Molecular and cellular adaptation of muscle in response to physical training

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ABSTRACT

Molecular biology tools can be used to answer questions as to how adaptations occur in skeletal muscle with training that could provide new frameworks to improve physical performance. A number of mRNAs for transfer of metabolic substrates into muscle cells increase after a single bout of exercise demonstrating the responsiveness of some gene expression to exercise. In stretch-induced hypertrophy SRE1 of the skeletal α-actin promoter is required to transactivate the promoter. Less retardation of SRF in crude nuclear extracts from the stretched muscle implies a conformational change in SRF because of the stretch. Transgenic animals will provide a tool to test questions concerned with how exercise signals adaptive changes in gene expression. Molecular biological approaches will be able to evaluate the interaction between physical activity levels and the expression of genes that modulate the susceptibility to many chronic diseases. Benefits of exercise extend beyond fitness to better health. Molecular biology is an important tool which should lead to improved physical performance and health in both elite athletes and the general public.

Keywords Exercise, plasticity, mechanism, chronic disease

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Exercise physiology is a discipline that is driven by questions as to how humans improve performance. If these questions are condensed to the fewest number of words, they might be: How does training make you (a) stronger? (b) move faster and longer without fatigue? and (c) improve motor skills? Molecular biology tools will help to answer these questions. If the reasons driving the asking of these questions were to be listed, they might be: (a) to improve the health and quality of life of frail or weak persons, or people with disabilities, as well as the normal person; (b) to improve performance of elite and recreational athletes; (c) for knowledge's sake; and (d) to increase strength in females in order to provide more equality with males in vocations requiring strength.

mRNA CHANGES WITH EXERCISE

Changes in some mRNAs by exercise are transient

The major use, till date, of molecular biology techniques in exercise physiology has been the quantification of mRNAs in skeletal muscles of animal models of exercise. A detailed list of these mRNA changes has been recently published (Booth & Baldwin 1996). As a general rule, changes in the concentrations of mRNAs [mRNAs] and the proteins [proteins] encoded by these mRNAs occur in the same direction. However, recent observations indicate that more than a single time point of [mRNA] is required to confirm this generality for each mRNA and to make valid interpretations of regulatory sites in gene expression. As a general example, when mRNA changes are very transient (0–4 h post-exercise), and changes in proteins are more long lasting (36 h post-exercise), a single time point at 18 h post-exercise would show no increase in mRNA. An erroneous interpretation could be made that all of the increased protein is due to a post-translational event, i.e., there was no pretranslational change, there being no change in mRNA at 18 h post-exercise. The term pretranslational is defined as events that alter the abundance of mRNA (which is the algebraic sum of transcription, mRNA processing, and mRNA stability); the term translational is defined as changes in the synthesis of protein per unit of mRNA (mRNA activ-
ity); and post-translational is defined as the modification of protein, such as phosphorylation, proteolytic clipping, or degradation.

Hexokinase II transcription rate has been found to increase immediately post-exercise, if the exercise duration is long enough. Immediately after 90 min of treadmill running by rats, the hexokinase II transcription rate increased by 300% (O'Doherty et al. 1996), but its [mRNA] increased by only 50% (O'Doherty et al. 1993). At 8 h post-exercise, hexokinase II [mRNA] increased by 250% while hexokinase activity increased by only 34% (O'Doherty et al. 1993). By 24 h post-exercise, hexokinase II mRNA was no different than normal and hexokinase activity increased by 42% (O'Doherty et al. 1993). Similar observations have been made by Neufert and Dohm (1993) for post-exercise glucose transporter 4 (GLUT4) expression. GLUT4 transcription increased by 80% three hours after a 40-min run on a motor-driven treadmill by rats that had been trained for 1 week, but remained unchanged from the controls both 30 min and 24 h after the exercise (Neufert & Dohm 1993). This again demonstrates the transient nature of transcription rate. However, [GLUT4 mRNA] did not increase 30 min, 3 h, or 24 h post-exercise (Neufert & Dohm 1993). Nevertheless, [GLUT4 protein] increased by 70% in the trained rats. Possibly, [GLUT4 mRNA] increased transiently between 3 and 24 h post-exercise. [Lipoprotein lipase (LPL) mRNA] in rats was found to be increased by 100% and 50% in the white and red vastus muscles immediately after swimming, but no difference was observed 24 h post-exercise (Ladu et al. 1991). Thus, changes in the mRNAs for hexokinase II, GLUT4, and LPL occur within hours. This information on the transient nature of metabolite expression may be used to plan human muscle biopsy studies and ultimately possibly to improve training protocols in the future.

Effect of training on mRNAs for mitochondrial proteins

[Mitochondrial protein] increases in endurance training (Booth & Baldwin 1996). Over 100 mitochondrial proteins are encoded in the nuclear genome, but about 20 of these are encoded in the mitochondrial genome. Williams (1986) showed that an increased amplification of mitochondrial DNA relative to nuclear DNA occurred in the skeletal muscle of rabbits during chronic stimulation when mitochondrial density increased. Recent data from biopsies of human skeletal muscle extend these findings of Williams to humans. Nuclear-encoded mRNAs for mitochondrial proteins (cytochrome oxidase subunit 1, fumarase, and succinic dehydrogenase) were at least 50% higher when expressed per unit of nuclear DNA in muscle biopsies from humans who were endurance-trained as compared to control subjects (Puntschart et al. 1995). On the other hand, no change in mitochondrial-encoded mRNAs for mitochondrial proteins (cytochrome oxidase subunit 1 and NADH dehydrogenase subunit 6) was noted when they were expressed per unit of mitochondrial DNA (Puntschart et al. 1995). Also, no change in mitochondrial genome-encoded 16S rRNA occurred when 16S rRNA was normalized for mitochondrial DNA in the same subjects (Puntschart et al. 1995). Mitochondrial-encoded messages translate proteins on mitochondrial-encoded ribosomes, which include 16S rRNA. These data show an increase in mitochondrial DNA copy number in endurance-trained human skeletal muscle. It is encouraging to find that an animal study (Williams 1986) done a decade earlier projected a confirmatory human study (Puntschart et al. 1995).

MUSCLE HYPERTROPHY

Background

Myofibre cross-sectional area increases during overload-induced hypertrophy of skeletal muscle. Radial enlargement of muscle fibres after resistance training or external loading confers to the muscle a greater potential for maximal force production. During load-induced myofibre hypertrophy there is an increased accumulation of contractile and noncontractile muscle proteins, and the synthesis and degradation rates of these proteins are critical for determining their net quantity (see Goldspink 1991 for a review). Protein synthesis and degradation rates have been shown to be altered in hypertrophying skeletal muscle (Goldberg 1969, Laurent et al. 1978). Molecular biology tools available today are mainly applied to examining the regulation of skeletal muscle protein synthesis; protein degradation and the specificity of this process remain not well understood (see Goldspink 1991, Jentsch & Schlenke 1995 for reviews).

Potential levels of regulation by which a muscle fibre can regulate its protein abundance include pretraslational, translational, and post-translational mechanisms. mRNA synthesis is regulated by the transcription rate of the gene encoding the mRNA. A gene's transcription rate is affected by cis-acting DNA sequences (the regulatory site is on the same gene) in nontranslated regions of the gene which can bind nuclear proteins with a high degree of specificity.

Overload-induced hypertrophy is a complex event but the research in this area supports a two-stage model of skeletal muscle adaptation to overload. Hypertrophying skeletal muscle appears to be sensitive to both loading conditions and the muscle fibre's microenvironment, both of which govern the degree of enlargement that the muscle fibre has achieved. Alterations in
cell shape are mechanical signals which have been shown to alter gene expression in the nucleus, and integrin receptors may play a prominent role in this pathway (see Schwartz & Ingber 1994 for a review). Integrins are proteins which connect the extracellular matrix to the cytoskeleton by spanning the sarcolemma.

**Stage 1: Regulation at the onset of hypertrophy**

Skeletal muscle protein synthesis increases during overload-induced skeletal muscle hypertrophy in both humans and animals (Goldspink 1977, Laurent et al. 1978, Wong & Booth 1990, MacDougall et al. 1995). Wong & Booth (1990) found that the major mediator of increased myofibril protein synthesis in the rat gastrocnemius muscle after acute isotonic resistance exercise was not RNA abundance, but most likely increased RNA activity (g protein/µg RNA). The implication being that translational or post-translational regulation is very important for increased protein synthesis after resistance exercise.

Chronic stretch overload induces a rapid and large increase in the mass of the chicken anterior latissimus dorsi muscle, and is accompanied by an increase in RNA activity at the onset of overload (Laurent et al. 1978). These data imply that increases in the translational capacity and/or translational efficiency are responsible for increased protein synthesis at the onset of hypertrophy due to chronic stretch. Increased RNA abundance does not appear to be a significant factor for increasing protein synthesis until later in the time course of stretch-induced hypertrophy.

The initial increase in muscle protein synthesis rates after overload appear to be regulated at the translational or post-translational level. Currently, understanding of translational regulation in overloaded skeletal muscle is not well understood. However, the understanding of translational regulation in striated muscle after physiological perturbations is growing rapidly. Translational efficiency is thought to be regulated by translational initiation factors whose activity can be modified by their phosphorylation state (Frederickson & Sonenberg 1993). Translational initiation factors regulate at the level of peptide chain initiation. The phosphorylation of eIF-4E (eukaryotic initiation factor-4E, which stimulates translation of mRNA) has been linked to increased protein synthesis in the pressure overloaded canine left ventricle (Wada et al. 1996). Decreased skeletal muscle protein synthesis rates during disuse atrophy are related to a decrease in the elongation rate of nascent polypeptide chains along the length of an mRNA (Ku & Thomason 1994). This decreased elongation rate in the atrophying soleus may be related to a decreased association of the 70-kD heat-shock cognate/heat shocking protein (HSP-70) with the polyosome (Ku et al. 1995).

![Figure 1](image)

**Figure 1** Schematic illustration of the decreased rate of elongation in an unloaded soleus muscle of the rat (Ku & Thomason 1994). In the left part of the figure, the ribosome at the right end of the mRNA from the control muscle has just begun translation and no translated protein is shown, the centre ribosome has produced half a nascent protein, and the ribosome at the left end of the mRNA has nearly completed translation as evidenced by the longer protein. After unloading (right part of the figure), twice as many ribosomes are translating from the mRNA. Ku and Thomason describe this change as similar to a ‘traffic jam’ of ribosomes on the mRNA. (Fig. 1). HSP-70 facilitates translation by stabilizing the translation complex with nascent polypeptide chains. Understanding the signalling involved in increasing translation at the onset of hypertrophy will provide important insight into the overall regulation of skeletal muscle hypertrophy.

**Stage 2: Regulation later in the time course of hypertrophy**

Myofibrillar protein mRNA levels increase later in the time course of overload-induced enlargement in most hypertrophy models. Skeletal α-actin mRNA has been shown to increase between the third and sixth days of chronic stretch overload (Carson et al. 1996). Increased mRNA levels in the hypertrophying muscle can reflect alterations in transcriptional efficiency, transcriptional capacity, and/or mRNA stability. During skeletal muscle hypertrophy several mechanisms could serve, either in concert or in stages, to increase the mRNA template available for translation and protein synthesis. Increased transcriptional efficiency would indicate an increased transcription rate of a given gene per myonuclei. Plasmid DNA injected into chicken anterior latissimus dorsi muscle has demonstrated that skeletal α-actin-directed reporter gene expression is increased at 6 days of stretch overload (Carson et al. 1996). However, the muscle also maintains other mechanisms for increasing mRNA abundance during rapid muscle growth.

The transcriptional capacity of the hypertrophying muscle can also be increased by the addition of satellite cell derived nuclei that fuse with the enlarging fibre. An enlarged fibre could reach a critical size threshold that induces intracellular signalling, and initiates regulation that increases the fibre's transcriptional capacity. Skeletal muscle myonuclei are thought to have a given cytosolic domain, so that the fibre maintains a certain nuclei:cytoplasm ratio (Check et al. 1971). Satellite cells are thought to be the source for additional myonuclei in growing and regenerating fibres (see Schultz & Mc-
Cormick (1994) for a review). Satellite cell nuclei may be recruited to enlarging fibres because the existing myonuclei's transcriptional machinery can only sustain a finite volume of muscle fibre (see Cheek et al. 1971 for a review). Hence, the nuclear domain hypothesis predicts that in order to sustain muscle fibre growth, the enlarging fibre must recruit additional nuclei. In support of this hypothesis, satellite cell activity in the compensatory overloaded mouse EDL muscle has been shown essential for hypertrophy (Rosenblatt & Parry 1992).

In summary, overloaded skeletal muscle undergoes a sequence of molecular events which drive increased protein synthesis and enlargement of the muscle. Translational and post-translational regulation appear critical at the onset of chronic loading and after acute resistance exercise. However, later in the time course of chronic loading it appears that mRNA synthesis is up-regulated. The increased mRNA template can be achieved by increasing the transcription rate of the given gene and/or the addition of satellite cell derived nuclei. Answering how overloaded skeletal muscle senses and signals these changes in the molecular regulation of myofibre growth should provide greater insights into the phenomena of overload-induced skeletal muscle hypertrophy.

**TRANSGENIC ANIMALS IN EXERCISE RESEARCH**

Considerable effort has been made in the study of gene regulation during the past two decades. Much of this progress is due to sophisticated molecular biology tools like transgenic technology. Transgenic technology was first successfully applied to mammals in the early 1980s, allowing permanent modification of an animal's genome, and molecular biologists were for the first time able to gain in depth knowledge about the biological function for the translation products of a series of genes (Palmiter et al. 1982, Gordon et al. 1993). The standard transgenic approach to study gene function includes animals engineered to either lack or over-express a functional gene. The outcome of such genetic manipulations on the development and differentiation of the transgenic animals is then monitored, and in many cases has given strong indications for the function of the target gene. However the construction of transgenic animals is not trivial (Tsika 1994) and complications often occur by the effects of the engineered genes on normal development (Miner et al. 1992, Olson et al. 1996). To date, subtle and precise alterations of the genome can be achieved with embryonic stem (ES) cell technology. However, to date, germline transmission using this ES technology has only been achieved with the mouse species, but considerable advances in this field for the production of larger transgenic animals - not, at least, to study physiological mechanisms - are expected (Mullins & Mullins 1996).

The potential for using transgenic technology to study gene regulation following a physiological stimulus like exercise has been recognised (Tsika 1994). Yet, the design of transgenic approaches to study the function of a specific gene in exercise physiology is challenging. Designs should be based on biochemical evidence for a role of the target gene (protein) in the physiological process to be studied (Kaplan et al. 1994). To fulfil the merit of this aim, the intention of a transgenic approach has to go beyond the gene level and must include potential molecular interactions (causalties) governing cellular physiology.

The power of a transgenic approach can be demonstrated by the molecular cause for a benign form of human erythrocytosis. This naturally occurring mutation in a Finnish family introduces an early stop codon in the erythropoietin receptor (EPO-R) (La Chapelle et al. 1993). Mutations of the EPO-R gene are autosomal dominant and lead to a C-terminal truncated EPO-R, lacking a domain that exerts negative control on erythropoiesis in erythroid progenitor cells. Cells expressing the mutant receptor are much more sensitive to erythropoietin and have higher than normal haemoglobin levels. The clinical condition is so mild that many of the affected individuals are themselves not aware of any abnormality, nor do they have any sense of illness. Indeed the life span is unaffected. The family's most famous member, Eero Maentyranta, whose blood carries 25–50% more haemoglobin than that of the average male, won three gold medals in cross-country skiing at the 1964 Winter Olympics in Innsbruck, Austria (Roush 1995)! As Lodish, researcher at Whitehead Institute for Biomedical Research, Cambridge, Massachusetts, says, "This is the first fully characterized mutation that enhances athletic performance". The molecular players involved in the negative control of EPO-R signalling have been identified, and involve Janus Kinase2 activating kinase (Jak2 kinase) and an 5r homology protein tyrosin phosphatase1 (SHP1) (Klingmüller et al. 1995), and researchers of the Whitehead group are now trying to produce a transgenic mouse that expresses the same nucleotide error as the hardy Finnish family.

Still, through 1996, there have been few physiological studies taking advantage of the advent of transgenic animals for the identification of gene regulation and function in developed animals. Ikemoto et al. (1995) successfully engineered a mouse harbouring a GLUT4 minigene the expression of which is restricted to skeletal muscle and adipose tissue. Expression of the GLUT4 minigene even at low levels caused large increases in the rate of glucose disposal in transgenic.
mice. Exercise training (swimming) markedly activated expression of the GLUT4 minigene.

Using reporter genes to study the influence of exercise on gene regulation of contractile proteins, signalling molecules or ion channels, and protein processing and sorting, is a promising area of research. Using the reporter CAT (chloramphenicol acetyltransferase) linked to the genes coding for muscle creatine kinase (MCK), β-myosin heavy chain (β-MHC) and slow myosin light chains (SMLC1 and SMLC2), Tsika and coworkers (Tsika et al. 1995, 1996, Wiedenman et al. 1996) have delineated the respective overload responsive regions within each promoter. However, this approach has yet to identify a discrete regulatory element within the identified regions.

When gene regulation is studied in mature, relatively slow growing animals, genetically engineered modifications of the target gene should not interfere with normal growth and differentiation since the aim is to study normally developed muscle. Hence techniques that allow blocking/modulating gene expression just prior to the exercise stimuli, would be ideal. Indeed within the past 5 years transgenic technology has advanced, and allows today the conditional targeting of transgene expression or gene-ablation to specific tissues at will (conditional control) (Barinaga 1994, Gu et al. 1994, Kitamura 1996).

The construction of transgenic animals with the help of advanced gene technology is demanding, and success of a strategy is often a matter of luck. A pragmatic approach to build information for the design of transgenes follows (Fig. 2). Somatic gene transfer techniques like plasmid injections or the use of viral vehicles can be used (Carson et al. 1995, Hofman et al. 1996, Walke et al. 1996). Plasmid methodology, as pioneered by Jon Wolff (Wolff et al. 1990), has identified serum response element 1 (SRE1) as a hypertrophy-response element in stretched muscle (Carson et al. 1996). Corin et al. (1995) conclude that somatic gene transfer can be used to rapidly define elements that direct myofiber-type-specific gene expression prior to the generation of transgenic mice.

With today’s advanced technology, experiments can be proposed that could turn off the tissue-specific function of genes. The initial experiments are promising and show that elegantly engineered transgenic animals are possible. These transgene animals would reduce the risk of interference with normal develop-
ment. These techniques could target potential members of exercise-responsive signal transduction pathways. A potential long-term goal of these experiments would be to describe signal elements of exercise-induced cascades. These experiments could include the use of transgenic animals containing muscle-specific and inducible antisense constructs that block kinase(s) modifying regulatory proteins in response to exercise (Moxham & Malhan 1996). A precautionary note would be in order. Exercise adaptations in mouse skeletal muscle need to be carefully characterized. It may not be valid to assume that adaptations in mouse occur to the same degree or follow the same time course as rat or human muscle adaptation to exercise. Such differences could affect interpretations in transgenic mouse experiments.

USAGE OF MOLECULAR BIOLOGY TOOLS IN CLINICAL EXERCISE PHYSIOLOGY

Incidence of chronic disease in the US and the potential role of physical activity in prevention

In the United States of America, the percentage of the population with chronic diseases (a medical condition lasting for a period of years) has been increasing and current estimates report that 45% of Americans have one or more chronic conditions and that these conditions account (in 1987) for 75% of US health care expenditures. The total costs projected in 1990 for people with chronic conditions amounted to $659bn. The US Government Centers for Disease Control and the American College of Sports Medicine (Pate et al. 1995), the Surgeon General of the United States (USDHHS 1996) and ourselves (Booth & Tseng 1995) have published elsewhere that appropriately performed physical exercise reduces the risk of breast and female reproductive cancers, claudication, coronary artery disease, depression, diseases arising from chronic bedrest, hypertension, non-insulin-dependent diabetes mellitus, obesity, post-menopausal osteoporosis, and sleep apnea associated with obesity, stroke, back pain, colon cancer, congestive heart failure, and loss of independent living. Mechanistic determination of how increased physical activity decreases the risk of chronic diseases will clearly fortify the rationales for health care professionals to prescribe exercise as a necessary part of their patients’ lives.

Potential role of exercise on disease associated genes

It has been long known that there is a genetic basis for disease. However, the number of human diseases that are purely heritable from a single gene are very uncommon relative to the number of multifactorial human diseases, e.g., diabetes, coronary artery disease and cancer. The recent increase in chronic diseases is not due to dramatic mutations in human DNA, but is due to dramatic changes in lifestyle (activity and diet). Multifactorial diseases, including all chronic diseases, are thought to be caused by environmental and temporal factors impinging on genetic factors. With the colossal effort of striving to define the entire human genome, we will soon be able to identify all genes and variant sequence(s) of specific genes that may correlate with susceptibility, severity and lethality of many human diseases. We expect virtually any multifactorial disease to involve multiple genes: some good and some bad. All humans have essentially the same genetic template, yet DNA sequence variations within individual genes (mutations) can also modulate predisposition to certain diseases. We define the term ‘disease-associated gene’ as a gene that increases or decreases the susceptibility or risk of developing a disease. Such a disease-associated gene cannot be attributed as the sole cause of disease but its expression clearly lowers or raises the likelihood threshold for a person to develop a disease. We predict that exercise can play a role as important as pharmaceutical medicines in the prevention and therapeutic management of chronic disease by activating or suppressing critical disease-associated genes.

Clinical utility of exercise with molecular biology

A recent molecular study has elucidated a novel mechanism by which a tobacco smoke byproduct can alter a critical cell cycle gene called p53 in lung cells and this alteration may cause or at least facilitate the development of some lung cancers (Denissenko et al. 1996). This most basic science enquiry has added strong evidence supporting the causal relationship between tobacco smoke and the development of some lung cancers. Other genetic and nongenetic factors are also certainly involved. Extrapolating from this specific example, the diagnostic, prognostic and therapeutic avenues afforded by basic research hold great promise for future clinical horizons. In a similar way, the causal relationship of certain lifestyles influencing the progression of various chronic diseases has been implied by seminal epidemiological human data, but it lacks the additional credibility that defined molecular mechanisms can provide in understanding pathophysiology, and ultimately, in orienting clinical endeavours such as prevention and therapy.

To address causal relationships of lifestyle with chronic disease, we postulate that toxins (including tobacco smoke), diet and activity levels interplay profoundly with the genetic backgrounds of each individual to determine the type, progression and severity of the specific disease(s). This paradigm provides a fertile
opportunity for exercise researchers to apply their skills to study the interaction of physical activity and healthy diets as environmental influences on the expression of specific disease-associated genes. It is imperative that we initially identify these disease-associated genes and then characterize how they are regulated by other genes and other nongenetic influences. Animal models of disease-associated genes should be developed and exercise studies performed on these animals might determine the mechanisms of how physical activity suppresses the expression of some ‘bad’ genes and activates other ‘good’ genes for modulating disease progression. This will be a significant new area of exercise research that would integrate molecular biology and clinical exercise physiology. We firmly believe that delineation of how lifestyle (activity and diet) influences the expression of both disease-susceptible and disease-resistant genes with molecular biological tools will provide a more scientific basis for the prescription of exercise and diet to decrease the risk of chronic disease. If more people increased their physical activity to appropriate levels, more chronic disease could be prevented, quality of life for individuals would improve, health care costs would be decreased, worker productivity would be increased, and fewer people would suffer from loss of independent living.

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