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Exercise Proteomics: Review

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Abstract

Aim: The aim of this review was to discuss the proteomics of exercise. **Results:** The sophisticated protein biochemical and mass spectrometric technologies can now be used to study subtle changes in protein concentration, isoform expression patterns, protein–protein interactions and/or post-translational modifications following physical exercise. **Conclusion:** Rapid advancements in molecular techniques and the streamlining of mass spectrometry-based proteomic workflows have enabled the establishment of global alterations in the concentration, isoform expression patterns, molecular interactions and post-translational modifications of muscle proteins following physical exercise. Recent genetic advances in the field of molecular exercise science will heavily influence the design of future proteomic studies in sports science and sports medicine.

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Introduction

Over the last decade, a large number of scientific breakthroughs have transformed the field of exercise science (Baldwin and Haddad 2010). Our understanding of gene regulation and protein alterations in response to physical exercise has dramatically improved through the application of molecular and cellular analyses of skeletal muscle adaptations. This has involved the clarification of novel structural, functional and metabolic aspects during force generation and physiological adaptability in response to different training regimes (Harridge 2007). Physical exercise triggers various physiological stimuli that involve neuronal, hormonal, metabolic and mechanical signals that are sensed, transduced and integrated in a highly coordinated manner (Flück 2006). The frequent recruitment of specific muscle groups causes long-term alterations in gene expression patterns and special changes in the concentration, isoform repertoire and/or post-translational modifications of skeletal muscle proteins (Booth and Laye 2010). During muscle adaptations, a vital relationship exists between contraction-induced signalling cascades and downstream effects in contractile fibres on the level of gene activation, mRNA processing, protein synthesis and protein assembly, as well as metabolic regulation. Novel integrative approaches attempt to study these effects of physical exercise-induced physiological disturbances on the level of the genome, transcriptome, proteome and metabolome (Petriz et al.2012; Burniston and Hoffman 2011; Hittel et al. 2007).

What is a Proteome?

A proteome indicates the quantitative protein expression profile of a cell, a tissue or an organism under exactly defined conditions. The human genome comprises about 100 000 genes and this gene inventory is applied for a cell type-specific expression of a set of 10 000 genes. One gene will result

in multiple protein products, on average 3 proteins per gene in S. cerevisiae, 1.3 proteins per gene in E. coli and perhaps more than 10 proteins per gene in humans if one also considers body fluids (Hochstrasser 1997). Thus, a proteome consists out of about 100 000 proteins. These simple numbers show the enormous variability in the composition of a proteome and the ability to form an infinite number of phenotypes. A small modifications in the expression parameters, due, for example, to physical exercise or stress or drug effects, will change the protein pattern and cause the presence or absence of a protein or gradual variations in abundances. Proteomics analyses and compares protein expression profiles and links the observed protein pattern changes to the causal effects.

From Genome to Proteome

The biochemical characterization of the proteins encoded by the approximately 20,300 human genes is complicated by the multi-functionality of many protein molecules, the highly diverse interactions within protein complexes and the dynamic nature of protein expression patterns (Paik et al. 2012). In contrast to the relatively stable genome, the global protein constellation of specific cell types or tissues is highly variably and constantly adapting to changed functional demands and environmental influences. The inconsistency between the extremely large number of individual protein species in the human body and the much lower number of identified genes is due to various regulatory mechanisms and extensive protein conversions. This includes the existence of alternative promoter repertoires, the alternative splicing of mRNAs and the enzymatic cleavage of some polypeptide chains into more than one subunit, as well as an extremely large variety of post-translational modifications (Altelaar et al. 2013).

Proteomics of Endurance Exercise

Exercise proteomics was used to study protein alterations in humans (Malm et al.2012; Egan et al. 2011; Norheim et al. 2011; Hody et al. 2011; Moriggi et al. 2010; Holloway et al. 2009) and animal models of physical activity (Gandra et al. 2012; Magherini et al. 2012; Macedo et al. 2012;Yamaguchi et al. 2010; Bouwman et al. 2010; Burniston 2008; Donoghue et al. 2007; Guelfi et al. 2006; Donoghue et al. 2005). The neuromuscular system has a vast capacity to become accustomed to a great variety of physical demands and differing training conditions by muscle remodelling involving changes in contractile properties, metabolic pathways and tissue mass (Bassel and Olson 2006). In this respect, human skeletal muscles demonstrate an extraordinary capacity to adjust to long-lasting endurance exercise by optimizing power output, increasing fatigue resistance and modifying metabolic processes to maximize aerobic capacity (Flueck and Eilers 2010). The physiological conditioning of fatigue resistance and the bio-energetic enhancement of aerobic performance are based on finely tuned physiological machinery that promotes endurance performance (Seene et al.2011). Proteomic profiling of the effect of endurance training on human *vastus lateralis* muscle revealed distinct adaptations in the expression profile of the mitochondrial proteome, especially affecting enzymes such as NADH dehydrogenase and ATP synthase (Egan et al. 2011). The mitochondria-enriched fraction from skeletal muscle biopsies showed differential expression patterns of enzymes of the citric acid cycle, oxidative phosphorylation, mitochondrial protein synthesis, oxygen transportation and antioxidant capacity following endurance training (Egan et al. 2011). A variety of proteomic surveys with animal models of endurance training have confirmed exercise-induced mitochondrial remodelling and an increased capacity for oxidative metabolism. A clear bioenergetic shift from glycolysis towards fatty acid oxidation exists in several trained animal species (Magherini et al 2012; Bouwman et al. 2010; Burniston 2008). Thus, the fact that endurance exercise results in mitochondrial remodelling and an increased oxidative capacity, rather than hypertrophy of muscle fibres, was confirmed by mass spectrometry-based proteomics. Interestingly, adenovirus-mediated delivery of cDNA encoding insulin-like growth

factor-I triggered neovascularization, muscular hypertrophy, fast-to-slow muscle transformation and a considerable endurance gain. This shows the crucial role of growth factors in metabolic and functional adaptations of the neuromuscular system during training. However, in the case of excessive physical exercise, muscle fibres might be challenged by the lack of a sufficient supply of oxygen. This makes the findings of proteomic analyses of muscle fibres under conditions of hypoxia relevant for sports medicine. Chronic hypoxia triggers functional adaptations in skeletal muscles and causes a metabolically compensatory enhancement of the glycolytic pathway to counteract the lack of oxygen (Viganò et al. 2008).

Proteomics of High-intensity Training

The global effects of high-intensity training during strenuous interval training or strength training have also been analysed by proteomics (Gandra et al. 2012; Norheim et al. 2011; Yamaguchi et al. 2010; Holloway et al. 2009; Guelfi et al. 2006). Human *vastus lateralis* muscle showed increased expression levels of the mitochondrial enzymes succinate dehydrogenase and ATP synthase in response to interval training, as well as post-translational modulations of troponin TnT and muscle creatine kinase (Holloway et al. 2009). Similar results were obtained with a rat model of high intensity exercise using swimming boats while carrying a weight (Yamaguchi et al. 2010) or treadmill training with incremental increases in speed until exhaustion (Gandra et al. 2012). The proteomic profiling of exercised rat *epitrochlearis* muscle revealed elevated levels of mitochondrial enzymes, especially NADH dehydrogenase. In contrast, the cytosolic Ca2+ binding protein parvalbumin was reduced following high intensity exercise (Yamaguchi et al. 2010). Changes in these muscle-associated proteins appear to represent distinct alterations in the fibre proteome following the stimulation of the AMP-activated protein kinase AMPK and elevation of sarcoplasmic Ca2+ levels during muscle contraction. Norheim and co-workers (Norheim et al. 2011) have initiated the proteomic identification of potential alterations in the secretion of signalling proteins from human skeletal muscle cells in response to strength training. Initial studies suggest that several types of myokines with paracrine or endocrine functions may be synthesized in myofibres and then being secreted for interactions with other tissues (Norheim et al. 2011). The exact activation process, release mechanisms and non-muscle targets of these novel protein factors remain to be determined.

Proteomics of Overtraining and Muscular Injury

Besides neuromuscular diseases, traumatic injury, toxic insults, alcohol abuse and pharmacological side effects, acute skeletal muscle damage can also be triggered by strenuous exercise. Vigorous strength training can put athletes at risk of severe fibre injury or even rhabdomyolysis. If muscle fibre breakdown triggers the extensive release of the intracellular muscle contents, the deleterious leakage of fibre proteins and ions may cause pathological fluid imbalances, disturbed electrolyte homeostasis, cardiac arrhythmia and acute kidney failure. In order to improve diagnostic methods to swiftly detect exercise-induced rhabdomyolysis and be able to better evaluate the degree of skeletal muscle damage, new and more reliable fibre-derived biomarkers are needed. Mass spectrometry-based proteomics presents an ideal analytical tool to establish a superior biomarker signature of exercise-related muscle damage (Ohlendieck et al. 2013). Besides the currently used serum biomarkers, creatine kinase and carbonic anhydrase, the application of proteomics promises to identify improved markers of rhabdomyolysis, as well as indicators of the natural secretion process that releases myokines and other fibre-associated indicators during exercise-induced adaptations. Proteomics has so far been applied to determine global changes in the case of delayed-onset muscle soreness in response to acute or repeated eccentric exercises (Hody et al. 2011) and skeletal muscle damage as a result of extensive downhill running in human athletes, as well as in an animal model of overtraining using an excessive treadmill endurance exercise (Gandra et al. 2012).

Surprisingly, myosin heavy chains and glycolytic enzymes decreased after eccentric tests, suggesting that eccentric training may trigger a switch to oxidative metabolism to protect against delayed-onset muscle soreness (Hody et al. 2011). Downhill running-associated skeletal muscle damage was found to induce increased levels of actin and desmin, but a reduction in the luminal Ca2+ binding protein calsequestrin of the sarcoplasmic reticulum. Hence, cytoskeletal functions, the assembly and stabilization of the Z-disc domain and calcium homeostasis seem to be affected in running-related muscle damage (Malm et al. 2012). The proteomic profiling of different parts of rat *gastrocnemius* muscle has shown that skeletal muscles with different fibre-type compositions respond differently in response to treadmill endurance overtraining (Gandra et al. 2012). The red portion of the over-trained *gastrocnemius* muscle exhibited an increased density of proteins involved in oxidative phosphorylation, lipid metabolism, antioxidant protection and the cellular stress response (Gandra et al. 2012), as usually observed during adaptations to endurance training. Interestingly, the white portion of the same muscle did not show these alterations following treadmill endurance overtraining (Gandra et al. 2012).

Conclusion

Rapid advancements in protein biochemical techniques and the streamlining of mass spectrometry-based proteomic workflows have enabled the establishment of global alterations in the concentration, isoform expression patterns, molecular interactions and post-translational modifications of muscle proteins following physical exercise. The systematic application of proteomics has identified adaptive changes to training in key proteins involved in excitation–contraction coupling, the contraction–relaxation cycle, metabolic pathways and the cellular stress response. These findings have both improved our general understanding of molecular and cellular mechanisms that underlie skeletal muscle transitions and identified interesting new biomarker candidates that are characteristic for exercise-induced muscle transformation. Recent physiological, biochemical and genetic advances in the field of exercise science will heavily influence the design of future proteomic and systems biological studies in sports science and sports medicine.

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