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RAPID COMMUNICATION

Improving human skeletal muscle myosin heavy chain fiber typing efficiency

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Abstract Single muscle fiber sodium dodecyl sulfate polyacrylamide gel-electrophoresis (SDS-PAGE) is a sensitive technique for determining skeletal muscle myosin heavy chain (MHC) composition of human biopsy samples. However, the number of fibers suitable to represent fiber type distribution via this method is undefined. Muscle biopsies were obtained from the vastus lateralis (VL) of nine resistance-trained males (25 ± 1 year, height = 179 ± 5 cm, mass = 82 ± 8 kg). Single fiber MHC composition was determined via SDS-PAGE. VL fiber type distribution [percent MHC I, I/IIa, IIa, IIa/IIx, and total "hybrids" (i.e. I/IIa + IIa/IIx)] was evaluated according to number of fibers analyzed per person (25 vs. 125). VL fiber type distribution did not differ according to number of fibers analyzed ($P > 0.05$). VL biopsy fiber type distribution of nine subjects is represented by analyzing 25 fibers per person. These data may help minimize cost, personnel-time, and materials associated with this technique, thereby improving fiber typing efficiency in humans.

Keywords SDS-PAGE · Muscle biopsy · Hybrid fibers · Vastus lateralis

Introduction

Single muscle fiber sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was introduced in 1988 as a method to determine skeletal muscle fiber type distribution of human biopsy samples (Biral et al. 1988). The method has since been adopted by numerous laboratories for fiber typing diverse populations under various conditions (Andersen et al. 1994a; Garner and Widrick 2003; Kesidis et al. 2008; Klitgaard et al. 1990; Kohn et al. 2007; Larsson and Moss 1993; Malisoux et al. 2006a; Parcell et al. 2003; Williamson et al. 2000; Zhou et al. 1995; Broos et al. 2012). It is well-established that different fiber types within a muscle vary considerably from a biochemical and functional perspective (Zierath and Hawley 2004) and that specific fiber types respond differentially to exercise, unloading, aging, and disease (Pette and Staron 1997). Thus, accurate and reliable fiber type-specific analyses are of great importance. Application of the SDS-PAGE technique to single muscle fiber segments (as opposed to homogenized tissue) has enabled high-fidelity and robust investigation of fiber type-specific gene expression, protein content/synthesis, contractile performance, and organelle anatomy (Daugaard et al. 2000; Dickinson et al. 2010; Galpin et al. 2012; Raue et al. 2012; Trappe et al. 2000; Yang et al. 2006; Ohira et al. 1999). Single fiber SDS-PAGE also permits sensitive detection and quantification of muscle fibers co-expressing multiple myosin heavy

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chain (MHC) isoforms (i.e. I/IIa, IIa/IIx, and I/IIa/IIx). These "hybrid" fibers have distinct functional (Trappe et al. 2004; Widrick et al. 2002) and metabolic (Galpin et al. 2012) profiles, may populate a third of healthy human muscle (Williamson et al. 2001), and manifest to a large extent in conditions such as unloading (Gallagher et al. 2005; Bagley et al. 2012), aging (Williamson et al. 2000), and spinal cord injury (Malisoux et al. 2007).

Single muscle fiber SDS-PAGE is diversely applicable and sensitive, but can also be time-consuming and resource-intensive if many hundreds to thousands of fibers are used for fiber-typing a cohort (Malisoux et al. 2006a; Williamson et al. 2000; Zhou et al. 1995; Andersen et al. 1999; Parcell et al. 2005; Murach et al. 2014). Perhaps as a consequence, most single fiber SDS-PAGE studies employ a relatively small number of individuals. Only five investigations to date have evaluated ≥9 individuals within a subject group of interest (Kohn et al. 2007, 2011; Malisoux et al. 2007; Parcell et al. 2003, 2005). However, to our knowledge, no study has examined the number of fibers suitable for fiber typing muscle samples in a larger cohort using this highly sensitive technique. We therefore sought to elucidate the number of isolated fibers that can represent the fiber type distribution of muscle samples using single muscle fiber SDS-PAGE. This information will help minimize cost, personnel-time, and materials associated with this method, potentially allow for analysis of larger subject groups, and provide guidance for researchers that employ the increasingly popular small-yield microbiopsy procedure (Hughes et al. 2015; Pietrangelo et al. 2011; Townsend et al. 2015; Krause et al. 2012).

Materials and methods

Nine resistance-trained males (25 ± 1 year, height = 179 ± 5 cm, mass = 82 ± 8 kg) were recruited by the California State University, Fullerton Center for Sport Performance. Subjects received written and oral information about experimental procedures and potential risks before giving written informed consent. Experimental procedures were in compliance with the Institutional Review Board of California State University, Fullerton. A mid-muscle belly biopsy from the vastus lateralis (VL) was obtained by the same technician using the Bergström technique (Bergström 1962). In order to facilitate smooth and verifiable fiber isolation from the muscle bundle, samples weighing ~15 mg were placed in cold skinning solution [(in mm): 125 K propionate, 2.0 EGTA, 4.0 ATP, 1.0 $MgCl_2$, 20.0 imidazole (pH 7.0), and 50 % (v/v) glycerol] and stored at 4 °C for at least 1 week prior to isolation. Fiber segments were randomly selected and extracted longitudinally in a physiological buffer using fine tweezers under a light microscope at room temperature and placed into 80 µL SDS buffer [10 % SDS, 6 mg mL^{-1} EDTA, 0.06 m Tris (pH 6.8), 2 mg mL^{-1} bromophenol blue, 15 % glycerol, and 5 % b-mercaptoethanol] for SDS-PAGE MHC identification, as previously described (Williamson et al. 2001). A total of 1,350 fibers were used for analysis.

Data were checked for normality and original or log transformed data were used for analysis. VL fiber type distribution (percent MHC I, I/IIa, IIa, IIa/IIx, and total hybrids) was evaluated using dependent t-tests for each fiber type (distribution from the first randomly selected 25 fibers typed versus the subsequent 125 typed). We chose 25–125 fibers as the range since this captures the number of fibers used by most laboratories that publish using single fiber SDS-PAGE (Andersen et al. 1994b; Biral et al. 1988; Gallagher et al. 2005; Klitgaard et al. 1990; Kohn et al. 2007; Larsson and Moss 1993; Malisoux et al. 2007; Parcell et al. 2003; Broos et al. 2012), represents a realistic and practical fiber typing workload, approximates findings from a less-sensitive technique in a small cohort (Henriksson-Larsen et al. 1983), and is representative of the average fiber yield from increasingly popular minimally invasive biopsy techniques (Townsend et al. 2015). False discovery rate using bootstrapping for the total number of fibers typed for each subject (5–145 fibers in 5 fiber increments, 5,000 random comparisons per sample) was analyzed using Matlab (Mathworks, Natick, MA, USA). Data are presented as mean ± SD.

Results

Average VL fiber type distribution from 125 fibers per person was: 17.3 ± 10.1 % MHC I, 10.4 ± 7.3 % MHC I/IIa, 60.0 ± 13.1 % MHC IIa, 11.3 ± 13.3 % MHC IIa/IIx, 22.0 ± 10.8 total hybrids (MHC I/IIa and IIa/IIx combined), and was the same regardless of the number of fibers analyzed per person ($P > 0.05$, Fig. 1). The false discovery rate fell to 0 % beyond 25 fibers, providing further evidence that 25 fibers can reliably estimate the fiber type distribution of a larger sampling of fibers if at least nine subjects are analyzed. Less than 1 % of all fibers in the 150 groupings were MHC IIx or I/IIa/IIx and are not presented in Fig. 1.

Discussion

Average VL fiber type distribution of muscle biopsy samples from 9 subjects is the same when analyzing 25 fibers versus five times that amount per individual. The largest difference in any MHC isoform percentage across the four groupings was 4.4 % (MHC IIa). In general, SDS-

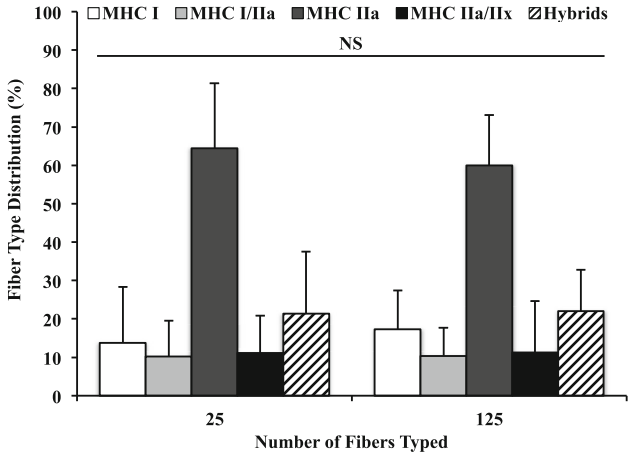

Fig. 1 Average MHC I, I/IIa, IIa, IIa/IIx, and hybrid fiber (I/IIa and IIa/IIx) distribution of all subjects when analyzing 25 versus 125 fibers per subject. Data presented as mean ± SD, *NS* not significant, $P > 0.05$

PAGE fiber typing nine subjects using ≥100 fibers per sample requires ≥30 personnel hours and ≥75 h of assay time. By contrast, fiber-typing nine subjects using 25 fibers per sample requires less than half the personnel and assay time and considerably fewer resources.

Most laboratories employing single fiber SDS-PAGE use ~100 fibers to represent fiber type distribution of a human skeletal muscle biopsy sample (Garner and Widrick 2003; Kesidis et al. 2008; Kohn et al. 2007; Broos et al. 2012; Parcell et al. 2005; Andersen et al. 1996; Raue et al. 2005; Malisoux et al. 2006b). However, numerous studies report using ~25–85 fibers to determine fiber type distribution (Andersen et al. 1994a; Klitgaard et al. 1990; Ohira et al. 1999; Harber et al. 2002; Parcell et al. 2000). The present findings confirm that a lower fiber count can be appropriate if subject sample size is sufficiently large. Worth noting is that greater variability is inherent when analyzing fewer fibers (i.e. the occurrence of one less MHC I fiber among 25 fibers means a 4 % change in MHC I versus a 1 % change when using 100 fibers). For example, one subject in this study showed 92.0 % MHC IIa in the 25 fiber grouping but 57.6 % in the 125 grouping. Greater variability with lower fiber count is reflected by noticeably larger standard deviations in the 25 fiber grouping (see Fig. 1). Subject population is also worth considering when choosing the number of fibers for analysis. For instance, evidence of fiber type grouping/clustering in the elderly and certain diseased populations due to progressive denervation likely decreases the chances of biopsying a heterogeneous portion of muscle. This phenomenon may influence the fiber-typing results (Nygaard and Sanchez 1982; Luciano et al. 1996). It is therefore beneficial to include more fibers for analysis if the sample size is less than nine subjects, when dealing with special populations, or when time and resources permit.

The data presented here can improve single muscle fiber SDS-PAGE fiber-typing efficiency in human studies. The possibility of including more subjects in an SDS-PAGE study due to reduced fiber typing workload is beneficial in regard to study design and statistical analysis. Furthermore, the knowledge that a smaller biopsy yield can still provide valid fiber type information is valuable for researchers that wish to couple low-yield minimally invasive microbiopsy procedures with SDS-PAGE (Pietrangelo et al. 2011; Vescovo et al. 1996). The fiber type-specific measures made possible by combining single fiber SDS-PAGE with molecular, protein, functional, and imaging procedures (Daugaard et al. 2000; Dickinson et al. 2010; Galpin et al. 2012; Raue et al. 2012; Trappe et al. 2000; Yang et al. 2006; Ohira et al. 1999) further underscores the utility of this technique and promotes its usage as a skeletal muscle phenotyping method.

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Compliance with ethical standards

Conflict of interest The authors have no conflicts to declare.

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