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Proteomic responses of skeletal and cardiac muscle to exercise

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Summary

Regular exercise is effective in the prevention of chronic diseases and confers a lower risk of death in individuals displaying risk factors such as hypertension and dyslipidaemia. Thus, knowledge of the molecular responses to exercise provides a valuable contrast for interpreting investigations of disease and can highlight novel therapeutic targets. While exercise is an everyday experience and can be conceptualized in simple terms, exercise is a complex physiological phenomena and investigation of exercise responses requires sophisticated analytical techniques and careful standardization of the exercise stimulus. Proteomic investigation of exercise is in its infancy but the ability to link changes in function with comprehensive changes in protein expression and post-translational modification holds great promise for advancing physiology. This review highlights recent pioneering work investigating the effects of exercise in skeletal and cardiac muscle that has uncovered novel mechanisms underling the benefits of physical activity.

Keywords

Skeletal muscle proteomics; cardiac muscle proteomics; exercise training; cardioprotection; 2-D gel electrophoresis; mass spectrometry; post-translational modification; heat shock proteins; ATP synthase beta.

Background

Regular exercise is fundamental to maintaining health throughout the lifespan and an individual's maximum aerobic capacity (i.e. VO_2max) is an independent indicator of their health status [1]. Low aerobic capacity is a risk factor for diseases, including type 2 diabetes mellitus and cardiovascular disease [2], and a stronger predictor of early mortality than other established risk factors such as cigarette smoking or hypertension [3]. Accordingly, regular exercise prevents chronic diseases and confers a lower risk of death in individuals displaying the established risk factors of cigarette smoking, hypertension and dyslipidaemia [4]. Whole-body aerobic capacity is determined by central (i.e. cardiovascular) and peripheral (i.e. skeletal muscle) components and regular physical activity produces specific molecular

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adaptations in both cardiac and skeletal muscles that confer protection against chronic disease.

A large body of literature exists regarding adaptations in striated muscle in response to exercise. In the majority this information arises from investigations testing specific hypotheses using reductionist approaches, and these works have illuminated many fundamental features of exercise-induced adaptation. More latterly, post-genomic techniques, such as gene expression profiling using microarrays, have been applied in exercise physiology. In particular, transcriptome analyses have illustrated extensive differences in mRNA expression between muscles of trained and untrained individuals [5, 6] and brought forth new insight of the complexity of molecular responses to exercise (e.g. [7–9]). In light of this knowledge it is clear physiological phenomena cannot be explained at the level of individual genes or signaling pathways, instead, networks of gene circuits interact to bring about changes in function. Thus, systems biology approaches embracing comprehensive analysis hold the potential to extend the boundaries of knowledge.

While transcriptome analysis is a powerful discovery tool the quantitative link between the abundance of a protein and the expression of its gene can be tenuous [10] and protein analysis is necessary to verify mRNA findings. Such validation may be especially important when investigating exercise because muscle activity affects the rate of protein turnover [11] and induces changes in post-transcriptional processing [12], translational regulation [13], and protein degradation [14] that may each influence the quantitative relationship between the abundance of a protein and the transcription of its gene. Further to validation of mRNA findings, proteomic analysis is necessary to discover post-translational modifications that can alter the functional characteristics of the cell in the absence of changes in gene transcription.

Technological developments including robust two-dimensional (2-D) gel electrophoresis and mass spectrometry (MS) now enable large-scale investigations and inductive ‘discovery science’ approaches to be applied at the protein level [15]. However, because proteins exhibit broad differences in physical-chemical properties and copy number, comprehensive analysis is difficult particularly in skeletal muscle, which expresses a relatively small number of myofibrillar proteins in high abundance. At present, proteome analysis cannot resolve the large numbers of features detected by microarray technology; in particular, low abundance proteins and hydrophobic membrane proteins may be absent in 2-D gel investigations. Nevertheless, the proteome is the interface between the genome and the environment, and is what defines a cell and dictates its functional characteristics. Thus, purposeful exploration of muscle proteome responses to exercise has the potential to advance physiology and our understanding of the health benefits of a physically active lifestyle. Excellent reviews of proteomic analyses of cardiac [16] and skeletal [17] muscle have recently been published in this journal and only a brief contextual introduction is included here prior to considering recent findings relating to exercise.

Proteomic Analysis of Striated Muscle

Skeletal muscle comprises a heterogeneous mixture of myofibres, in human muscle 3 fibre subtypes are recognized based on their contractile and metabolic properties [18]. Fast-twitch fatigable fibres rely predominantly on glycolytic metabolism and are designated FG (fast glycolytic), whereas, fast-twitch fatigue-resistant and slow-twitch fibres have relatively greater mitochondrial content and are designated FOG (fast oxidative glycolytic) and SO (slow oxidative), respectively. The fibre type-specific differences in contractile function are due to differential expression of a diverse range of isoforms of each myofibrillar protein [19]. Myosin heavy chain (MyHC) isoforms are intimately associated with myofibre contractile and energetic properties and are commonly used molecular markers: FG fibres express MyHC IIx/d, FOG fibres express MyHC IIa and SO fibres express MyHC I, which is also the predominant isoform in adult human myocardium. Phenotyping of muscle based on its profile of FG, FOG and SO fibres or relative expression of MyHC isoforms has provided important insights regarding muscle function and plasticity, but such characterization is relatively simplistic. For example, changes in isoform expression, splice variation or post-translational modification of other myofibrillar proteins, such as troponins and myosin light chains, can alter contractile characteristics in the absence of changes in MyHC. Moreover, each myofibre type is metabolically heterogeneous and can exhibit a broad spectrum of different metabolic enzyme concentrations that determines substrate utilisation and resistance to fatigue.

Proteomics affords the capability to analyse large numbers of contractile and metabolic proteins simultaneously and, therefore, develop a more sophisticated understanding of muscle diversity and plasticity. Early investigations produced 2-D gel maps of rodent heart [20] and mixed-fibre gastrocnemius muscle [21], and compared [22–25] archetypal muscles such as rat extensor digitorum longus (EDL) and soleus that comprise predominantly fast- or slow-twitch fibres, respectively. These and other proteome mining works provide important catalogues of accessible muscle proteins and extend earlier biochemical and histochemical studies describing muscle diversity. Indeed, proteome mining of human muscle [26] reveals the relative expression of many myofibrillar and metabolic proteins lies within the extremes observed in archetypal rodent fast- and slow-twitch muscle, which is consistent with the differences in myofibre profile between rodents and humans [18].

The plasticity of muscle in response to changes in activity is elegantly demonstrated by chronic low-frequency stimulation (CLFS), which is capable of transforming fast-twitch muscle to a slow contractile phenotype [27]. Proteome studies [28, 29] employing CLFS of rabbit tibialis anterior clearly illustrate the spectrum of muscle plasticity and have identified novel biomarkers of muscle transformation. However, voluntary muscle contraction involves ordered recruitment of muscle motorunits [30] corresponding with exercise intensity (as opposed to CLFS, which stimulates the muscle motorunit pool uniformly) and even the most rigorous program of sports training cannot approach the level of activity imposed by CLFS. Nevertheless, voluntary activity results in overt changes in muscle function, which are associated with protection from disease. For example, proteomic analysis [31] of muscle from elderly adults living a physically active lifestyle reveals robust differences in the expression of mitochondrial enzymes and preservation of insulin sensitivity compared to

sedentary counterparts. Furthermore, comparative analysis of human upper-and lower-limb muscles [32] reveals that enzymes involved in energy metabolism are more abundant in vastus lateralis than deltoid, despite only moderate differences in MyHC isoform expression between the two muscles. This difference, which is consistent with the role of vastus lateralis in locomotion, clearly demonstrates that the muscle proteome is more closely related to muscle function than is myofibre profile.

Adaptation of the skeletal muscle proteome to exercise

Responsiveness to exercise training can vary widely in humans [33, 34] and this imparts considerable difficulty to investigating exercise-induced adaptations. Moreover, the implausibility of sampling some tissues from healthy humans necessitates the use of animal models to enable access to organs for molecular investigation and, to date, the majority of proteomic investigations of exercise have used laboratory rats and employed exercise models including forced treadmill running, swimming and voluntary wheel-running (Table 1). Muscle adaptation in response to exercise training is specific to the training stimulus, which comprises 3 interrelated variables: (i) intensity, (ii) duration and (iii) frequency. Exercise intensity is arguably the predominant component of the training stimulus, but swimming and free wheel-running models do not afford control of exercise intensity and this imparts difficulties in comparing data across these studies. In contrast, the a rodent model of intensity-controlled treadmill running [35, 36] enables exercise to be prescribed at intensities relative to each animal's VO_2max, which is consistent with best practice in human studies. Proteomics has been used to investigate skeletal muscle responses after either an acute exercise stress (Table 2) or a chronic period of training (Table 3). We will consider these two experiment designs separately because proteins that change immediately after acute exercise may be particularly involved in supporting muscle activity or the processes of recovery and adaptation, whereas, protein differences between trained and untrained muscles may relate more closely with differences in muscle function.

The first studies in exercise proteomics investigated the acute effects of swimming. For example, Takahashi et al. [37] performed 2-D electrophoresis (2-DE) on muscle pooled from rats killed immediately after 150 minutes swimming and reports lesser expression of a gel spot containing a putative transcription factor, AI854635, which has since been identified as zinc finger protein 3. In C2C12 myoblasts, the mRNA expression of zinc finger protein 3 decreases after serum deprivation [37], implicating this protein in the process of myoblast differentiation. However, as yet, the role of zinc finger protein 3 in the adaptation of differentiated adult muscle has not been elucidated. In a similar study Guelfi et al. [38] isolated gastrocnemius muscles from rats either immediately after or 30 min after a 3-min bout of swimming. Rather than pooled samples, biological replicates were analysed which enabled statistically significant differences to be identified in the expression of troponin T, creatine kinase and a spot containing both adenylate kinase 1 and heat shock protein 20 (hsp20). More recently, Gandra et al [39] investigated changes in rat gastrocnemius muscles isolated either 3 h or 24 h after an incremental treadmill test to exhaustion and reports significant changes in glycolytic enzymes, heat shock cognate protein 70 and carnitine palmitoyltransferase II. These changes observed in proteins involved in high-energy phosphate [38] and glycolytic [39] metabolism are consistent with the energy demands of

intense exercise. However, because muscles were isolated at different time points or after differing bouts of acute exercise there is no direct identity among the findings (Table 2) of these studies.

Changes in spot expression in the minutes to hours after exercise may be too rapid to be accounted for by differences in protein synthesis or degradation. Instead, post-translational modification may be the mechanism underlying the changes in spot expression. Covalent modifications, including phosphorylation and acetylation, alter the isoelectric point (p*I*) of a protein without significantly altering its M_r and result in the appearance of spots with patterns of migration that are markedly different from the non-modified form. Indeed, proteome mining studies consistently identify many muscle proteins as series of spots of similar M_r but different p*I*. Besides providing separation for MS analysis, this enables shifts in post-translational state to be quantified based on relative changes in spot volume (Figure 1).

Recently, we [35] found a conspicuous effect of endurance training was to alter the relative expression of individual spots within multi-spot series; thus linking changes in the spot profile of individual proteins with differences in muscle function. Using image analysis we compared the relative expression of 187 protein spots matched across 2-D gels of muscles from 6 endurance-trained and 6 sedentary rats [35]. Proteins were unambiguously identified in 80 gel spots using peptide mass fingerprinting and confirmatory MALDI-ToF MS/MS. Statistical analysis detected significant changes in the expression of 15 gel spots, which represented the products of 11 individual genes. Six of the 11 differentially expressed genes were present as multiple protein spots. Endurance training altered the expression of single spots from each of the multi-spot series identified as transferrin (total of 3 spots), albumin (total of 4 spots) and lactate dehydrogenase A (total of 2 spots), and induced changes in multiple spots from series identified as phosphoglucomutase 1 (PGM1), triosephosphate isomerase and mitochondrial aconitase (ACON2).

When a protein is present in multiple gel spots, it can be erroneous to interpret a change in expression of an individual spot as being indicative of a change in the total abundance of the gene product. For example, ACON2 resolved as 5 discrete spots (Mr ~ 85 kDa, pI 7.5 - 8.0) and endurance training decreased the expression of 2 spots and increased the expression of a third [35]. Despite the marked differences in the spot profile, the sum volume of ACON2 was not different between sedentary and exercised muscle. Nonetheless, the changes in ACON2 expression may be biologically significant. Muscle activity is intimately associated with production of reactive oxygen species and ACON2 is particularly susceptible to metal-catalyzed oxidation. Using 2-DE and western blotting of derivatised proteins we found the shift in ACON2 spot pattern correlated with an increase in carbonylation, which previously has been associated with a loss of enzyme activity. Exercise-induced carbonylation of ACON2 seems inconsistent with its role in the tricarboxylic acid (TCA) cycle but inactivation of aconitase by oxidation may result in its association with mitochondrial DNA nucleoids, which stabilizes the mitochondrial genome during adverse conditions [40].

In the same experiment [35], PGM1 was resolved as 4 spots, and the statistically significant decrease in the normalised volume of 2 spots was accompanied by lesser (non statistically

significant) decreases in the 2 other isoelectric species. In this case, the changes in spot expression indicate a general decrease in protein abundance in exercised muscle, and corresponded with a significant decrease in enzyme activity. PGM1 catalyses the conversion of glucose 1-phosphate and glucose 6-phosphate, important in glycogen metabolism, and this finding is consistent with a fundamental adaptation of muscle to endurance training. That is, trained muscle has a greater ability to utilize fatty acids, which spares the muscle's limited glycogen stores, thus offsetting fatigue and prolonging endurance. Using histochemistry, we found the decreases in enzyme activity and expression of PGM1 were associated with greater glycogen content particularly in SO and FOG myofibres which were hypertrophied in exercise-trained muscle. Interestingly, the effect of exercise on PGM1 is opposite to the increase in PGM1 expression detected by proteomic analysis of muscle from type 2 diabetes patients [41]. So, although, PGM1 is not traditionally considered as a point of control in glycogen metabolism, these findings from unbiased proteomic investigations suggest modulation of PGM1 is a prominent feature that correlates with differences in muscle metabolism and health status.

The incorporation of high-intensity intervals within a training program is associated with more rapid and pronounced improvements in aerobic capacity and enhanced health-related benefits compared to continuous moderate exercise [42]. Yamaguchi et al. [43] investigated the effects in rat epitrochlearis muscle of 5 days of high-intensity interval training incorporating multiple 20-s bouts of weighted swimming interspersed by 10-s recovery periods. Difference in-gel electrophoresis (DIGE) was used to analyse muscle from 4 control and 4 exercise-trained animals. Using DIGE, independent samples labeled with Cy3 or Cy5 canine fluorescent dyes and a pooled internal standard labeled with Cy2 dye are combined equivalently and separated in each gel. This approach minimizes technical variation and affords quantitative expression analysis because each spot is normalised to a consistent internal standard. Yamaguchi et al. [43] reports significant increases in ATP synthase beta and subunits of Complex I in exercise-trained muscle, while, spots containing the soluble calcium-binding protein, parvalbumin, and fast isoforms of myosin regulatory and essential light chains were less abundant, consistent with a shift toward a more fatigue resistance aerobic phenotype.

Recently, we [44] also used high-intensity interval training to conduct the first proteomic investigation of the effects of exercise in human muscle. Healthy adult males completed a 6-wk training programme involving three sessions per week, utilizing six 1-min bouts at VO_2max interspersed with 4 min at 50% VO_2max. Using 2-DE, we resolved 256 protein spots in small (~15 mg) biopsy samples of vastus lateralis taken before and after the training regimen and, as expected, 2-DE resolved many proteins as multiple isoelectric species, offering the opportunity to learn new information about exercise-induced adaptation in humans. Myosin light chains were clearly resolved but were not differentially expressed as reported [43] in rat muscle. Instead, interval exercise in humans affected both post-transcriptional processing and post-translational modification of slow- and fast-isogenes of troponin T, which may relate to augmented excitation-contraction coupling specifically associated with interval training [42]. Of all myofibrillar proteins, troponin T exhibits the greatest diversity [19] and changes in expression, splice variation and post-translational modification of troponin T isogenes can alter myofibril Ca^{++}-sensitivity and affect muscle

contractility [45]. Based on time-series data [46] describing alterations in rat myofibrillar proteins during hindlimb suspension, changes in troponin T are an early event in muscle adaptation and a sensitive indicator of changes in function. Indeed, interval exercise resulted in robust modulation of troponin T proteins in the absence of significant changes in myofibre profile assessed by traditional MyHC isoform analysis [44]. In addition, we discovered interval training increases phosphorylation of muscle creatine kinase (KCRM). Enhanced phosphocreatine metabolism is consistent with the adaptation to sprint training [47], but the significance of post-translational modification of KCRM is not yet clear. During the initial seconds of exercise, the KCRM reaction is the main contributor to ATP re-synthesis and can affect the rate of increase in oxygen consumption [48], as well as modulate the activation of glycogenolysis and glycolysis. The change in post-translational state of KCRM associated with interval training suggests regulatory mechanisms exist that may influence muscle energy metabolism at the onset of exercise by modifying KCRM.

To date, proteomic analyses of exercise-trained skeletal muscle (Table 3) have mostly detected changes in sarcomeric proteins, metabolic enzymes and molecular chaperones. Some of the proteins differentially expressed after acute exercise (i.e. triosephosphate isomerase, troponin T and muscle creatine kinase; Table 2) are also modulated in exercise-trained muscle (Table 3). In addition, exercise training is associated with modulation of the tricarboxylic acid cycle and electron transport chain enzymes, which is not evident after acute exercise. The metabolic enzymes listed in Table 3 are not regarded as being rate-limiting in their respective metabolic pathways but, nonetheless, these unbiased proteomic findings may point to important information regarding changes in muscle metabolism or other biological processes. In particular, 2 proteins, ATP synthase subunit-β and albumin, have been highlighted in studies of animal and human muscle and this places special significance on these findings. Heat shock proteins (hsp) also represent a notable portion of the proteins identified in proteomic analyses of skeletal (Tables 2 and 3). Exercise-induced changes in hsp expression are commonly observed in skeletal muscle [49] but the exact mechanistic role of such modifications remains to be fully elucidated. The ability of proteomics to measure changes in expression and post-translational modification across numerous hsp simultaneously will hopefully assist in deciphering their specific roles in exercise-induced adaptation.

Adaptation of the cardiac proteome to exercise

Boluyt et al. [50] reports the first MS-based proteomic investigation of the cardiac response to exercise. Rats underwent a 6-week regimen of endurance training incorporating progressive increments in exercise duration, which resulted in a 16 % increase in cardiac mass (i.e. adaptive cardiac hypertrophy). Boluyt et al. [50] investigated hearts isolated 24 h after cessation of exercise and 2-D gel expression profiling detected 26 statistically significant differences between sedentary and exercised animals, including 12 spots that were expressed exclusively in endurance-trained hearts. In particular, endurance training resulted in the expression of hsp20 that was not evident in hearts of sedentary rats. Furthermore, cardiac expression of hsp20 did not occur in response to a bout of unaccustomed exercise in previously sedentary animals, suggesting hsp20 expression is a

component of exercise-induced adaptation rather than an acute stress response to treadmill running.

Reproducibility is a fundamental tenet of scientific experimentation and corresponding data from independent work can provide robust evidence that novel discoveries represent important biological phenomena. Using the rat model of intensity-controlled treadmill running we [36] investigated the effects of a 6-week training regimen consisting of 4, 30-minute sessions per week at ~75 % VO_2max. To standardize the progression of the training stimulus each animal's VO_2max was measured at 2-week intervals during the training period and their absolute training intensity was increased inline with the increments in performance. This programme of endurance running increased the animals' VO_2max by an average of 23 % and was associated with an 11 % increase in cardiac mass. Consistent with Boluyt et al [50], a spot identified as hsp20 was significantly increased in hearts of endurance-trained animals. However, in our work [36], the hsp20 spot that was increased in endurance-trained hearts exhibited a lower than predicted p*I*, consistent with post-translational modification. Accordingly, MS/MS analysis unambiguously identified phosphorylation of hsp20 at serine 16, which is important in the interpretation of these findings.

Together, these independent proteomic investigations suggest endurance training is associated with an adaptive increase in cardiac expression of hsp20 [50], and endurance-trained hearts are characterized by greater transient phosphorylation of hsp20 in the hours immediately after exercise [36]. This may be an important mechanism underlying exercise-induced cardioprotection and phosphorylation of hsp20 at serine 16 has been highlighted as a therapeutic target in heart disease [51]. Introduction of a phosphopeptide analogue of hsp20 in vitro increases cardiomyocyte rate of shortening [52], and expression of a constitutively phosphorylated form of hsp20 protects cardiomyocytes from apoptosis induced by the protein kinase A pathway [53]. Moreover, transgenic overexpression of hsp20 enhances basal cardiac function, and can attenuate ischaemia/reperfusion injury [54], whereas, if phosphorylation of hsp20 is prevented the heart is more sensitive to ischaemia/reperfusion injury [55]. Pharmacological manipulation of hsp20 phosphorylation in humans has not yet been reported, nevertheless, moderate endurance exercise may represent a cost-efficient approach to achieving this beneficial molecular adaptation in a clinical setting.

Endurance training is associated with robust changes in the expression of enzymes involved in cardiac energy metabolism, in particular fatty acid oxidation (Table 4). Sun et al [56] first reported changes in the expression of β-oxidation enzymes, including short-chain acyl-CoA dehydrogenase, and components of the TCA cycle in the hearts of rats exposed to high-intensity swimming. Later, we [36] used moderate intensity treadmill running and measured increases in heart fatty acid binding protein (HFABP), short- and long-chain acyl-CoA dehydrogenases and mitochondrial thioesterase-1. Thus despite cardiac muscle being chronically active and characterized by high mitochondrial content, endurance training further increases the heart's ability to oxidize fatty acids similar to the effect observed in skeletal muscle. Modulation of malate dehydrogenase and aspartate aminotransferase is a second prominent metabolic feature of the exercise-trained cardiac proteome (Table 4). These enzymes form the malate-aspartate shuttle that transports electrons to within the

mitochondria and is also enhanced in exercise-trained skeletal muscle [57]. In addition, aspartate aminotransferase is identical to the plasma membrane fatty acid transporter (FABPpm) [58] so changes in this enzyme could also relate to long-chain fatty acid uptake.

Adaptations in cardiac energy metabolism, in particular mitochondrial fatty acid oxidation, may be a prominent aspect of exercise-mediated cardioprotection. For example, the changes in mitochondrial enzymes induced by endurance training are generally opposite to the changes in the cardiac proteome associated with ageing [59, 60] or heart failure induced by pressure overload [61, 62]. Striated muscle mitochondria can be distinguished as sub-sarcolemmal (SS) and intermyofibrillar (IMF) populations and more recent proteomic work [63] has investigated exercise-induced changes in cardiac SS and IMF mitochondria, separately. iTRAQ analysis of mitochondrial peptides resolved using orthogonal 2-D HPLC-MS/MS detected increases in the expression of mitochondrial enzymes associated with fatty acid oxidation and the TCA cycle specifically in IMF mitochondria. In addition, Kavazis et al [63] discovered endurance training is associated with decreased expression of monoamine oxidase in both SS and IMF populations. Monoamine oxidase generates hydrogen peroxide through oxidation of noradrenaline and other neurotransmitters, and is implicated in pressure-induced maladaptive hypertrophy [64]. The reduction in monoamine oxidase may represent further a potential mechanism of cardioprotection against hypertension relating to enhanced cardiac β-adrenergic receptor responsiveness associated with endurance training [65].

Endurance exercise is beneficial in restoring cardiac function after myocardial infarction induced by coronary artery ligation [66]. Bansal et al [67] reports proteomic analysis of cardiac infarct regions from rats that either remained sedentary or performed a programme of endurance training in the weeks after infarction. Endurance exercise significantly improved cardiac function and was associated with greater expression of glutathione peroxidase-1 and manganese superoxide dismutase, which is consistent with literature linking redox-based mechanisms and exercise-induced cardioprotection [68]. However, heart failure in humans is a progressive and complex condition involving both inherited and environmental factors that co-occur with other complicated risks including hypertension, obesity, and insulin resistance. Thus, the use of acute models of cardiac failure (e.g. myocardial infarction induced by coronary artery ligation) is limited and genetic models involving overexpression or deletion of individual genes do not encompass the complexity of chronic disease. Some years ago, Koch and Britton began artificial selection of rats for low or high aerobic exercise capacity [69]. This work has produced two populations of rats: high capacity runners (HCR) and low capacity runners (LCR) that have the potential to illuminate complex mechanisms underlying diseases associated with low cardiorespiratory fitness. Sedentary HCR and LCR exhibit substantial differences in aerobic capacity, disease susceptibility and mortality rate. For example, LCR exhibit features of metabolic syndrome [70] and are more susceptible to environmental challenges such as ischaemia/reperfusion [71], high-fat diet [72] or hypoxia [73]. In contrast, HCR closely resemble endurance-trained animals and are protected from environmental insults despite living a habitually sedentary lifestyle.

At the transcriptome level, LCR hearts exhibit greater expression of genes associated with glucose metabolism, whereas, genes involved in lipid oxidation are enriched in HCR [74]. This mimics the dichotomy observed between maladaptive and adaptive cardiac hypertrophy [75], but it is important to verify which of these features also occur at the protein level and to this end we recently conducted proteomic analysis of HCR-LCR hearts using DIGE. In many aspects the proteome and transcriptome findings were highly complementary but we found limited direct correspondence between the proteome and transcriptome data. This may be due to the greater resolution of microarray compared to DIGE or the fact that differences detected by DIGE may include post-translational modifications not evident at the transcriptome level. In agreement with the microarray findings each enzyme of the β-oxidation pathway was significantly more abundant in HCR hearts but contrary to the transcriptome dataset there was limited evidence to suggest up-regulation of glycolytic metabolism in LCR. Instead, changes in enzymes associated with ketone body and amino acid metabolism were enriched in LCR. Several features of the HCR cardiac proteome are identical to the effects of endurance training (Table 3) adding further evidence that modulation of cardiac energy metabolism is one of the key benefits of exercise. Interestingly, selection on high running capacity was not associated with elevated expression or phosphorylation of hsp20. Thus, despite similarities in cardiac energy metabolism, differences exist between the hsp response associated with acquired and innate increases in aerobic capacity, and phosphorylation of Hsp20 seems to be a specific training-induced adaptation.

Expert commentary

Exercise proteomics is an emerging discipline, first highlighted in a prospective of research in exercise sciences published in 2000 [76]. Since this time, a small number of original articles have been published reporting proteomic analysis of exercise (Table 1). These pioneering works provide important new information regarding exercise-induced adaptation. In the majority, proteomic analyses of skeletal (Tables 2 and 3) and cardiac (Table 4) muscle have detected changes in metabolic enzymes, myofibrillar components, chaperones and anti-oxidant enzymes. The conclusions from these discovery-based investigations align closely with existing knowledge from hypothesis-led research. Moreover, it is encouraging to note that proteomic analysis of exercise-induced muscle adaptation has uncovered new information whilst providing confirmation, at the protein level, for previously reported changes in gene transcription and simultaneously presenting well-established adaptations that allow the novel findings to be aligned with the wider literature.

Muscle biochemistry has a long history and it may be tempting to assume knowledge regarding abundant muscle proteins is complete. However, shifts in the 2-DE profile of multi-spot proteins are a conspicuous response to exercise and have enabled proteomic investigations to uncover novel information at the level of post-translational modification. The combination of 2-DE and MS-based protein identification, is a powerful investigative tool capable of detecting detailed information without knowledge *a priori* regarding the identity of the protein or the type of covalent modification. As yet, this approach has not been fully exploited and the identification of state-specific post-translational modifications represents a key area where 2-DE and functional proteomics can contribute knowledge.

However, to achieve this goal, sophisticated MS/MS analysis is necessary to map covalent modifications to specific residues, which is crucial to correctly interpret the biological significance of these discoveries.

Phosphorylation is a key regulatory mechanism in striated muscle and is a commonly studied post-translational modification (e.g. [77–82]). Nevertheless, MS/MS mapping of phosphopeptides is challenging because loss of the relatively labile phosphate during fragmentation using collision-induced dissociation (CID) makes it difficult to determine the site of the modification [83]. Electron-transfer dissociation (ETD) is a complementary fragmentation technique that is highly specific for the N-Cα bond and produces MS/MS spectra composed of c- and z-ions. Importantly, covalent modifications to side-chains, such as phosphorylation of serine, threonine and tyrosine residues, remain intact during ETD fragmentation of the peptide backbone and this greatly facilitates unambiguous identification of the specific site of modification. For example, ETD analysis was crucial in mapping phosphorylation of ventricular myosin regulatory light chain (MLRV) to serine 15 (Figure 1). Phosphorylation of MLRV is necessary for normal cardiac function and responsiveness to β1-AR stimulation [84]. Specifically, phosphorylation of MRLV determines the kinetics of force development and Ca^{++}-sensitivity of force production in cardiomyocytes [85]. Proteomic analysis of hearts from animals used in [35] revealed endurance training decreases MLRV phosphorylation, which may relate to the improved myocardial Ca^{++}-handling associated with endurance training [86].

To make full use of the information from functional proteomics studies, new practices and bioinformatic tools are required to integrate 2-DE multi-spot profiles and MS/MS-based identification of site-specific modifications with information regarding changes in biological function. Presently, the majority of 2-D gel investigations only report the identity of spots highlighted as significantly altered by differential image analysis software. In many cases, these individual spots belong to multi-spot series and, as we have discussed, the practice of reporting changes in the expression of individual spots as being synonymous with a change in the total abundance of that protein may be unjustified. Proteome mining and greater use of 2-D gel databases, such as World-2DPAGE; http://world-2dpage.expasy.org/repository/ will help to highlight which spots are commonly observed as multiple isoelectric species and this should guide the interpretation of new findings. Furthermore, integration of 2-D gel maps with available databases of MS-based protein identifications (e.g. PRIDE; http://www.ebi.ac.uk/pride/) and post-translational modifications (e.g. PhosphoSite; http://www.phosphosite.org/) will enable more complete interpretation that will lead to greater insight. Presently, bioinformatic tools specifically suited to functional proteomics are relatively underdeveloped and this has lead to the application of gene pathway analysis tools to proteomic data. However, unlike mRNA expression, 2-D gel data reporting changes across isoelectric subspecies of a gene product may not be dichotomous (i.e. do not depict either an increase or decrease in total protein abundance). Moreover, an increase in a particular gel spot may relate to relatively greater abundance of an inactive form of that protein, and represent a decrease in biological activity. Because of these potential false assumptions, interpretation of proteomics data using current pathway analysis suites is fundamentally flawed and further development of protein ontology and pathway analysis

tools (e.g. iProClass; http://pir.georgetown.edu/iproclass/) is required in order to fully exploit functional proteomics data.

Implicit in the term “proteomics” is the desire to analyse the entire protein complement of a cell or tissue. In the case of striated muscle, this objective is not readily achieved and current exercise-proteomics literature is based on separations of about 60 [38] to 1200 [56] protein spots. DIGE, which is considered the gold standard in 2-D gel proteomics and can resolve about 800 [43] to 3500 [87] spots and offers the opportunity to delve deeper in to the proteome. Nonetheless, because 2-DE separates many proteins as multi-spot series the number of non-redundant identifications may be little more than one-half of this number and, at best, the current literature considers only the top 5 % of the most abundant muscle protein species. Besides limited dynamic range, hydrophobic membrane proteins and proteins at the extremes of Mr and p*I* are poorly resolved using 2-DE. In an attempt to overcome these difficulties we [44, 88, 89] and others [90, 91] have employed size (M_r) fractionation by 1-DE and solution-based separation of tryptic peptides using reverse-phase HPLC. For example, by combining this approach with iTRAQ labelling we [44] were able to detect significant changes in the expression of mitochondrial enzymes in exercise-trained human muscle, which were not observed using 2-DE.

When coupled with high mass-accuracy MS, solution-based approaches can identify almost 5000 proteins in cardiac muscle [92] and about 1000 [90] to 2000 [91] skeletal muscle proteins and provide a tantalizing preview of in-depth muscle proteomics, including regulatory proteins (e.g protein kinase A, calcium calmodulin dependent protein kinase, 5‘AMP activated kinase) important in exercise-induced adaptation. However, while solution-based approaches excel in proteome mining, their reproducibility is relatively poor and, in the majority, peptides that can be detected across multiple samples (i.e. can be included in statistical analyses) represent proteotypic peptides of a protein, which are less likely to provide information regarding post-translational modification. Specifically, solution-based workflows often encounter problems of missing data because, despite analysing multiple replicates, many proteins are identified with low sequence coverage. Thus, when employed in differential expression profiling, less than one-third of the proteins identified can be compared across groups. For example, Hwang et al. [93], identified > 1200 proteins in muscle from lean, obese and type 2 diabetic patients but only 400 of these were present in at least half of the samples investigated and statistical analysis was conducted on only 92 proteins. Nonetheless, Hwang et al. [93] reports novel differences in intermediate filament and microtubule proteins providing new mechanistic insight that may not have been uncovered based on hypothesis-led reasoning. Thus, further development of these techniques is warranted.

In one respect, the limited resolution of striated muscle proteomics is an obstacle to understanding the intricacies of muscle adaptation. However, this feature, along with the reasonable accessibility of skeletal muscle, makes muscle proteomics attractive for biomarker discovery because proteins important in muscle function and whole-body metabolism (i.e. sarcomeric components and metabolic enzymes) are highly enriched. Individually, mechanistic markers such as ‘key’ rate-limiting enzymes may be of limited use, and imbalances within metabolic pathways caused by disease may alter which

enzymatic reactions predominate resulting peculiar changes in enzyme stoichiometry. Proteomics affords unbiased and comprehensive analysis extending beyond traditional myofibre phenotyping and proteomic data may highlight new features of interest. Considering the central role of oxygen metabolism in biological complexity [94] and the close correlation between aerobic capacity and health, molecular features associated with endurance exercise should be useful in the selection of biomarkers that distinguish between health and disease.

Tables 2-4 summarize proteins modulated in skeletal and cardiac muscle in response to exercise. Notably few proteins have been identified in more than one investigation, particularly in skeletal muscle, where differences in exercise intensity affect the nature of adaptation more so than in the heart. Nevertheless, some common features emerge and the unbiased nature by which these data were collected provides some validation of their biological importance. These markers may provide key mechanistic insight or be surrogates for mechanisms that are more difficult to detect. For example, changes in subunits of NADH dehydrogenase and F1-ATPase are regularly detected in exercised heart and skeletal muscle. The subunits of NADH dehydrogenase and F1-ATPase highlighted in proteomic studies each protrude in to the mitochondrial matrix and may simply be accessible biomarkers for general changes in electron transport chain proteins, most of which are integral to the inner mitochondrial membrane and may not be resolved using standard 2-DE. Alternatively, these findings may represent new mechanistic insight. For example, ATP synthase is specifically regulated in health vs disease [95] and exercise-induced changes in skeletal muscle ATP synthase-β (ATPB) [43, 44] oppose those reported [41] in proteomic analysis of muscle from diabetes patients. Changes in ATPB expression may correspond with enhanced Ca^{++}-calmodulin-dependent kinase II activity [96] and cardiac preconditioning is associated with specific phosphorylation of ATPB [97, 98], which could regulate the activity of the F1-ATPase. Therefore, post-translational modification of the β-subunit of the F1-ATP synthase may be an important mechanism underling muscle metabolic disease.

Five-year view

Proteomics is a technology-driven discipline and immediate advances in exercise proteomics will come from the further application of DIGE in combination with sub-cellular fractionation, or more sophisticated solution-based proteomics that incorporate enrichment of specific post-translational modifications. For example, phosphorylation events can directly modulate electron transport chain activity and LC-MS/MS analysis of TiO2-enriched fractions [82] can detect more than 200 phosphorylation sites in mitochondrial proteins. Future combination of such enrichment strategies with iTRAQ labelling could be used to address some of the current limitations and enable exercise-induced post-translational modifications to be quantified using solution-based techniques.

The comparison of results between laboratory rodents and humans is confounded by differences in myofibre phenotype and basal metabolic rate [18]. Therefore, while obtaining human skeletal muscle by percutaneous needle biopsy may be regarded as more difficult, the importance of understanding the health benefits of exercise and the complexity of exercise-induced adaptation warrant further carefully conducted studies directly in humans rather

than translation of data from laboratory rodents. In that regard, proteomic techniques offer the advantage of comprehensive analysis despite limited sample volume and are a highly efficient means of investigating the protective effects of exercise against human diseases. Likewise, proteomic analysis of human body fluids offers an exciting opportunity to develop accessible biomarkers directly in humans that may be relevant in clinical and sports training scenarios. For example, proteome profiling of blood [99, 100], saliva [101] or urine [102] may reveal novel diagnostic markers indicative of the health-related benefits of physical activity or the effects of doping or underperformance syndrome in elite athletic populations.

Muscle proteome changes in response to ageing and disease have recently been reviewed [16, 17] and herein we have considered responses to exercise. The health benefits of exercise are irrefutable and an important next step will be to compare the effects of different exercise regimens in healthy and diseased populations. To date, proteomic investigations have focused on striated muscle responses to endurance training, which is associated with health benefits underpinned by improvements in aerobic capacity. Training involving resistance exercise also benefits health [103] and helps to preserve muscle function during ageing [104]. As yet, the effects of resistance training have not been investigated using proteomics and this is a clear priority for future work. Proteomic data exist regarding postnatal muscle growth [105] and hypertrophy associated with pharmacological agents [106] or experimental interventions [107] but resistance exercise comprises complex mechanical, metabolic and hormonal signals and is unlikely to be recapitulated by such models. Moreover, many athletic disciplines and recreational activities involve a mixture of both endurance and resistance work that affect muscle differently [108]. The comprehensive nature of proteomic analysis may bring new insight regarding optimization of such concurrent training programs, which remains a current issue in sports physiology and health-based exercise prescription.

Key Issues

Regular exercise brings about adaptations in heart and skeletal muscles that enhance aerobic capacity and are associated with protection against cardio-metabolic diseases. Post-genomic research has illustrated the complexity underlying physiological adaptation; in particular, microarray analysis has uncovered comprehensive changes in muscle gene transcription induced by exercise.

Proteomic techniques, including 2-D electrophoresis and mass spectrometry, have enabled a discovery science approach to be applied at the protein level, and have uncovered novel exercise-induced post-translational modifications that may affect the functional properties of muscle in the absence of changes in gene transcription.

Few of the proteins modulated by exercise have been identified in more than one investigation, particularly in skeletal muscle, where differences in exercise intensity affect the nature of adaptation more so than in the heart. Nonetheless, some common features emerge and the unbiased nature by which these data were collected provides some validation of their biological importance. In skeletal muscle, the most commonly modulated proteins include sarcomeric proteins, glycolytic enzymes and chaperone proteins, whereas cardiac

adaptations include comprehensive changes in mitochondrial enzymes involved in fatty acid oxidation.

Proteomic analysis of cardiac muscle discovered that exercise enhances the expression and phosphorylation of heat shock protein 20, which has been associated with enhanced myocardial contractility and protection from cardiac insults such as catecholamine excess and ischemia/reperfusion injury.

Some of the changes highlighted in exercised muscle are opposite to the changes reported from proteomic analysis of muscle metabolic disease or cardiomyopathy, in particular, post-translational modification of F1-ATPase β-subunit is consistent across both cardiac and skeletal muscle.

Exercise proteomics is an emerging discipline and immediate advances in this field will come from further application of advanced analytical techniques including DIGE in combination with sub-cellular fractionation and further development of in-solution techniques such as 2-D LC-MS/MS. To date, proteomic investigations have focused on striated muscle responses to endurance training, which is associated with health benefits underpinned by improvements in aerobic capacity. Training involving resistance exercise also benefits health but, as yet, muscle adaptation in response to resistance training has not been investigated using proteomics and this is a clear priority for future work.

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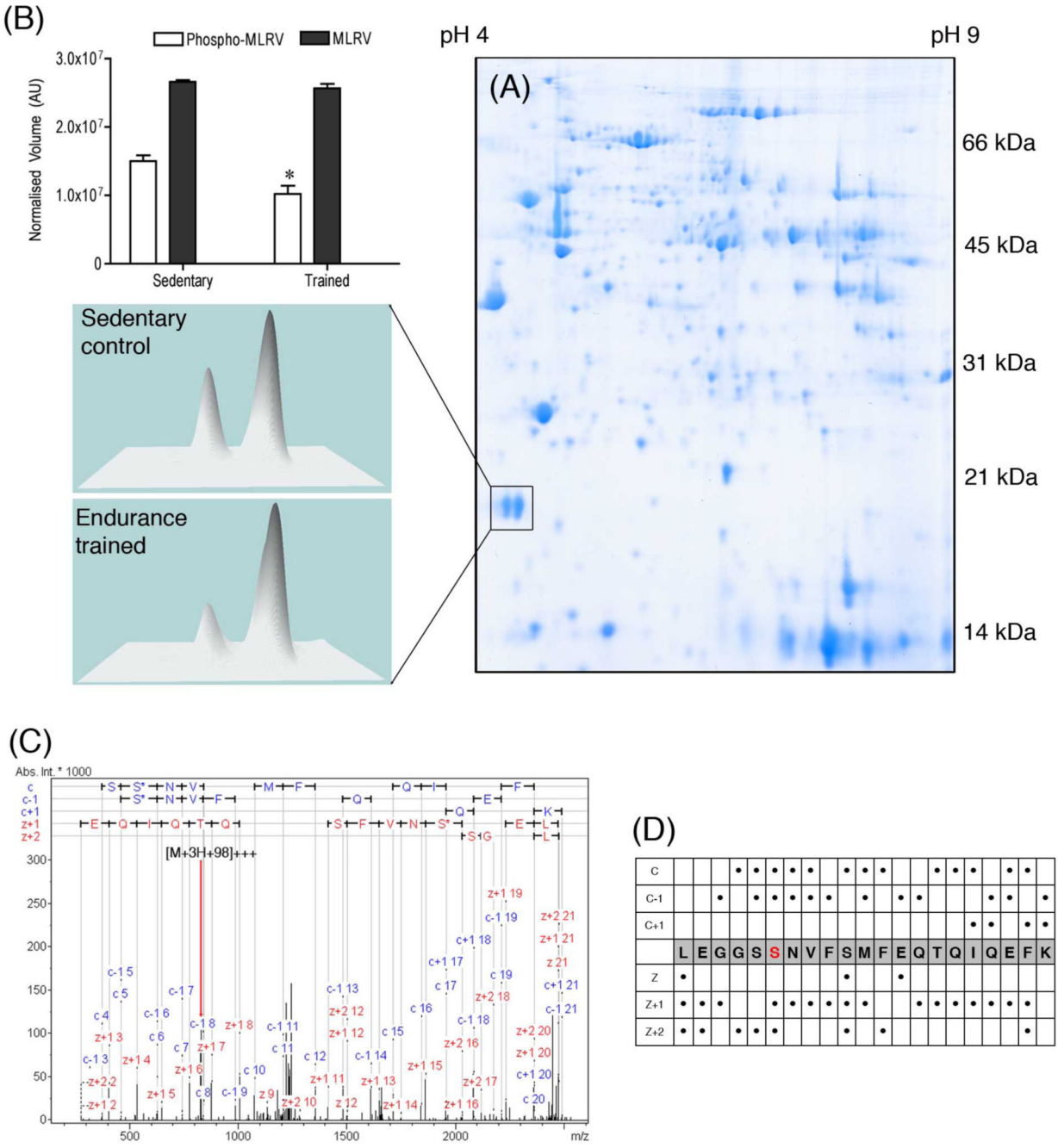

Figure 1. Myosin regulatory light chain phosphorylation

(A) Cardiac samples from sedentary and endurance-trained rats, used in [35], were separated using broad-range (pH 3-10) isoelectric focusing and denaturing polyacrylamide gel electrophoresis. (B) The normalised volume of spot, Mr ~20 kDa, pI ~4.7 was significantly less in endurance-trained hearts, while, no change occurred in the expression of the neighboring spot Mr ~ 20 kDa pI ~4.9. MS analysis of in-gel tryptic digests by nLC-ESI-MS/MS unambiguously identified each of these spots as the ventricular isoform of myosin light chain 2 (MLRV_RAT; P08733). Complementary CID and ETD analysis detected 90 %

of the protein sequence and Mascot MOWSE MudPIT scores were 6,933 and 8,187 for the acidic and basic species, respectively. (C) Phosphorylated peptides (residues 10-30: LEGGSSNVFSMFEQTQIQEFK) were only detected in the relatively acidic isoelectric species (pI ~4.7), which was less abundant in endurance-trained hearts. (D) Interpretation of ETD spectra (dots represent observed c, c -1, c +1, z, z +1 or z +2 fragment ions) enabled the specific site of phosphorylation to be mapped to serine 15.

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Table 1

Proteomic investigations of exercise.

Species	Exercise mode	Tissue	Exercise regimen: intensity, duration and frequency.	Ref.
Rat	Running	Heart	Progressive endurance training (6 wk): 1-min intervals at treadmill speeds of 24 m/min to 66 m/min, 5 days per week.	[50]
			Progressive endurance training (6 wk) at 70-75 % VO2max for 30 min, 4 days per week.	[36]
			Endurance training: 30 m/min at 0 % grade, 60 min per day for 5 days.	[63]
			Eight weeks endurance training progressing from 10 m/min, 5 % grade for 10 min to 16 m/min for 50 min performed 5 days per week.	[67]
		Plantaris muscle	Endurance training (5 wk) at 70-75 % VO2max for 30 min, 4 days per week.	[35]
		Gastrocnem ius muscle	Graded exercise test commencing at 12 m/min and encompassing incremental (1-3 min stages) increases in running speed until exhaustion.	[39]
		Hippocamp us	Voluntary wheel running for 5 consecutive nights.	[109]
			Treadmill running, two 20-min bouts per day at 20 m/min or Voluntary wheel running.	[110]
		Cerebellum	Progressive endurance training (3 wk) commencing at 6 m/min and increasing to 15 m/min for 20 min, 7 day per week.	[111]
	Swimming	Gastrocnem ius muscle	Three 30-min bouts interspersed by 10 min rest, total 150 min exercise.	[37]
			Single 3-min bout of weighted (10 % body weight) swimming.	[38]
		Epitrochlea ris muscle	Fourteen 20-sec bouts of weighted (14 % body weight) swimming interspersed by 10-sec rest periods.	[43]
		Heart	Progressive training (8 wk): two bouts per day of 15 min to 120 min duration, 6 days per week.	[56]
Human	Running	Vastus lateralis muscle	High-intensity interval training (6 wk): six 1-min bouts at 100 % VO2max interspersed by 4 min at 50 % VO2max, 3 days per week.	[44]
	Vibration	Vastus lateralis and soleus muscle	Two 6-min bouts per day (ranging from 19 Hz to 25 Hz) for 89 sessions.	[112]
	Mixed	Urine	In-competition doping control samples from athletes participating in endurance, strength or team sports.	[102]
	Tai Chi Chuan	Blood	Tai Chi Chuan (tai chi 37 forms) performed 3 times a week for 12 weeks.	[113]
	Eccentric resistance		Eccentric contractions of biceps femoris muscle: 3 sets of 10 repetitions using a load equivalent to 8-10 repetition maximum.	[100]
Horse	Running	Gluteal muscle	Three 5-min bouts of exercise progressing from 60 % to 100 % VO2max over a 15-wk period.	[114]
		Vastus lateralis muscle	Progressive training (18 wk) from 60 % to 80 % of estimated heart rate maximum, 4 days per week.	[115]
Rabbit	Running	Renal cortex	Endurance training (12 wk): treadmill speed of 18 m/min, 0 % grade for 60 min periods 5 day per week.	[116]
Pig	Running	Blood	Endurance training (14 wk): 65-75 % maximum hear rate for 30 min, 4 days per week.	[99]
Mouse	Running	Midbrain	Voluntary wheel running.	[117]

Table 2
Skeletal muscle proteins modulated by acute exercise.

Protein description	UniProt accession number	Publication reference
Glycolysis/ Glycogenolysis		
Triosephosphate isomerase *	P48500	[39]
Glyceraldehyde-3-phosphate dehydrogenase	P04797	[39]
Pyruvate dehydrogenase E1 component beta	P49432	[39]
Sarcomeric proteins		
Troponin T, slow skeletal muscle *	Q7TNB2	[38]
Alpha actin, skeletal muscle	P68136	[39]
High-energy phosphate metabolism		
Muscle creatine kinase *	P00564	[38]
Adenylate kinase 1	P39069	[38]
Chaperone and heat shock proteins		
Heat shock protein 20	P97541	[38]
Heat shock cognate 71 kDa protein	P63018	[39]
Lipid metabolism & β-oxidation		
Carnitine O-plamitoyltransferase II	P18886	[39]
Transcriptional regulation		
zinc finger protein 3	Q8BLB0	[37]

Description and UniProt Knowledgebase accession number of proteins identified as differentially expressed in skeletal muscle after an acute bout of exercise.

* Proteins that are also modulated by chronic exercise training (Table 3).

Table 3
Skeletal muscle proteins modulated by chronic exercise training.

Protein description	UniProt accession number	Publication reference
Sarcomeric and calcium handling proteins		
Troponin T, slow skeletal muscle *	Q7TNB2	[44]
Troponin T, fast skeletal muscle	P50753	[44]
Myosin light chain MLC1-f	P02600	[43]
Myosin light chain, phosphorylatable, fast skeletal muscle	P04466	[43]
Myosin regulatory light chain 2v	P08733	[35]
Parvalbumin	P02625	[43]
Glycolysis/ Glycogenolysis		
Alpha enolase	P04764	[35]
Beta enolase	P13929	[44]
Phosphoglucomutase 1	P38652	[35]
Triosephosphate isomerase *	P48500	[35]
Muscle glycogen phosphorylase	P09811	[43]
Lactate dehydrogenase A	P04642	[35]
Tricarboxylic acid cycle		
Acontiase 2	Q63270	[35]
Maltate dehydrogenase, mitochondrial	P04636	[43]
Succinate CoA	B2GV06	[35]
Succinate dehydrogenase [ubiquinone] flavoprotein subunit	P31040	[44]
Oxoglutarate (alpha-ketoglutarate) dehydrogenase	Q5XI78	[43]
Electron transport chain		
ATP synthase alpha subunit	P25705	[44]
ATP synthase beta subunit	P06576/ P10719	[43, 44]
NADH dehydrogenase (ubiquinone) Fe-S protein 1, 75 kDa	Q66HF1	[43]
NADH dehydrogenase (ubiquinone) Fe-S protein 2	Q641Y2	[43]
Chaperone and heat shock proteins		
Alpha B-crystallin	P23928	[35]
60 kDa heat shock protein	P10809	[44]
Heat shock protein beta 1, 27 kDa heat shock protein	P04792	[44]
Stress-70 protein, mitochondrial	P48721	[43]
Transport proteins		
Albumin	P02770/P02768	[35, 44]
Transferin	P12346	[35]
myoglobin	Q9QZ76	[35]
Lipid metabolism & β-oxidation		
Trifunctional enzyme subunit alpha	P40939	[44]
High-energy phosphate metabolism		
Muscle creatine kinase *	P06732	[44]

Description and UniProt Knowledgebase accession number of proteins identified as differentially expressed in skeletal muscle after a period of endurance training. Proteins are classified and ranked according to biological process.

*Proteins that are also modulated after an acute bout of exercise (Table 2).

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Table 4
Cardiac muscle proteins modulated by endurance training.

Protein description	UniProt accession number	Publication reference
Fatty acid transport and beta-oxidation		
Heart Fatty acid binding protein	P07483	[36]
Trifunctional enzyme subunit alpha *	P48500	[63]
Long-chain acyl-CoA dehydrogenase	P15650	[36, 63]
Short-chain Acyl-CoA dehydrogenase	P15651	[36, 56]
Enoyl-CoA hydratase	P14604	[56]
Hydroxyacyl-CoA dehydrogenase	QPWVK7	[63]
Delta(3,5)-Delta(2,4)-dienoyl-CoA isomerase	Q62651	[63]
Electron transport chain		
NADH dehydrogenase (ubiquinone) alpha subcomplex	Q561S0	[36, 56]
NADH dehydrogenase (ubiquinone) flavoprotein 1	Q5XIH3	[36]
ATP synthase D-chain	P31399	[50]
ATP synthase O-subunit	Q06647	[63]
ATP synthase subunit alpha *	P15999	[56]
Electron transfer flavoprotein subunit alpha	P13803	[36]
Electron transfer flavoprotein subunit beta	Q68FU3	[56]
Malate-aspartate shuttle and tricarboxylic acid cycle		
Malate dehydrogenase, cytoplasmic	O88989	[36, 56, 63]
Aspartate aminotransferase	P00507	[36, 63]
Citrate synthase	Q8VHF5	[63]
Aconitase *	Q63270	[36]
Isocitrate dehydrogenase [NAD] subunit alpha	Q99NA5	[56]
Dihydrolipoamide S-succinyltransferase	Q01205	[50]
Antioxidant and heat shock proteins		
Heat shock protein 20	P97541	[36, 50]
Similar to amine oxidase	P21396	[63]
Stress-70 Protein	P48721	[56]
Antioxidant protein 2	O35244	[56]
Glutathione peroxidase 1	P04041	[56]
Thioredoxin-dependent peroxidase reductase	Q9Z0V6	[63]
Sarcomeric proteins		
Alpha actin	P68035	[36]
Alpha cardiac myosin heavy chain	P02563	[36]
Myosin light polypeptide 3	P16409	[56]
Nebulette	D4A4G1	[36]
Glycolysis		
Triosephosphate isomerase *	P48500	[50, 56]
Similar to glyceraldehyde-3-phosphate dehydrogenase	Q498M9	[50]

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Protein description	UniProt accession number	Publication reference
High energy phosphate metabolism		
Creatine kinase Basic-type	P07335	[36]
Creatine kinase M-type *	P00564	[36]
Miscellaneous		
26S proteasome non-ATPase regulatory subunit 1	O88761	[36]
A kinase (PRKA) anchor protein 3	Q66HC6	[56]
Aldehyde reductase	P51635	[56]
Alpha-2-HS-glycoprotein precursor (Fetuin-A)	P24090	[36]
Four and a half LIM domains protein 2	O35115	[36]
Methylmalonate-semialdehyde dehydrogenase [acylating]	Q02253	[63]
Prohibitin	P67779	[56]
Similar to hydroxysteroid dehydrogenase like 2	Q4V8F9	[56]

Description and UniProt Knowledgebase accession number of proteins identified as differentially expressed in cardiac muscle after a period of endurance training. Proteins are classified and ranked according to biological process.

* Proteins that are also modulated in skeletal muscle after chronic exercise training (Table 3).