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The number of satellite cells in slow and fast fibres from human vastus lateralis muscle

Abstract The aim of this investigation was to study the distribution of satellite cells in slow (type I fibres) and fast (type II fibres) fibres from human vastus lateralis muscle. This muscle is characterised by a mixed fibre type composition and is considered as the site of choice for biopsies in research work and for clinical diagnosis. Biopsy samples were obtained from five healthy young volunteers and a total of 1,747 type I fibres and 1,760 type II fibres were assessed. Satellite cells and fibre type composition were studied on serial muscle cross-sections stained with specific monoclonal antibodies. From a total of 218 satellite cells, 116 satellite cells were found in contact with type I fibres ($53.6 \pm 8\%$ of the satellite cells associated to type I fibres) and 102 satellite cells in contact with type II fibres ($46.4 \pm 8\%$ of the satellite cells associated to type II fibres). There was no significant difference ($P = 0.4$) between the percentages of satellite cells in contact with type I and with type II fibres. Additionally, there was no relationship between the mean number of satellite cells per fibre and the mean cross-sectional area of muscle fibres. In conclusion, our results show that there is no fibre type-specific distribution of satellite cells in a human skeletal muscle with mixed fibre type composition.

Keywords Satellite cell · Myonuclei · Muscle fibre type · Phenotype

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Introduction

Satellite cells are undifferentiated myogenic precursors described in detail by Mauro (1961). Following minor or severe injury to muscle fibres, satellite cells become activated and start to proliferate in order to repair the damaged area (Grounds 1998). Therefore, satellite cells are considered as important structures involved in the maintenance of normal muscle function. The distribution of satellite cells in slow and fast skeletal muscles has been investigated mainly in animal studies. It has been shown that satellite cells occur more frequently in slow than in fast muscles (Gibson and Schultz 1983; Schmalbruch and Hellhammer 1977). It is currently suggested that in a muscle of mixed fibre types, the percentage of satellite cells in contact with slow fibres (type I fibres) is higher than that in contact with fast fibres (type II fibres). Slow fibres would contain more satellite cells than fast fibres as they are the first and most frequently recruited during muscle activity (for review see Hawke and Garry 2001; Schultz and McCormick 1993; Schultz 1989).

The aim of the present study is to investigate the distribution of satellite cells in slow (type I fibres) and fast fibres (type II fibres) from human vastus lateralis, a muscle with a mixed fibre type composition, which is considered as the site of choice for biopsies in research work and for clinical diagnosis.

Material and methods

Subjects

Biopsy samples were obtained from the vastus lateralis muscle of five healthy young volunteers (age 26 ± 5 years, weight 74 ± 9 kg, height 172 ± 3 cm). None of the subjects was engaged in any intensive exercise training programs, apart from their habitual physical activity. The study protocol was approved by the Re-

gional Committee on Research Ethics at the Karolinska Institute, and informed consent was obtained from all subjects prior to participation.

Muscle biopsies

Biopsies were obtained from the quadriceps femoris muscle (vastus lateralis), using the Weil–Blakesley's conchotome technique. After local anaesthesia (1 ml of a 5 mg/ml solution of Carbocain, Astra, Södertälje, Sweden), an incision, 8-mm long and 10-mm deep, was made in the skin and penetrating the underlying fascia. The muscle tissue was embedded in an embedding medium (O.C.T. Compound, Company will be added) and frozen in isopentane cooled in liquid nitrogen and stored at –80°C until analysed.

Immunohistochemistry

Thick serial cross-sections (5 μm) were cut at –20°C using a cryostat microtome (Zeiss, HM560), mounted on glass slides, and air dried at room temperature. Satellite cells were visualised using a monoclonal antibody against CD56 (dilution 1:100, Cat no. 347740, Becton Dickinson) (Crameri et al. 2004; Kadi et al. 2004a, b; Maier and Bornemann 1999; Renault et al. 2002). The anti-CD56, which is similar to the anti-leu19 antigen, recognizes the neural cell adhesion molecule (NCAM), a cell–cell recognition glycoprotein expressed during the early stages of myogenesis and in satellite cells. Muscle cross sections were air dried, rinsed for 20 min in phosphate-buffered saline (PBS), and incubated for 20 min with diluted normal blocking goat serum. Sections are then incubated for 2 h at 37° with primary mouse antibody diluted in bovine serum albumin. Slides are washed in PBS for 15 min and incubated for 30 min with diluted biotinylated goat anti-mouse secondary antibody (dilution 1:200 in normal serum, Vector Laboratories, BA-9200). The slides are then washed for 20 min in PBS and incubated for 30 min with Vectastain ABC reagent (Vector Laboratories, PK-6100). For the visualisation of primary antibody binding, the DAB substrate kit for peroxidase (Vector Laboratories, SK-4100) was used. Sections are then rinsed, counterstained with Mayer's hematoxylin, cleared and mounted using mountex. Myonuclei were blue and satellite cells were stained brown (Fig. 1a). Only satellite cell profiles comprising a nucleus were counted. The visualization of satellite cells and myonuclei was done at high magnification (objective ×40 or ×60). To study the distribution of satellite cells in slow (type I fibres) and fast fibres (type II fibres), sections were also stained with the monoclonal antibody A4840 (dilution 1:100, Developmental Studies Hybridoma Bank) (Kadi et al. 1998). Using the A4840 antibody, slow muscle fibres (type I fibres) are stained and fast fibres (type II fibres, both subtypes IIA and IIX) are unstained with the A4840 antibody (Fig. 1b). The area of muscle fibres and the percentages of type I and type II fibres were calculated on the whole muscle cross-section. To study the distribution of satellite cells associated with specific fibre types, images from serial sections stained with the CD-56 and with the A4840 antibodies were matched and acquired using SPOT digital camera and SPOT software version 3.4 (SPOT Insight; Diagnostic Instruments, Sterling Heights, MI, USA) connected to the microscope (Nikon Eclipse E400, Badhoevedorp, The Netherlands). We first identified all satellite cells present in a cross section. Then, for each satellite cell, we checked whether it was associated with a type I or with a type II fibre. We then calculated the percentage of satellite cells in association with type I and with type II fibres. A total of 1,747 type I fibres and 1,760 type II fibres were assessed. The total number of satellite cells (SC_{tot}), the number of satellite cells associated with type I fibres (SC_I) and the number of satellite cells associated with type II fibres (SC_{II}) were counted. The percentages of satellite cells associated with type I (SC_I/SC_{tot} in percentage) and with type II fibres (SC_{II}/SC_{tot} in percentage) were calculated. The number of satellite cells per fibre type I (SC_I/F_I) and the number of satellite cell per fibre type II (SC_{II}/F_{II}) were also calculated.

Statistical analysis

Data are presented as means and standard deviations. The Mann–Whitney test was used to compare the percentage of satellite cells associated with type I fibres to that associated with type II fibres. The product-moment correlation coefficient (r) was used to determine the relationship between two morphological parameters. P values below 0.05 were considered statistically significant.

Results

As expected, the analysis of fibre type composition showed that vastus lateralis muscle is a mixed skeletal muscle containing both type I and type II muscle fibres ($51 \pm 12\%$ type I fibres and $49 \pm 12\%$ type II fibres). The mean cross-sectional area of type I fibres was $5,472 \pm 964$ μm^2 and that of type II fibres was $6,116 \pm 1,217$ μm^2.

On serial muscle cross sections, we found that satellite cells were in contact with both type I and type II fibres (Fig. 1a,b). Of a total of 218 satellite cells, 116 satellite cells were found in contact with type I fibres and 102 satellite cells in contact with type II fibres. This analysis included 1,747 type I fibres and 1,760 type II fibres. The analysis of satellite cell distribution in vastus lateralis muscle showed that there was no statistically significant difference ($P = 0.4$) between the proportion of satellite cells associated with type I muscle fibres ($53.6 \pm 8\%$ of the satellite cells associated with type I fibres) and that associated with type II muscle fibres

(46.4 ± 8% of satellite cells associated with type II fibres). Similarly, there was no significant difference ($P = 0.4$) between the number of satellite cells per fibre type I ($0.067 ± 0.01$) and the number of satellite cells per fibre type II ($0.063 ± 0.01$).

In this study, we investigated the question of whether the mean number of satellite cells per fibre is correlated to the area of muscle fibres. We found no relationship between the number of satellite cells per fibre and the cross-sectional area of muscle fibres ($r^2 = 0.2$; $P = 0.5$).

On the contrary, we found a significant correlation ($r^2 = 0.87$; $P = 0.0004$) (Fig. 2) between the mean number of myonuclei per fibre and the mean cross-sectional area of muscle fibres.

Discussion

Our results showed that satellite cell distrubution in human vastus lateralis is not dependent on fibre type composition. In human vastus lateralis, a major knee extensor involved in human locomotion, satellite cells are equally distributed in type I and type II fibres. In previous studies on animal models, it has been shown that in a muscle of mixed fibre types and in uniform muscles, satellite cells are found predominantly in slow fibres (Schmalbruch and Hellhammer 1977; Gibson and Schultz 1983). It has been suggested that since slow fibres tend to be recruited with greatest frequency during muscular activities, a larger satellite cell pool may be required to supply the demands of daily micro-injuries occurring in this specific fibre type (Schultz 1989). The results of our study suggest that the loading pattern of human vastus lateralis muscle does not require a specific distribution of satellite cells among types I and II muscle fibres. Thus, in a human muscle with mixed fibre types, both slow and fast fibres would be equally equipped in term of satellite cells in order to ensure adequate reparation of segmental injuries of the muscle fibre. It is important to note that our results are

Fig. 1 Serial muscle cross-sections stained with **a** the antibody CD56 and counterstained with Mayer's hematoxylin and **b** with the antibody A4840. In (**a**), arrowheads point to satellite cells. In (**b**), type I fibres are stained whereas type II fibres are unstained. Note the presence of three satellite cells (two satellite cells in contact with two type II fibres and one satellite cell in contact with one type I fibre).
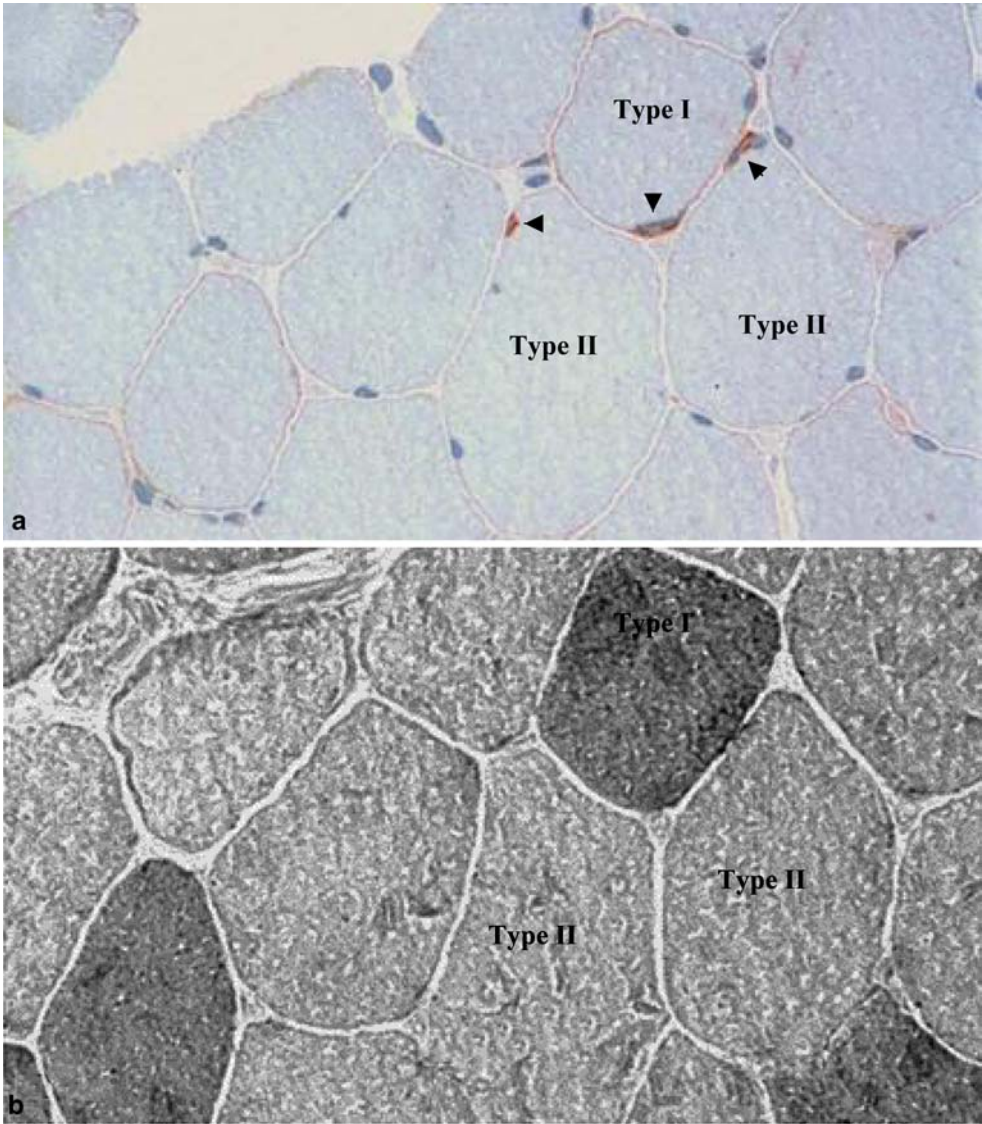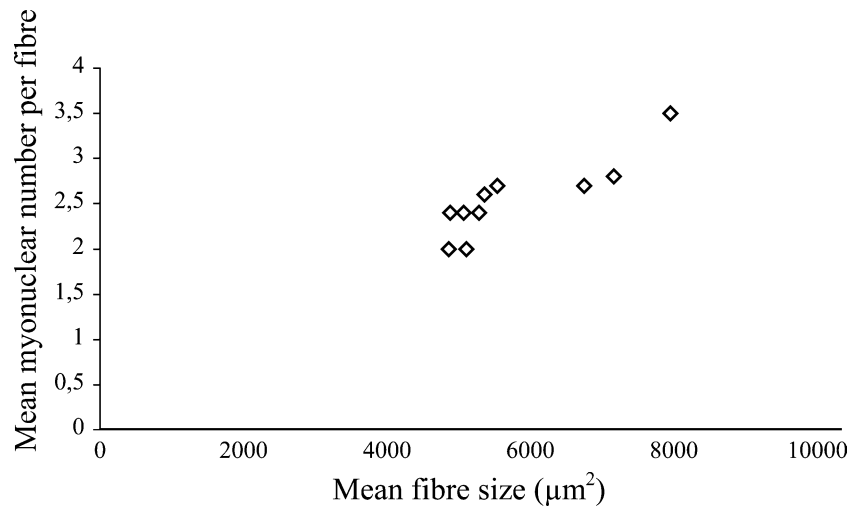
Fig. 2 The relationship between the mean myonuclear number per fibre and the mean cross-sectional area of muscle fibres

based on the analysis of a total of 3,507 muscle fibres and 218 satellite cell profiles. It remains unknown whether a higher number of biological observations might reveal a trend toward a preferential association of satellite cells with a specific fibre type. The quantitative determination of satellite cells on muscle biopsies is a sophisticated task. Satellite cells are traditionally counted on muscle cross-sections or longitudinal sections using light microscopy, confocal microscopy and electron microscopy. Sajko et al. (2004) compared the results obtained by counting satellite cells per fibre profile and per unit of fibre length using the optical dissector principle. Satellite cells were expressed as a number of satellite cell per fibre length, number of satellite cell per number of myonuclei, number of satellite cell per 1 cm^3 and number of satellite cell per fibre profile. Similar trends were found using all parameters. The question of whether satellite cells are preferentially associated with a specific fibre type was not addressed in the study by Sajko et al. (2004).

As satellite cell frequency was not influenced by the fibre type composition, we assessed the question of whether the size of muscle fibres can influence satellite cell distribution in human vastus lateralis. Our results showed that satellite cells are not associated with a particular muscle fibre size. This is in line with the results of a previous work on animals (Giddings and Gonyea 1992) showing that there is no preferential distribution of satellite cell among a specific fibre size. On the contrary, we found that the number of myonuclei per fibre and the size of muscle fibres are closely interdependent. In humans, the relationship between the area of muscle fibres and their myonuclear content has been previously studied in relation to muscle fibre hypertrophy upon strength training (for review see Kadi 2000). It has been shown that very large muscle fibres contain more myonuclei than smaller muscle fibres (Hikida et al. 1998; Kadi et al. 1999a, b). The findings from human vastus lateralis support the concept of the "nuclear domains" (Cheek 1985), in which each myonucleus controls a defined cytoplasmic volume. However, it is also important to note that small changes in fibre area can be sustained by adjustment of the transcriptional capacity of existing myonuclei without the addition of new myonuclei (Kadi et al. 2004b).

In conclusion, contrary to results found in animal uniform skeletal muscles, we found no fibre type-specific distribution of satellite cells in a human skeletal muscle with mixed fibre type composition.

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