Letter to the editor: Comments on Stuart et al. (2016): “Myosin content of individual human muscle fibers isolated by laser capture microdissection”

Stefano Schiaffino,1 Marta Murgia,2,3 Leslie A. Leinwand,4 and Carlo Reggiani2

1Venetian Institute of Molecular Medicine, Padua, Italy; 2Department of Biomedical Sciences, University of Padova, Padua, Italy; 3Department of Proteomics and Signal Transduction, Max-Planck-Institute of Biochemistry, Martinsried, Germany; and 4Department of Molecular, Cellular and Developmental Biology and BioFrontiers Institute, University of Colorado, Boulder, Colorado

TO THE EDITOR: In a recent paper, Stuart et al. (7) conclude that muscle fibers isolated by laser capture microdissection (LCM) from the human vastus lateralis muscle I) are all hybrid fibers containing mixtures of fast and slow myosin heavy chain (MYH) isoforms, and 2) contain significant levels of MYH4 (type 2B) and MYH6 (α-cardiac). We believe that both conclusions of this paper are unfounded and are inconsistent with a large number of previous studies. Given the relevance of this issue for muscle physiology, any new or controversial report would require citation of the papers reaching different conclusions and discussion of the possible reasons for the discrepancies.

Stuart’s study is based on samples from a very heterogeneous group of subjects, including young and old, men and women, lean or metabolic syndrome subjects, and even type 1 and type 2 diabetes patients. However, it is not specified which are the subjects actually analyzed in this study, and how many single fibers were examined for each type of analysis. Each single fiber was isolated by LCM from ~300 sections, half of which were unstained before LCM and mRNA analyses and half that were stained with two antibodies specific for fast or slow myosins before LCM and protein analyses (Western blotting and mass spectrometry). RNA analysis of fibers isolated by LCM showed that type I/slow fibers contain only ~50% MYH7 (type I/slow) mRNA, in addition to large amounts of MYH1 (type 2A) and MYH2 (type 2A) transcripts, and that 2A and 2X fibers likewise contain a mixture of MYH1 and MYH2, as well as significant amounts (>10%) of MYH7 transcripts (Fig. 5C). This result is in contrast with a previous in situ hybridization study with probes validated to be specific to each human MYH isoform, which showed that most fibers in human vastus lateralis contain exclusively either MYH7, MYH2, or MYH1 transcripts, with two minor populations of hybrid fibers coexpressing MYH1 with MYH2 or MYH2 with MYH7 transcripts (6).

The MYH isoform-specific antibodies used by Stuart et al. for Western blotting analyses are commercial antibodies, whose specificities are not documented in this or previous studies and some of which are certainly nonspecific. For example, the antibody used to detect MYH6 (HPA001349) was raised against a peptide whose sequence is 100% identical in human MYH6 and MYH7. The reactivity detected with this antibody likely reflects the presence of MYH7 rather than MAY6. Indeed, it is known that MYH6 is expressed in human jaw muscles, but not in most other muscles (see ref. 5) and RNA databases do not demonstrate the presence of MYH6 mRNA in limb muscles of any mammals (see http://www.gtexportal.org/home/). The antibody used to detect MYH4 (SAB4301129) was raised according to Sigma against a “synthesized peptide derived from the COOH-terminal of human MYH4.” The sequence of this peptide is not specified, however, it is known that the COOH-terminal regions of fast MYHs are extremely similar (8), therefore cross-reactivity is likely and should be verified.

The hybrid nature of human muscle fibers was supported in Stuart’s paper by proteomic analyses showing coexistence of multiple MYHs within the same fiber, with the surprising finding that most type 1 fibers contain <50% MYH7, most 2A fibers <50% MYH2, and most 2X fibers <50% MYH1 (Fig. 6, A–C). Mass spectrometry also revealed that significant amounts of MYH4, MYH6, and MYH8 proteins are present in human muscle fibers (Fig. 5A), although, surprisingly, only traces of the corresponding transcripts were detected by mRNA analysis (Fig. 5C). However, these conclusions are not supported, as the amount of each MYH was determined using the total number of peptides and not unique peptides. Given the high sequence identity of MYHs, this approach does not provide reliable results, as clearly pointed out in previous muscle proteome studies (1, 2). We have recently described a novel procedure for single muscle fiber proteomics, using mechanically dissected single mouse muscle fibers processed for mass spectrometry by a shotgun approach (4). In this study we confirmed that MYH proteins must be quantified by the intensities of peptides unique for each isoform. Indeed, total peptide analysis leads to the false conclusion that muscle fibers contain multiple MYHs, including significant amounts of MYH7 and MYH4 within the same fiber (Table 1).

The view that human muscle fibers are all hybrid fibers containing a mixture of fast and slow myosins is also in contrast with electrophoretic studies, showing that the majority of isolated human muscle fibers contain a single MYH band, only a minor proportion of fibers containing two bands, either MYH2 with MYH7 or MYH1 with MYH2 (see ref. 3). In conclusion, we believe that available evidence based on different approaches indicates that most of the fibers in human skeletal muscle contain either fast or slow MYHs, with only a minor proportion of fibers containing a mixture of fast and slow MYHs, and that MYH4 and MYH6 are not present in most skeletal muscles. It is possible that pathological samples display atypical patterns of MYH gene expression, therefore control and pathological samples should always be clearly identified.

Finally, Stuart et al. try to correlate their results with the ATPase histochemical reaction used for muscle fiber typing.
and refer to the “pH optimum” of the ATPase. However, statements such as “the type I fiber ATPase pH optimum of 4.6 is attributed to a complex of MYH6 and MYH7” or “the pH optimum of 9.4 in fast-twitch fibers is determined by a complex of MYH1, MYH2, MYH4, and MYH8” are misleading because the ATPase histochemical method reveals specific resistance of myosin isoforms to nonphysiological pH values, as determined by acid or, respectively, alkali preincubation, and not the pH of the ATPase reaction itself.

DISCLOSURES

L. A. Leinwand is a co-founder of MyoKardia, Inc. and has ownership of stock and has a sponsored research agreement with MyoKardia.

AUTHOR CONTRIBUTIONS


REFERENCES