How many phosphoproteins does it take to make muscle grow?

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Perspective

Understanding how exercise causes muscles to adapt is fundamental to improving our health, quality-of-life and longevity. Exercise capacity is strongly and inversely related with all-cause mortality and offsetting the age-associated loss of muscle mass is a key component of this protective effect. In adults, muscle is the most abundant tissue in the body, it is the largest reservoir of amino acids that can be used to support metabolism and repair other tissues, and in healthy individuals it is the primary site of postprandial glucose disposal. Clearly there is an intimate and reciprocal relationship between exercise and muscle; without muscle we could not exercise – and – without exercise our muscles deteriorate and become dysfunctional. Resistance exercises involving high-force maximal contractions can stimulate muscle growth and are recommended (alongside endurance activities) in national guidelines for health, particularly as a countermeasure against age-associated declines in physical function. Despite the clear importance of skeletal muscle and the key role of resistance exercise to human health we know surprisingly little about the molecular events that link muscle contraction to muscle adaptation.

Muscle growth induced by exercise is underpinned by myofibre hypertrophy and protein accretion, which in turn results from changes in the net balance between protein synthesis and degradation. The recent focus of interest has been on the regulation of protein synthesis and it is now well-established that resistance training increases ribosomal translation, and that signalling via the mammalian target of rapamycin (mTOR) is a major effector of this process. The rapamycin-sensitive mTOR complex (mTORC1) is a principal regulator of cell growth induced by growth factors such as insulin-like growth factor I (IGF-I), but high-force contractions of skeletal muscle can stimulate mTORC1 through a mechanism that is distinct from the classical growth-factor pathway. The group of Troy Hornberger have largely driven developments in this area to focus attention to the activation of mTORC1 by contraction-induced increases in phosphatidic acid (1). Nonetheless, important gaps in knowledge still exist between the action of muscle contraction and the stimulation of mTORC1 by phosphatidic acid, and other as yet unidentified regulators may also be involved. To some extent traditional hypothesis-led research is limited in its ability to tackle this issue – largely because we may not know the identity of all of the relevant players. In this issue of the Journal of Physiology, Potts et al (2) addresses the need for new insight in this area by using proteomic techniques to investigate changes in phosphorylation that co-occur with the activation of mTORC1 in mouse muscle subjected to a bout of maximal-intensity contractions.

Proteomics, probably more than any other -omic discipline, was once regarded derogatorily as a ‘fishing expedition’ but non-targeted proteomic profiling has now become a proven discovery technique and is increasingly being adopted to find novel avenues of exploration and generate new hypotheses. Proteomics is arguably more challenging than other -omic endeavours because proteins

exhibit a more diverse range of physiochemical properties compared to other macromolecules. Furthermore, proteins commonly exist as different species which can be the product of combinations of different splicing events and post-translational modifications, and the number of protein species outweighs the number of genes by many orders of magnitude (3). Notwithstanding these challenges, proteins are the closest molecular link to cellular function and important biological processes involved in signal transduction can only be studied at the protein level. We (4) reported 'top-down' analysis of protein species using 2-dimensional gel electrophoresis in the first application of proteomics in human exercise physiology. However, muscle is a challenging substrate for protein-level separation because it is dominated by a small number of highly abundant myofibrillar proteins and nowadays bottom-up workflows involving digestion of proteins in to peptides prior to analysis are more often favoured. Indeed, the co-evolution of peptide mass spectrometry and its data analysis techniques have been paramount to the advancement of the field, and tandem mass spectrometry (MS/MS) of protein digests is now unrivalled in its ability to discover new site-specific covalent modifications such as phosphorylation.

Phosphorylation causes changes in protein conformation that alter functional characteristics (e.g. enzymatic activity, protein-protein interactions and subcellular localisation) of the protein and also change its peptide MS/MS spectra, which can be used to map modifications to specific residues (5). Potts et al (2) reports almost 6,000 phosphorylation sites on more than 4,800 proteins including low-abundance proteins involved in signal transduction. One-hour after a bout of maximal-intensity contractions there were more than 600 differences in phosphorylation status spread across more than 300 proteins. Less than half of the exercise-responsive phosphorylation sites have been previously detected and in most cases the kinases responsible for phosphorylation of these sites have not been defined. Therefore, this work represents a substantial addition to the body of information on muscle responses to resistance exercise. Deciphering which of these signals link contraction to adaptation will be the next challenge and this may not be a straight-forward process. For instance, exercise is associated with widespread perturbations to homeostasis, therefore molecular events detected in exercised muscle could be associated with restoration of cellular homeostasis rather than, or as well as, being the signalling events that instigate adaptation. Moreover, such comprehensive information on phosphopeptides is not equivalent to knowing the protein species which are the entities that are actually responsible for biological processes. Indeed, it is uncommon for a protein to be modified at just one site or by just one type of modification (5). So, at some point the field will also have to start trying to 'put humpty-dumpty back together again' in order to uncover the true nature of the protein species that dictate muscle adaptation.

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Additional Information

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