When less is more: a simple Western blotting amendment allowing data acquisition on human single fibers

Thomas E. Jensen¹,² and Erik A. Richter¹

¹Molecular Physiology Group, Department of Exercise and Sport Sciences, University of Copenhagen, Copenhagen, Denmark; and ²Program in Cell Biology, The Hospital for Sick Children, Toronto, Ontario, Canada

The subdivision of skeletal muscle into distinct fiber types based on myosin heavy chain (MHC) isoform expression [I, Ia, and IIx in humans; I, Ila, IIx, and IIB in rodents (9)], has proven to be a practical and lasting working concept not only in studies of contractile properties but in the general study of muscle physiology. Hence, in the past 30–40 years, a wealth of rodent data, interspersed with occasional human studies, has demonstrated that both the expression and regulation of a multitude of proteins can vary markedly depending on the muscle fiber type. In rodents, protein expression appears in some cases to represent an activation pattern-dependent continuum, as seen e.g., for glucose transporter 4 (GLUT4) (11). Other proteins in rodents have been reported to be more or less exclusively expressed in muscles dominated by a subset of fiber types [e.g., mouse TBC1D1 and TBC1D4 (6)]. Muscle type-specific protein signaling also appears to occur with some stimuli in rodents. For instance, Akt is phosphorylated in response to passive stretch in ex vivo-incubated MHC type II isoform-dominated mouse extensor digitorum longus (EDL) muscle but not in the more MHC type I isoform-containing soleus muscle (8). Ultimately, these distinct protein expression and/or activation profiles may regulate end-point biological processes in a muscle fiber type-specific manner. In rodent muscles, this seems again to represent a continuum in some cases while being confined to certain muscle fiber types in others. Using the rat glucose uptake response as an example, insulin generally stimulates an increase in this process in skeletal muscle, but the response is many-fold higher in MHC type I-dominated muscles compared with muscles with predominantly MHC type II isoforms (7). In contrast, the AMP-mimetic AMPK activator, aminoimidazole carboxamide ribonucleotide (AICAR), has in rats been shown to increase glucose uptake in MHC type II isoform-dominated muscles but not in MHC type I isoform-dominated muscles, despite activating AMPK in both muscle types (1). This distinct muscle difference suggests that some proteins required for AICAR to stimulate glucose uptake into muscle are expressed in a fiber type-specific manner.

Human skeletal muscle fiber type composition is in general very heterogeneous with for instance the vastus lateralis muscle, the muscle most commonly biopsied, containing ~50% of MHC type I and 50% of MHC type II fibers (2). Human studies have confirmed that both protein expression and regulation can differ depending on the fiber type (e.g., 2, 4). If a given investigated response occurs in a subset of muscle fibers, then Western blotting of protein obtained from a whole muscle biopsy lysate could conceal a fiber type-specific expression or activation. The risk of underestimating effect sizes or even false negative conclusions has obvious implications to scientific advancement in muscle physiology and drug development. With this in mind, why then are scientific questions not more commonly asked and answered at the fiber type level in humans? To a large part this may stem from the fact that the currently published techniques, which are Western blotting on pooled single fibers after fiber type determination (2) or immunofluorescence microscopy on muscle biopsy sections (3, 4), are either very labor intensive or technically difficult. Also, in the case of immunofluorescence microscopy the reliability of the results depends critically on the antibodies being ultraspecific for the investigated protein. As a result, only a limited number of labs have taken these methods on, while the majority still process and analyze human muscle biopsies in a fashion that does not permit fiber type-specific investigation.

In this issue of the Journal of Applied Physiology (JAP), Murphy et al. (5) describe a simple but valuable alteration of the immunoblotting of freeze-dried human muscle tissue already routinely performed by many laboratories that study human muscle physiology. By simply dissolving freeze-dried single fibers directly in sample buffer, and performing all normalization steps and fiber typing after blotting, this development greatly speeds up the acquisition of data at the fiber type level. To demonstrate the technique’s usefulness, Murphy et al. employed this method to demonstrate the fiber type-specific expression of α1- and β1-subunits of the heterotrimeric protein AMPK in human skeletal muscle. A great asset of this procedure no doubt lies in its straightforwardness and in the modification of an existing laboratory workhorse technique. This should increase the likelihood that this add-on will be tried and tested by the scientific community. Its main drawback may lie in the limited amount of protein that can be analyzed from single fibers, but how much of a limitation this is remains to be seen (see Fig. 1).

The immediate applications for this technique are readily apparent, including the examination of fiber type-specific protein expression patterns, posttranslational protein modifications with various interventions such as different exercise regimens, stimulation with various hormones, and pharmacological compounds. In conjunction with a 2008 JAP Innovative Methodology paper by Wacker et al. showing that quantitative RT-PCR is likewise possible at the single-fiber level (10), the techniques now seem to be in place to enable characterization of both mRNA and proteins obtained from human muscle biopsies at fiber-type resolution.

DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).
Fig. 1. Overview of previously published and featured methods for fiber type-specific protein measurement in human muscle-biopsies: 1, immunohistochemistry on muscle cross-sectional slices; 2, pooling of single fibers for Western blotting after fiber typing; 3, direct lysis and Western blotting of single fibers. See text for details.

REFERENCES


