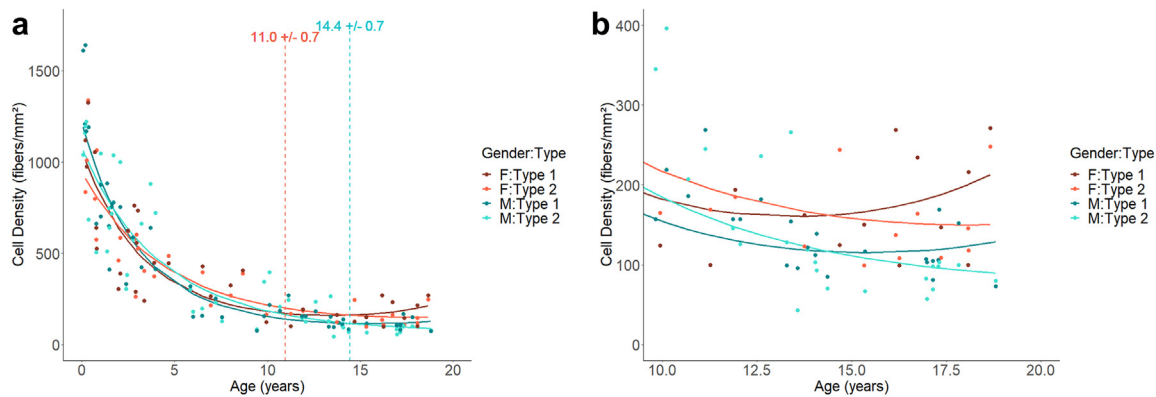
Fig. 7: Evolution of fibre density *vs* age and gender. Mean values for the number of fibres per mm² by type and gender. **a.** The vertical dotted lines indicate the breakpoints derived from a bi-linear regression model of the data from each type calculated individually (*cf.* Supplemental Material). **b.** focus on age 10–18 years.

specimens were processed in a standardised way and in accordance to the current best practices for muscle biopsy processing. Finally, only histologically normal biopsies were considered, as described in the methods section.[7]

Another limitation is that, due to the use of historical samples, we lack information on biometric parameters such as weight and height as well as lifestyle. It is possible that these variables will influence the values and be responsible for the heterogeneity of the data after puberty. It is recommended to systematically collect biometric parameters at the time of the biopsy. If this is not possible, means of capturing these data from electronic health records should be put in place.

Dependency of parameters on gender, fibre type and age, before and after the onset of puberty

A fundamental observation from this comprehensive study is the consistent inflexion of trends (slopes) around age 10 in the evolution of all parameters from birth to age 18. This underlines the strong dependency of muscle morphology on age and, notably, the major transformations that are occurring during puberty. Fig. 9 summarises the dependency of parameters on gender, age and fibre type. Below age 10, all parameters measured are independent of gender. With puberty, gender-dependent differences are only observed in parameters indicative of, or relating to, fibre size such as MinFeret and CSA as well as in the number of fibres/mm² (Fig. 10).
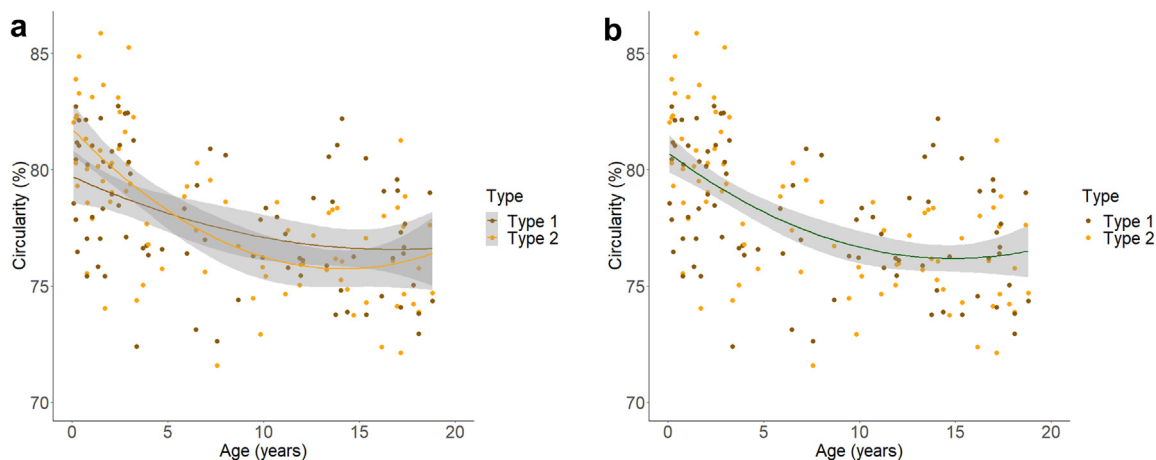

Fig. 8: Evolution of fibre circularity as a function of age. **a.** Mean values for the circularity of type 1 (Ochre) and type 2 (Brown) fibres, calculated for each individual were used to determine the optimal model curves representing the evolution of the mean for this parameter using a polynomial model fit in R[8] (*cf.* Supplemental Material). No significant difference was observed for a given fibre type between females and males, thus allowing grouping in a curve representing the mean for each type. **b.** Consolidation of the data into one model. The grey zone represents the 95% confidence interval for each curve.
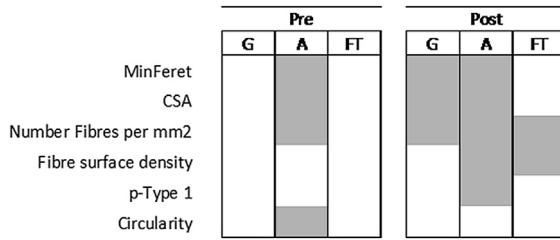
Fig. 9: Dependency of parameters on gender, age and fibre type, pre- and post-onset of puberty. Visual representation illustrating the evolution of parameter dependency on gender (G), age (A) and fibre type (FT) before (Pre) and after (Post) the onset of puberty, as determined from the graphs presented. White: Independent; Grey: Dependent.

The size of Type 1 and type 2 fibres as revealed by the MinFeret or CSA, is essentially identical within each gender at every age within this study window. Prior to age 10 there is no difference in size between genders, while the overall size of all fibres becomes greater in males than females, after the onset of puberty. As example, for females, the MinFeret plateaus at approximately 45 μm around age 11, whereas in males it continues to increase, reaching a plateau of approximately 52 μm around age 16. This is to be integrated with the fact that, while the percentage of type 1 and type 2 fibres is constant and equivalent (approximately 50% each) from birth up to age 11 for both sexes, the percentage of type 1 fibres starts to diminish at this point, reaching approximately 40% by age 18.8

independently of gender. This results in the lower density of fibres per unit area of muscle for males *vs* females. According to a recent paper,[12] during the transition from childhood to adulthood there is development of sex-specific patterns in CSA of the muscle fibres. The most probable cause for the gender-dependent difference is the tendency for males to develop higher degrees of muscle strength due to hormonal changes, namely the peak of testosterone.[12,13]

Research that has measured the fibre type of multiple muscles has found that the deltoid muscle is relatively slow twitch.[13] Some authors[14] state that in the deltoid, one of the most commonly biopsied muscles, there is an equivalent proportion of type 1, 2a and 2b fibres (1/3 type 1 and 2/3 type 2). Other authors[15] point to an equivalent number of type 1 and 2 fibres in males. The relative number of each fibre type is highly dependent on exercise, age[16] and gender.[17]

Surface area occupied by type 1 and 2 fibres

From birth until onset of puberty, there is a 5-fold decrease in the mean number of fibres per unit of surface (*i.e.* per mm^2). During this time, the mean cross-section area per fibre is identical for both type 1 and type 2 fibres, and increases 5-fold. This results in an essentially constant fibre surface density in this period for both fibre types. In this observation window (birth to 10 years), the relative percentage of type 1 *vs* total fibres is constant at approximately 50% (Fig. 2). After 10 years of age, in both males and females, there is a decrease in the number of type 1 fibres per unit of surface (*i.e.* per
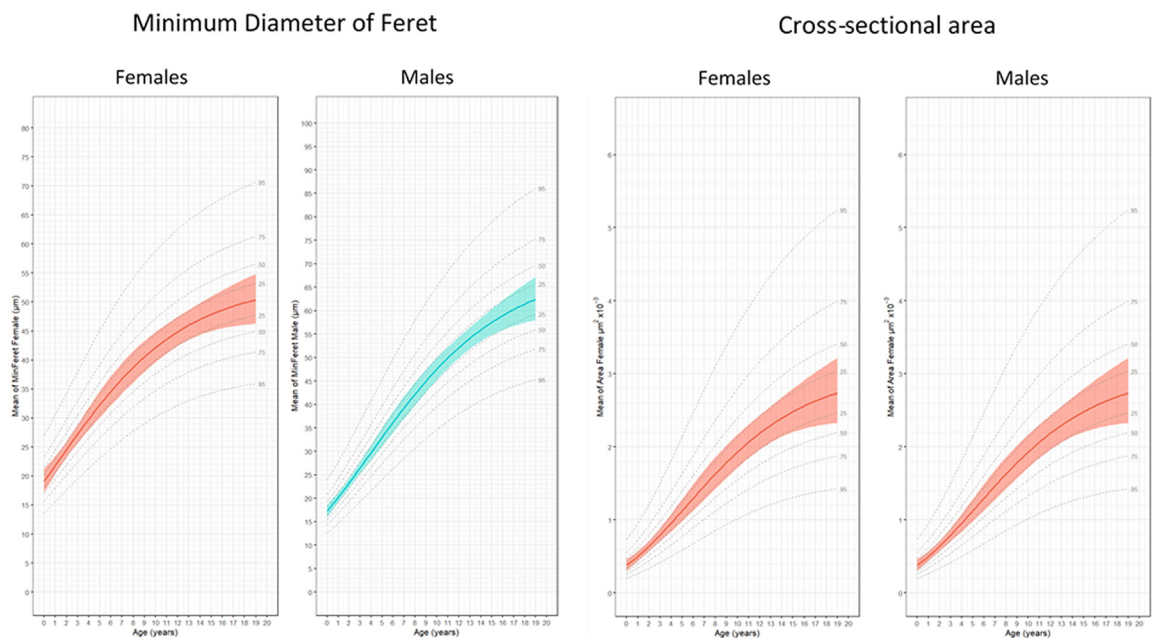

Fig. 10: Reference charts for MinFeret & Cross-Sectional Area.

mm^2), whereas the number of type 2 per unit of surface (*i.e.* per mm^2) increases.

This trend is also reflected in the surface area occupied by type 1 and type 2 fibres (Mean fibre surface density), *i.e.* the surface of a muscle occupied by type 1 fibres decreases while the surface occupied by type 2 fibres increases after age 10 (Fig. 6).

It would appear therefore, that there is an enhanced generation of type 2 fibres when compared with type 1 fibres. The main hormones that influence skeletal muscle maturation are pituitary growth hormone, thyroid hormone, and the sex hormones.[18,19] We hypothesise that the differences observed at the onset of puberty are most probably due to the changes in the hormonal background. It is known that with ageing, the inactivity and the progressive loss of motor units are responsible for the changes in the fibre CSA, with a greater reduction of the CSA of type 2 fibres than for type 1 fibres. The opposite can be true for children before and after puberty when global activity levels increase and the peripheral nervous system reaches maturity.[20,21]

Fibre circularity

Fibre circularity is essentially identical for both fibre types in a given individual, independent of gender. It decreases as the children mature, until the onset of puberty, then remains constant to the end of the observation window. Fibre circularity is one of the parameters that can help pathologists establishing a diagnosis. For example, a high degree of circularity is observed in muscle biopsies from patients with congenital myopathies. The finding that the circularity of fibres tends to decrease and reach a plateau around age 10, gives important information to the pathologists even in the presence of minimal changes and in particular when analysed together with other characteristics such as the fibre size and type.

Generalisability

This study allows us to offer the graphs below as reference for pathology laboratories analysing biopsies of deltoid muscle. In addition to the templates, a link is offered to download reference tables with the corresponding values in Supplemental Materials.

Conclusion

This study offers effective insight into the evolution of normal morphometric parameters according to fibre types from birth to age 18 in females and males. We have established the normative values that can be used to assess the characteristics of the muscle fibre types in this age range. Reference charts have been generated to allow comparison of data from patients in pathology laboratories working on neuromuscular diseases.

Data sharing statement
The data collected during this study will be made available to others upon request. The normative values that were obtained from the analysis will be publicly-available on the institutional site of the AIM (Association de L'Institut de Myologie) https://www.institut-myologie.org/recherche/centre-dexploration-et-devaluation-neuromusculaire/laboratoire-dhistopathologie/and through the website of the ERN EURO-NMD https://ern-euro-nmd.eu/.

The access to the iconography that was necessary to generate the data will be granted always in a de-identified way and by request to one of the authors (TE). The data will be available upon publication with a signed data access agreement.

Declaration of interests
The authors have no conflicts of interests regarding this manuscript. BBR is an inventor on the patent describing the algorithms used in this study.

Appendix A. Supplementary data
Supplementary data related to this article can be found at https://doi.org/10.1016/j.ebiom.2022.104367.

References
1 Brooke MH, Engel WK. The histographic analysis of human muscle biopsies with regard to fiber types. 1. Adult male and female. *Neurology.* 1969;19(3):221–233.
2 Brooke MH, Engel WK. The histographic analysis of human muscle biopsies with regard to fiber types. 4. Children's biopsies. *Neurology.* 1969;19(6):591–605.
3 Moore MJ, Rebeiz JJ, Holden M, Adams RD. Biometric analyses of normal skeletal muscle. *Acta Neuropathol.* 1971;19(1):51–69.
4 Meltzer HY, Rastogi S, Ellison J. Quantitative histochemical evaluation of normal human skeletal muscle. *Neurology.* 1976;26(9): 849–852.
5 Jansson E, Hedberg GE. Skeletal muscle fibre types in teenagers: relationship to physical performance and activity. *Scand J Med Sci Sports.* 1991;1(1):31–44.
6 Staron RS, Hagerman FC, Hikida RS, et al. Fiber type composition of the vastus lateralis muscle of young men and women. *J Histochem Cytochem.* 2000;48(5):623–629.

7   Udd B, Stenzel W, Oldfors A, et al. 1st ENMC European meeting: the EURO-NMD pathology working group recommended standards for muscle pathology Amsterdam, The Netherlands, 7 December 2018. *Neuromuscul Disord*. 2019;29(6):483–485.

8   R Core Team. R. *A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing; 2021.

9   RStudioTeam. In: *RStudio: Integrated Development for R. 2021 (1.4. 1106)*. Boston, MA: RStudio, PBC; 2021.

10   Venables WNR BD. *Modern applied Statistics with S*. 4th ed. New York: Springer; 2002.

11   Oldfors A, Dubowitz V, Sewry CA. *Muscle Biopsy E-Book: A Practical Approach*. 5th ed. The Netherlands: Elsevier Health Sciences; 2020.

12   Esbjornsson ME, Dahlstrom MS, Gierup JW, Jansson EC. Muscle fiber size in healthy children and adults in relation to sex and fiber types. *Muscle Nerve*. 2021;63(4):586–592.

13   Oertel G. Morphometric analysis of normal skeletal muscles in infancy, childhood and adolescence. An autopsy study. *J Neurol Sci*. 1988;88(1–3):303–313.

14   Swash M, Schwartz M,S. *Biopsy Pathology of Muscle*. Boston, MA: Springer; 1991.

15   Johnson MA, Polgar J, Weightman D, Appleton D. Data on the distribution of fibre types in thirty-six human muscles. An autopsy study. *J Neurol Sci*. 1973;18(1):111–129.

16   Bougea A, Papadimas G, Papadopoulos C, et al. An age-related morphometric profile of skeletal muscle in healthy untrained women. *J Clin Med*. 2016;5(11).

17   Miller AE, MacDougall JD, Tarnopolsky MA, Sale DG. Gender differences in strength and muscle fiber characteristics. *Eur J Appl Physiol Occup Physiol*. 1993;66(3):254–262.

18   Haizlip KM, Harrison BC, Leinwand LA. Sex-based differences in skeletal muscle kinetics and fiber-type composition. *Physiology*. 2015;30(1):30–39.

19   Welle S, Tawil R, Thornton CA. Sex-related differences in gene expression in human skeletal muscle. *PLoS One*. 2008;3(1):e1385.

20   Lexell J, Taylor CC. Variability in muscle fibre areas in whole human quadriceps muscle: effects of increasing age. *J Anat*. 1991;174:239–249.

21   Garcia-Garcia A, Calleja-Fernandez J. [Neurophysiology of the development and maturation of the peripheral nervous system]. *Rev Neurol*. 2004;38(1):79–83.