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Integrative Biology of Exercise

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Exercise represents a major challenge to whole-body homeostasis provoking widespread perturbations in numerous cells, tissues, and organs that are caused by or are a response to the increased metabolic activity of contracting skeletal muscles. To meet this challenge, multiple integrated and often redundant responses operate to blunt the homeostatic threats generated by exercise-induced increases in muscle energy and oxygen demand. The application of molecular techniques to exercise biology has provided greater understanding of the multiplicity and complexity of cellular networks involved in exercise responses, and recent discoveries offer perspectives on the mechanisms by which muscle "communicates" with other organs and mediates the beneficial effects of exercise on health and performance.

Introduction

Superior locomotive ability was once essential for human survival and a fundamental reason that *Homo sapiens* evolved and prospered. Physical activity was obligatory for evading predators and food procurement. Evolutionary theory describes the mechanism of natural selection as "survival of the fittest," the underlying supposition being that the "fit," as opposed to the "unfit," had a greater likelihood of survival. Modern day humans run faster, jump higher, and are stronger than at any time in history. Yet exercise, particularly when undertaken to an individual's maximum, is a complex process involving the synchronized and integrated activation of multiple tissues and organs at the cellular and systemic level. Though the reductionist approach of dissecting biological systems into their constituent parts has been valuable in explaining the basis of many biochemical processes, for exercise biologists, this approach has severe limitations: the integrative biology of exercise is extremely complex and can be neither explained nor predicted by studying the individual components of various entities.

Exercise represents a major challenge to whole-body homeostasis, and in an attempt to meet this challenge, myriad acute and adaptive responses take place at the cellular and systemic levels that function to minimize these widespread disruptions. Previous reviews have considered the metabolic responses to exercise and the cellular mechanisms that underpin skeletal muscle adaptation to exercise training (Bassel-Duby and Olson, 2006; Coffey and Hawley 2007; Egan and Zierath, 2013; Hoppeler et al., 2011). Here, we highlight that voluntary, dynamic, whole-body exercise provokes widespread changes in numerous cells, tissues, and organs that are caused by or are a response to the increased metabolic activity of contracting skeletal muscle. To meet this challenge, multiple integrated and redundant responses operate to blunt the homeostatic threats generated by the increased energy and O_2 demand. In this "muscle-centric" view of exercise, the systemic (cardiovascular, respiratory, neural, and hormonal) responses are viewed as "service functions," supplying the contracting muscles with fuel and O_2 to sustain a given level of activity. The fundamental premise is that multiscale and redundant responses simultaneously operate to blunt the many challenges to whole-body homeostasis caused by the demands of the contracting muscles. The application of molecular biology techniques to exercise biology has provided a better understanding of the multiplicity and complexity of cellular pathways involved in these exercise responses. Recent discoveries offer perspectives on the role played by skeletal muscle in numerous homeostatic processes and on the mechanisms by which muscle "communicates" with other organs such as adipose tissue, liver, pancreas, bone, and brain.

Why Study Exercise?

There are several broad reasons to study exercise. Hypotheses generated over the last two decades from comparative physiologists (Hochachka et al., 1999) and anthropologists (Bramble and Lieberman, 2004) suggest that the combined traits of superior endurance capacity and an impressive ability to thermoregulate permitted ancestral humans from the high plains of East Africa to succeed as game hunters and thereby obtain high-protein sources of food that were essential for the emergence of larger brains and complex cooperative behavior. Human skeletal muscles, limbs, and the supporting ventilatory, cardiovascular, and metabolic systems were well suited for upright locomotion,

with economy of movement for bipedal walking and running far exceeding that of other primates (Bramble and Lieberman, 2004; Brooks, 2012). At this time, lifestyle and energy availability were inextricably linked to the periodic cycles of feasts and famines, with certain genes evolving to regulate efficient storage and utilization of endogenous fuel stores, the so-called "thrifty genes" (Neel, 1962). Expanding on Neel's original concept, survival during feast-famine cycles throughout the hunter-gatherer period was accompanied by the selection of genes and traits to support a "physical activity cycle" (Booth et al., 2002; Chakravarthy and Booth, 2004), and under these constraints most of the present human genome evolved. During modern times, those alleles and traits that evolved for energy storage and locomotion are now exposed to an inactive lifestyle and access to energy-dense foods over an extended lifespan, thereby increasing the risk of chronic disease. Therefore, the first reason to study exercise is to provide insight into the pathogenic processes underpinning the numerous contemporary physical inactivity-mediated disorders.

The recent emergence of noncommunicable diseases as major killers in industrialized nations (Bauer et al., 2014) and the role of physical activity in preventing and/or treating these conditions is a second reason to study exercise. A sedentary life is now so prevalent that it has become common to refer to exercise as having "healthy benefits" even though the exercise-trained state is the biologically normal condition. It is a lack of exercise that is abnormal and carries health risks (Booth and Lees, 2006). Physical inactivity increases the incidence of at least 17 unhealthy conditions and related chronic diseases (Booth et al., 2000), whereas a low exercise capacity is an independent predictor of all-cause mortality (Blair et al., 1996; Myers et al., 2002) and morbidity (Willis et al., 2012). Yet exercise in both biological research and as primary preventative therapy continues to be undervalued and underutilized by the scientific and medical communities. Consequently, a third reason to study exercise is to determine the precise mechanisms by which it promotes whole-body health and to establish molecular links between specific exercise interventions and disease prevention. Although the last decade has seen major advances in unraveling the mechanism(s) by which cellular, molecular, and biochemical pathways are affected by exercise, the understanding of how these effects are linked to health benefits is still lacking. In this context, epidemiological evidence suggests that only half of the protective effects of exercise can be explained on the basis of traditional risk factors like reductions in blood pressure (BP) and blood lipids (Joyner and Green, 2009).

A fourth reason to study exercise is to understand the capacity of various mammalian species to function in extreme environments and to test hypotheses about physiological regulation under such conditions. In this context, humans are competent athletes, but our capacity for locomotion is paltry compared with that of other species that are more powerful and faster and possess greater endurance. With respect to speed, the cheetah (*Acinonyx jubatus*) reigns supreme among terrestrial mammals, achieving maximum velocities of 113 km/hr (Sharp, 1997), making the world's fastest human (with a top speed of 48 km/hr) seem rather pedestrian. The pronghorn antelope (*Antilocapra Americana*) can sustain speeds of >80 km/hr for 4–5 km, and the Greyhound and sled dog are similarly capable of extraordinary bursts of speed (Poole and Erickson, 2011). Notwithstanding such comparisons, research into the "limits" of athletic capacity provides insight into the roles of various organ systems involved in maximizing human performance (Joyner and Coyle, 2008). Such enquiry is not new. In 1925, Nobel Laureate A.V. Hill published a paper on the physiological basis of athletic records (Hill, 1925) and was the first to describe the concept of an individual's maximum oxygen uptake (VO_{2max}) as an index of the highest energy demand that can be met aerobically while exercising. Hill proposed that an individual's VO_{2max} was the single best measure of cardio-respiratory performance and could be used for quantifying the adaptation of many organ systems to physical activity or inactivity (Bassett, 2002). Perhaps fittingly, the test for VO_{2max} for the assessment of athletic potential originally proposed by Hill is now recognized as a better predictor of mortality than any other established risk factor or biomarker for cardiovascular disease (Myers et al., 2002). Clearly the biology underlying maximal exercise performance confers advantages beyond the athletic arena!

Voluntary Exercise: More Than Muscle Contraction

What We Mean When We Talk about Exercise

Exercise is the voluntary activation of skeletal muscle for recreational, sporting, or occupational activities. The distinction between voluntary, whole-body in vivo responses to exercise versus those evoked by other experimental models is important. Ex vivo electrical stimulation of an isolated skeletal muscle, for instance, evokes an action potential and "contraction" and triggers intracellular pathways with putative roles in training adaptation (Fitts and Holloszy, 1978). However, whole-body, voluntary exercise induces a range of additional physiological responses that are critical for muscle performance (and movement). Accordingly, many effects observed in animals and isolated systems frequently differ from those seen in humans in vivo, and care should be taken when extrapolating responses from one set of conditions or a given experimental model to another (Schlegel and Stainier, 2007).

Voluntary exercise encompasses many elements beyond simple muscle contraction. Volitional effort generated in the motor cortex of the brain drives the spinal cord to recruit motor units, resulting in specific movement patterns. In parallel with neural signals to skeletal muscle, there are also powerful neural feedforward signals to the cardiovascular, respiratory, and metabolic and hormonal systems, along with neural feedback from the contracting skeletal muscles, that generally permit metabolic demands to be met with limited disruption of homeostasis (Figure 1).

Numerous issues relating to the speed, force, duration, and intensity of muscle contractions, along with the total muscle mass engaged in the activity, are important for a complete understanding of the physiological responses to exercise. An isometric or static contraction of high force but short duration compresses blood vessels in the contracting musculature and limits blood flow and O_2 delivery to those muscles while simultaneously increasing BP. In contrast, during sustained rhythmic exercise like cycling or running, the contraction times are short, there is little disruption of muscle blood flow, and perturbations in BP

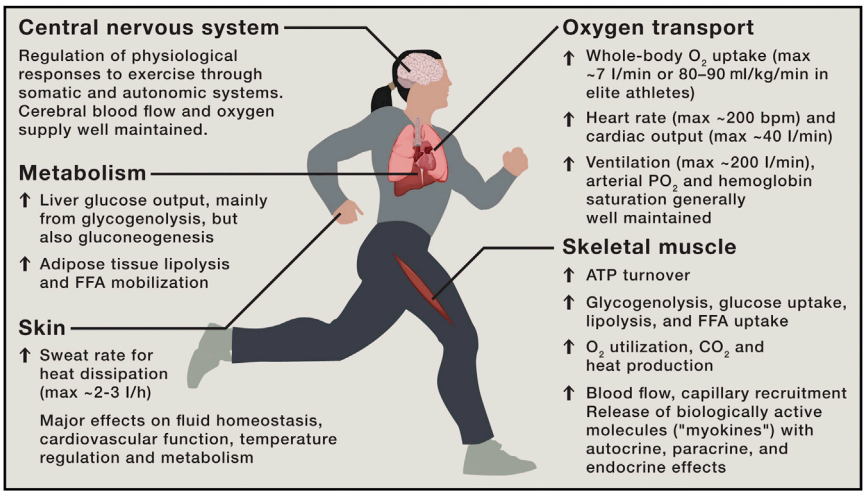

Figure 1. The Physiological Responses to Voluntary, Dynamic Exercise
Multiple organ systems are affected by exercise, initiating diverse homeostatic responses.

are minimized. The muscle mass engaged in exercise is critical, as it determines both the absolute O_2 flux and total fuel requirements. For most aerobic-based activities, such as running or cycling, active muscle mass amounts to ~15 kg in a 70 kg athlete (Coyle et al., 1991), although for rowing and cross-country skiing (for which the athlete is substantially taller and heavier), this is markedly higher (Hagerman 1984). The nomenclature relating to the quantification of exercise intensity is also relevant because the prevailing work rate exerts a major role in determining the overall physiological responses to exercise. For exercise lasting >5 min, intensity is typically expressed as a percentage of an individual's VO_{2max}. Low-, moderate-, and high-intensity exercise correspond to <45%, 45%–75%, and >75% of individual VO_{2max}, respectively.

Skeletal Muscle Energy Metabolism

ATP is required to fuel the cellular processes supporting muscle contraction. These include the maintenance of sarcolemmal excitability (Na^+/K^+ ATPase), reuptake of Ca^{2+} into the sarcoplasmic reticulum (Ca^{2+} ATPase), and force generation via actin-myosin cross-bridge cycling (myosin ATPase). Intramuscular [ATP] is remarkably well maintained over a wide range of exercise intensities and durations, and while [ATP] declines under certain exercise and/or environmental conditions, the magnitude of change is small when considered against the total turnover of ATP within active myocytes. During sprint exercise, ATP turnover can increase 100-fold above rest (Gaitanos et al., 1993; Parolin et al., 1999), a range of metabolic activity exceeding that in all other tissues and one that poses a major energetic challenge to the contracting myofibers. Given that intramuscular [ATP] is relatively small, metabolic pathways responsible for ATP resynthesis are rapidly activated. During short-term (~30–60 s) maximal exercise, this is achieved primarily through substrate-level phosphorylation via the breakdown of creatine phosphate and during the conversion of glucose units, derived almost entirely from intramuscular glycogen, to lactate (Gaitanos et al., 1993; Parolin et al., 1999). The mobilization of extramuscular substrates is also critical to maintain skeletal muscle metabolism during prolonged exercise (van Loon et al., 2005; Wasserman, 2009). Thus, the liver increases the release of glucose into the circulation (initially derived from glycogenolysis and later from gluconeogenesis), and the adipocyte increases the hydrolysis of its triglyceride stores and the release of long-chain nonesterified fatty acids into the bloodstream.

The relative contribution of carbohydrate and lipid to oxidative metabolism is determined primarily by the prevailing exercise intensity (Romijn et al., 1993) and is influenced by prior diet, training status, sex, and environmental conditions (Jeukendrup 2003). The contribution from the oxidation of carbohydrate-based fuels rises with increasing exercise intensity, with a concomitant reduction in lipid oxidation. Conversely, during prolonged exercise at a fixed level of moderate intensity, rates of carbohydrate oxidation decline as lipolysis and fat oxidation increase. The regulation of fuel mobilization and utilization involves a combination of local factors such as sarcoplasmic $[Ca^{2+}]$, intramuscular levels of ATP breakdown products (ADP, AMP, IMP, Pi), and muscle temperature and intramuscular substrate availability, as well as systemic factors such as the plasma level of key hormones (epinephrine, insulin, and glucagon) and circulating metabolites (Hawley et al., 2006). These factors are not only involved in mediating the acute response to exercise, but also activate signaling pathways critical for many of the chronic adaptations to regular exercise training. Recent reviews have summarized the various cellular and molecular factors involved in the regulation of skeletal muscle carbohydrate (Jensen and Richter, 2012; Richter and Hargreaves, 2013) and lipid (Jeppesen and Kiens, 2012) metabolism during exercise, as well as the interactions between them (Spriet, 2014).

Oxygen Transport System

At rest, whole-body O_2 consumption in healthy, young, adult humans averages about 3.5 ml/kg/min, with ~20%–25% of this used by resting skeletal muscle. Thus, for a 70 kg person, resting O_2 consumption is ~250 ml/min, with 50 ml/min taken up by skeletal muscle. In lean, healthy, untrained adults, VO_{2max} is typically 10–15 times resting values. In elite endurance-trained athletes, VO_2 max values can exceed 85 ml/kg/min (Saltin and Åstrand, 1967). Though O_2 fluxes in humans are high, they are marginal compared to values achieved by elite racehorses with VO_{2max} values of 110 l/min, equating to 220 ml/kg/min (Poole and Erickson, 2011).

VO_{2max} is determined by the combined capacities of the central nervous system to recruit motor units, the pulmonary and cardiovascular systems to deliver O_2 to contracting skeletal muscles, and the ability of those muscles to consume O_2 in the oxidative, metabolic pathways. Associated with large increases in O_2 consumption during maximal exercise in humans are peak values for cardiac output (Q) and ventilation of 40 and 200 l/min,

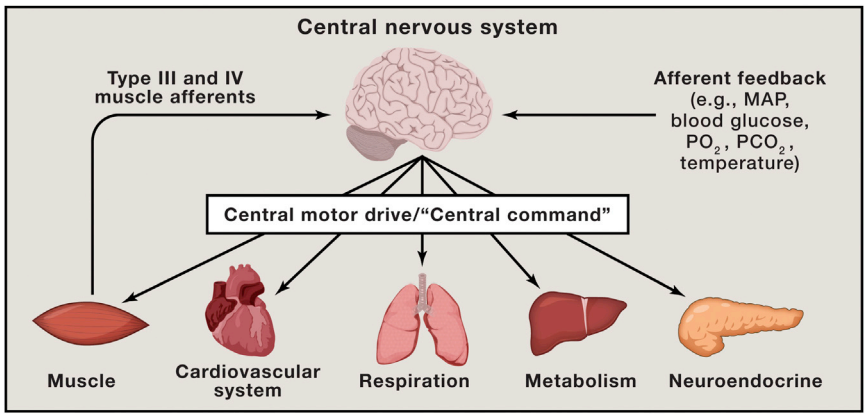

Figure 2. Complex and Redundant Physiological Control Mechanisms during Voluntary, Dynamic Exercise

Motor cortical drive leads to skeletal muscle contraction, as well as parallel activation ("central command") of key neuro-endocrine responses, fuel mobilization, and support systems that increase oxygen and substrate delivery to contracting skeletal muscle. The integrated response is fine-tuned by afferent feedback, involving mechano- and chemo-sensitive type III and IV afferents in active skeletal muscle, but also by critical sensors that monitor various parameters, including mean arterial blood pressure (MAP), blood glucose concentration, oxygen and carbon dioxide levels, body temperature, and blood volume.

representing 8- and 20-fold increases, respectively, above rest. In addition, blood flow to active skeletal muscle can increase ~100 times above basal levels, accounting for up to 80%–90% of Q. Notably, there is only a modest (~20%) increase in mean arterial blood pressure (MAP), whereas values for arterial PO_2, PCO_2, and pH remain essentially identical to rest until maximal exercise intensities are reached.

The cardiovascular adjustments to exercise require an intact autonomic nervous system and are driven by three signals: (1) feedforward "central command" related to motor output, which activate selected areas in the brainstem cardiovascular (and respiratory) centers to stimulate increases in heart rate (HR), BP, and ventilation; (2) afferent feedback from thinly myelinated and unmyelinated type III and IV afferents in contracting muscles that increase sympathetic activation; and (3) baroreceptors in the carotid sinus and aortic arch that provide feedback on BP to the brainstem cardiovascular centers. The HR response to exercise is driven primarily by central command-mediated vagal withdrawal and activation of sympathetic outflow to the heart. Both factors also augment cardiac stroke volume, and the action of the so-called "muscle pump" ensures that venous return from the active muscle vasculature maintains diastolic filling and stroke volume (SV). The central motor drive and central command are subject to "fine-tuning" via feedback signals that monitor substrate levels, MAP, blood gases and pH, fluid status, and body temperature despite the marked homeostatic challenges associated with high-intensity exercise (Figure 2).

The primary mechanism responsible for skeletal muscle hyperemia during exercise is vasodilation in the active skeletal muscle, most notably in the small arterioles. Mechanical, neural, and humoral factors, including those released from contracting skeletal muscle, have been implicated in this response. Because the rise in muscle blood flow is closely coupled to metabolic rate, vasodilating signal(s) released from contracting skeletal muscles roughly in proportion to their O_2 demand is(are) responsible (Hellsten et al., 2012). Candidate dilator substances and mechanisms include inward rectifying K^+ channels, adenosine, ATP from various sources, products of skeletal muscle metabolism, and reactive O_2 species. However, no single substance can fully account for the increases in muscle blood flow, and the molecular identity of one or more of these signals is unknown.

Blood flow is redistributed away from the kidney, liver, other visceral organs, and inactive muscle via vasoconstriction in these vascular beds, secondary to increased sympathetic activity during exercise. This permits a higher fraction of Q to be delivered to active skeletal muscle and partially offsets the fall in total peripheral resistance as a result of skeletal muscle vasodilation. Blood flow to the central nervous system remains either unchanged or increases slightly, and coronary blood flow increases. Because evaporation of sweat is the major mechanism for dissipation of heat during exercise, especially at higher environmental temperatures, there is an increase in skin blood flow and sweating-induced fluid loss with exercise (González-Alonso et al., 2008). With increased exercise intensity, the skin becomes a target for vasoconstriction as skeletal muscle blood flow increases despite increased metabolic heat production. To maintain MAP, skeletal muscle takes priority over skin blood flow. As exercise approaches VO_{2max}, the finite cardiac pumping capacity means that active skeletal muscle is also subject to vasoconstriction (Calbet et al., 2004). With increased environmental stress, the combination of progressive hyperthermia and dehydration further challenges the cardiovascular system during prolonged, strenuous exercise (González-Alonso et al., 2008).

The critical functions of the pulmonary system are to maintain arterial oxygenation and to facilitate the removal of CO_2 produced during oxidative metabolism. This is achieved by increased ventilation in proportion to exercise intensity, and arterial PO_2 and PCO_2 are generally maintained at resting levels until heavy exercise. The factors responsible for the marked increase in ventilation include descending central command in parallel with motor cortical activation of skeletal muscle that stimulates the brainstem respiratory centers and feedback stimulation from type III and IV afferents (Dempsey et al., 2014). In most healthy individuals exercising at sea level, arterial oxyhemoglobin saturation (SaO_2) is well maintained. However, in some highly trained endurance athletes, high-intensity exercise results in a significant drop in SaO_2 that impairs O_2 delivery to contracting skeletal muscle and results in impaired exercise capacity (Amann et al., 2006). Another threat to locomotor muscle O_2 delivery and performance during high-intensity exercise is reflex

sympathetic vasoconstriction of the limb skeletal muscle vasculature, secondary to activation of type III and IV afferents in respiratory muscles (Dempsey et al., 2002). Increases in respiratory muscle work during heavy exercise result in increased metabolite accumulation and activation of these afferents. This reflex serves to direct a greater proportion of the limited Q to the respiratory muscles but at the expense of the locomotor muscles and exercise performance.

The acute cardiovascular adaptations to both dynamic and isometric exercise lead to patterns of long-term remodeling and adaptations that increase VO_{2max} and minimize disruptions in whole-body homeostasis. The autonomic and sensory feedback systems described previously are subject to chronic resetting so that, during dynamic exercise, somewhat lower BPs are tolerated, thus permitting greater increases in skeletal muscle blood flow. This is accompanied by cellular changes in the brainstem cardiovascular center that tend to be pro-vagal and sympathoinhibitory. These changes partly explain why exercise at any given submaximal work rate after training is accompanied by a lower HR and BP. They also contribute to the chronic BP lowering effects of exercise in general. Adaptations to resistance training are less well characterized. However, during maximal weight lifting, BP can exceed 480/350 mmHg (MacDougall et al., 1985), consistent with the idea that compression of the blood vessels in the contracting skeletal muscles evokes responses designed to overcome "under perfusion" by substantially increasing BP.

With dynamic exercise, there is considerable remodeling of the vascular system, especially in the skeletal muscles subjected to training, including an increase in the diameter of large conducting vessels like the femoral artery for leg exercise (Green et al., 2012). There is also an increase in the number of arterioles and increased capillary density in the trained musculature. This structural remodeling is driven by a complex and redundant sequence of events that includes NO, prostaglandins, and vascular endothelial growth factor (VEGF) signaling pathways (Hoier and Hellsten, 2014). The time course of remodeling also varies by blood vessel size. Early in exercise training, there is a marked increase in nitric oxide synthase (NOS) expression in the large conducting vessels in response to increased shear stress. However, as the caliber of the vessel increases with training, the shear stress normalizes and NOS expression returns to baseline values. Though many of these adaptations are restricted to the vascular beds of the working muscle, improved endothelial function appears to be a whole-body response to exercise training.

Dynamic exercise training is associated with an increase in cardiac chamber size, but not wall thickness, that facilitates the increase in SV caused by this mode of training. Endurance training promotes volume hypertrophy, whereas resistance training does not cause major changes in the thickness of cardiac muscle. The stimulus for cardiac volume hypertrophy with dynamic exercise training is stretch of the ventricle caused by the increased venous return from the periphery. This stretch is facilitated by training-induced increases in blood volume and catecholamine concentrations. The cellular mechanisms responsible for cardiac hypertrophy with exercise training involve activation of a number of pathways, including the insulin-like growth factor 1 (IGF-1)-phosphatidylinositide 3-kinase (PI3K)-Akt/protein kinase B axis (Ellison et al., 2012), in particular PI3K (p110α). Downstream of Akt, exercise-induced cardiomyocyte hypertrophy and proliferation appears to be associated with reduced C/EBPβ expression and a concomitant increase in CITED4 expression (Boström et al., 2010). Cardiac hypertrophy also involves de novo cardiomyocyte formation by activation of both circulating and tissue-specific cardiac progenitor cells.

In highly motivated young, healthy individuals, VO_{2max} does not appear to be limited by muscle mitochondrial oxidative capacity (Boushel et al., 2011). Rather, O_2 delivery to skeletal muscle is rate limiting, and although this is determined by both convective and diffusive mechanisms, central cardiovascular function and the ability to increase active skeletal muscle blood flow appear to be critical (González-Alonso and Calbet, 2003). However, muscle mitochondrial oxidative capacity does appear to be an important determinant of endurance exercise performance (Joyner and Coyle, 2008). Thus, treadmill running time at submaximal exercise intensity is used as a physiological correlate of transgenic interventions that impact muscle oxidative capacity (Potthoff et al., 2007; Wang et al., 2004).

Skeletal Muscle Matters

Skeletal Muscle Fiber Type and Adaptation Plasticity

The application of surgical techniques to exercise biochemistry in the 1960s (Bergström and Hultman 1966) made it possible to obtain small (100–150 mg) samples of human skeletal muscle for histological and biochemical studies to identify specific morphological, contractile, and metabolic properties. Using these approaches, different fiber types have been identified along with their contractile characteristics, and these have been related to functional and metabolic properties of skeletal muscle during exercise (Saltin et al., 1977). The metabolic potential of muscle has also been evaluated by determining different substrate and enzyme activities. Comprehensive discussion of skeletal muscle fiber types and the gene programs responsible for fiber-specific properties are beyond the scope of this Review and have been summarized elsewhere (Bassel-Duby and Olson 2006; Saltin et al., 1977; Schiaffino and Reggiani 1996; Zierath and Hawley 2004). However, a brief overview of the classification of human muscle fiber types and their metabolic potential is warranted.

Histologically, skeletal muscle appears uniform but is comprised of myofibers that are heterogeneous with respect to size, metabolism, and contractile function. On the basis of specific myosin heavy-chain isoform expression, myofibers can be classified into type I, type IIa, type IId/x, and type IIb fibers, with types I and IIa exhibiting high oxidative potential and capillary supply and with types IIx and IIb fiber being primarily glycolytic (Pette and Staron 2000; Saltin et al., 1977; Schiaffino and Reggiani 1996). Type I myofibers are typically referred to as "slow-twitch fibers" because they exert slow contraction time to peak tension, owing to the ATPase activity associated with the type I myosin, whereas type II fibers are termed 'fast-twitch' myofibers and have quicker contraction time but a rapid fatigue profile (Bassel-Duby and Olson 2006; Saltin et al., 1977). With endurance training, the enhancement of the oxidative potential of type IIx and IIb fibers is markedly increased, resulting in a

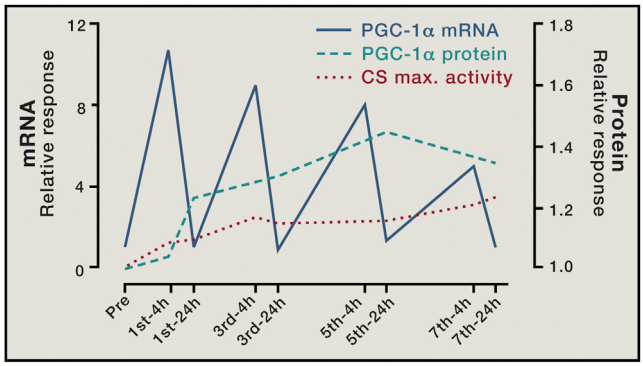

Figure 3. Repeated Transient Bursts in Messenger Ribonucleic Acid Precede Increases in Transcriptional and Mitochondrial Proteins in Response to Short-Term Training

Subjects (n = 9) completed seven sessions of high-intensity interval training during a 2 week intervention. Skeletal muscle biopsies from the vastus lateralis were obtained 4 and 24 hr after the first, third, fifth, and seventh training session. PGC-1α, peroxisome-proliferator-activated receptor γ coactivator α; CS, citrate synthase. Data are redrawn from Perry et al. (2010).

potential for oxidation that markedly surpasses the aerobic capacity of type I fibers of untrained individuals (Saltin et al., 1977). Indeed, the absolute level for the activities of both oxidative and glycolytic enzymes in all fiber types in humans is large enough to accommodate a substantial range of aerobic and anaerobic metabolism.

Whether endurance- or resistance-based exercise training in humans can result in fiber type "reprogramming" remains open to debate. Certainly endurance training induces changes in the metabolic properties of skeletal muscle by conferring an increased oxidative profile to the trained myofibers (Saltin et al., 1977). Such effects are likely to involve a plethora of signaling cascades and transcription factors including, but not limited to, calcium signaling pathways involving calcineurin, calcium-calmodulin-dependent kinase, and the transcriptional cofactors peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α) and PPARδ (Olson and Williams 2000; Lin et al., 2002; Wang et al., 2004; Wu et al., 2002). However, the specific transcriptional factors directly involved in the control of the muscle phenotype and fiber-specific contractile properties remain to be fully characterized.

Human skeletal muscle displays remarkable plasticity, with the capacity to alter both the type and amount of protein in response to disruptions in cellular homeostasis induced by the habitual level of contractile activity, the prevailing substrate availability, and environmental conditions (Hawley et al., 2011; Zierath and Hawley 2004). Though this phenomenon of "adaptation plasticity" is common to all vertebrates, a large variation in the degree of adaptability between humans is evident. This partly explains the large interindividual responses to standardized exercise training interventions and the striking differences in performance between individuals (Bouchard et al., 2011). The functional consequences of adaptation plasticity are specific to the mode of exercise and are influenced by the volume, intensity, and frequency of the contractile stimuli along with the half-life of specific exercise-induced proteins (Hawley 2002). Prolonged endurance-based exercise training elicits changes that increase the mitochondrial protein content and respiratory capacity of the trained myofibers. These adaptations underpin the altered patterns of substrate oxidation during submaximal exercise (from carbohydrate- to fat-based fuels) that result in less lactate production at a given submaximal power output or speed (Holloszy 1967). In contrast, strength and resistance-based training stimulates the myofibrillar proteins responsible for muscle hypertrophy, culminating in increases in maximal contractile force output (Phillips 2014) without substantial changes in fuel use during exercise. Concomitant with the vastly different functional outcomes induced by these diverse exercise modes, the genetic and molecular machinery affecting these adaptations are distinct.

Adaptations to Exercise Training: The Cumulative Effect of Repeated Exercise Bouts

The conversion of various chemical, electrical, and mechanical signals generated during muscle contraction to molecular events promoting physiological responses and subsequent adaptations involves a cascade of events resulting in activation and/or repression of specific signaling pathways regulating exercise-induced gene expression and protein synthesis/degradation. These pathways are numerous and have been reviewed elsewhere (Bassel-Duby and Olson 2006; Coffey and Hawley 2007; Egan and Zierath, 2013; Hood et al., 2006). Potential signals during contractile activity include, but are not limited to, increased sarcoplasmic [Ca^{2+}], increased AMP and/or an increased ADP/ATP ratio, reduced creatine phosphate and glycogen levels, increased fatty acid and ROS levels, acidosis, and altered redox state, including NAD/NADH, and hyperthermia (Hawley et al., 2006). Redundancy and compensatory regulation are key characteristics of biological systems that act to preserve physiological responses and adaptations to a variety of "threats" to cellular homeostasis. Indeed, some gene deletions or mutations have little effect on metabolic adaptation, highlighting the potential caveats involved in utilizing transgenic or knockout models to examine mechanisms of muscle adaptation (McGee et al., 2014).

Key signaling pathways involve Ca^{2+}/calmodulin-dependent kinases (CaMK), calcineurin, AMP-activated protein kinase (AMPK), mitogen-activated protein kinases (ERK1/2, p38 MAPK), and mammalian target of rapamycin (mTOR). The targets of these signaling pathways include many transcription factors, coactivators, and repressors. Exercise increases activation of CaMKII (Rose and Hargreaves, 2003), AMPK (Winder and Hardie 1996; Fujii et al., 2000), and MAPKs (Widegren et al., 1998). As previously noted, contraction-induced alterations in intracellular [Ca^{2+}] are linked to distinctive programs of gene expression that establish phenotypic diversity among skeletal myofibers (Chin, 2005; Tavi and Westerblad, 2011). In addition, activation of AMPK by exercise-induced alterations in muscle energy status increases gene transcription in skeletal muscle (McGee and Hargreaves, 2010).

Adaptations to exercise training result from the cumulative effect of transient increases in mRNA transcripts that encode for various proteins after each successive exercise bout. These repeated bursts in mRNA expression appear to be essential to drive the intracellular adaptive response to exercise training (Neufer and Dohm 1993; Perry et al., 2010) (Figure 3). The timing

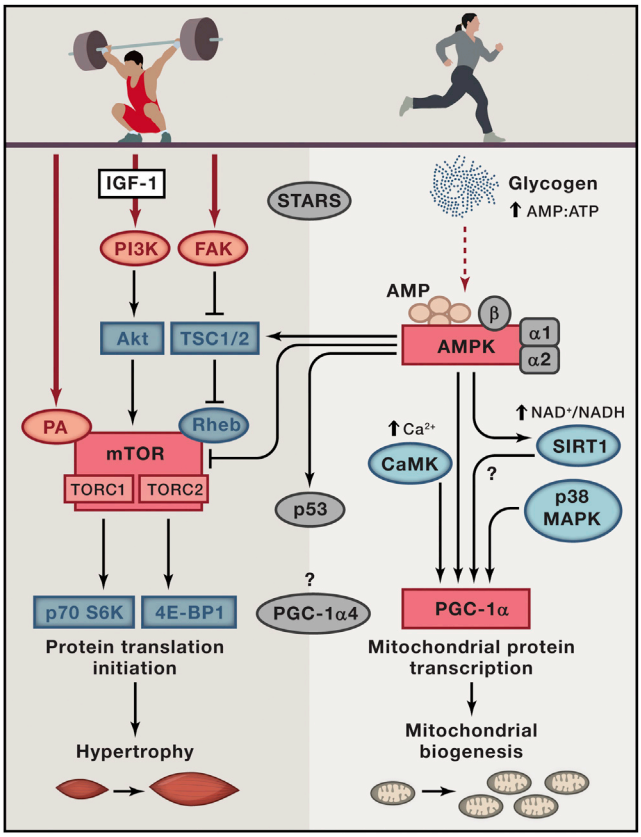

Figure 4. Schematic of the Major Signaling Pathways Involved in the Control of Skeletal Muscle Hypertrophy and Mitochondrial Biogenesis

Multiple primary signals, including, but not limited to, mechanical stretch, calcium, pH, redox state, hypoxia, and muscle energy status, are altered with voluntary dynamic exercise. Following initiation of one or more of these primary signals, additional kinases/phosphatases are activated to mediate a specific exercise-induced signal. In mammalian cells, numerous signaling cascades exist. These pathways are regulated at multiple sites, with substantial crosstalk between pathways producing a highly sensitive, complex transduction network.

and responsiveness of individual mRNA species to different types of contractile activity is variable, but peak induction for both "metabolic" and "myogenic" genes generally occur 4–8 hr after an exercise bout, with mRNA levels returning to pre-exercise levels within 24 hr (Yang et al., 2005). Another level of regulation of mRNA and protein abundance by exercise involves alterations in DNA methylation status (Barrès et al., 2012), histone modifications (McGee and Hargreaves, 2011), and microRNA expression (Zacharewicz et al., 2013). Ultimately the ability of a given muscle cell to alter the type and quantity of protein is a function of its half-life. Proteins that turn over rapidly and have high rates of synthesis are capable of attaining a new steady-state level faster than those that turn over slowly during adaptation to contractile and other stimuli.

Mitochondrial Biogenesis and Endurance Training Adaptation

Mitochondrial biogenesis requires the coordination of multiple cellular events, including transcription of two genomes, synthesis of lipids and proteins, and the stoichiometric assembly of multisubunit protein complexes into a functional respiratory chain (Hood et al., 2006). Impairments at any step can lead to defective electron transport, failure of ATP production, and an inability to maintain energy homeostasis. Since the seminal work of Holloszy (1967), who discovered that muscles of treadmill-trained rats exhibited higher levels of mitochondrial proteins than those of untrained animals, major breakthroughs in unraveling the cellular events controlling skeletal muscle mitochondrial biogenesis have occurred. Several transcription factors that regulate the expression of the nuclear genes encoding mitochondrial proteins were discovered (Scarpulla 2006). These include nuclear respiratory factors 1 and 2 (NRF-1, NRF-2) that bind to the promoters and activate transcription of genes that encode mitochondrial respiratory chain proteins (Kelly and Scarpulla 2004). NRF-1 also activates expression of the nuclear gene that encodes mitochondrial transcription factor A (TFAM), which moves to the mitochondria and regulates transcription of the mitochondrial DNA (i.e., the mitochondrial genome). Because not all promoters of genes transcribing mitochondrial proteins have NRF-1-binding sites, other transcription factors are involved in contractile-modulated mitochondrial biogenesis, including the estrogen-receptor-related receptors (ERR) α and δ and the peroxisome proliferator-activated receptor coactivators (PPARs), which regulate expression of the mitochondrial fatty acid oxidative enzymes (Kelly and Scarpulla, 2004; Scarpulla, 2006).

Another major breakthrough in unraveling the cellular events that promote mitochondrial biogenesis was the discovery of PGC-1α, an inducible coactivator that regulates the coordinated expression of mitochondrial proteins encoded in the nuclear and mitochondrial genomes (Lin et al., 2005). A critical feature of the PGC-1 coactivators is that they are highly versatile and interact with many different transcription factors to activate distinct biological programs in different tissues (Lin et al., 2005). In skeletal muscle, PGC-1α has emerged as a key regulator of mitochondrial biogenesis that responds to neuromuscular input and the prevailing contractile activity. A single bout of endurance exercise induces a rapid and sustained increase in PGC-1α gene and protein in skeletal muscle (Mathai et al., 2008), whereas muscle-specific overexpression of PGC-1α results in a large increase in functional mitochondria (Lin et al., 2002), improvements in whole-body VO_{2max}, a shift from carbohydrate to fat fuels during submaximal exercise, and improved endurance performance (Calvo et al., 2008). Gain-of-function studies reveal that expression of PGC-1α at or near physiological levels leads to activation of genetic programs characteristic of slow-twitch muscle fibers (Lin et al., 2002), with the muscles of these transgenic mice resistant to contraction-induced fatigue. Loss of function studies challenge the absolute requirement of PGC-1α for exercise training-induced changes in muscle mitochondrial biogenesis, angiogenesis, and fiber type changes (Geng et al., 2010; Rowe et al., 2012). On balance, current observations place PGC-1α as a central player in orchestrating many of the oxidative adaptations to exercise.

AMPK and p38 MAPK are two important signaling cascades that converge upon the regulation of PGC-1α and consequently the regulation of mitochondrial biogenesis (Figure 4). AMPK

induces mitochondrial biogenesis partly by directly phosphorylating and activating PGC-1α (Jäger et al., 2007), but also by phosphorylating the transcriptional repressor HDAC5, which relieves inhibition of the transcription factor myocyte enhancer factor 2 (MEF2), a known regulator of PGC-1α (McGee and Hargreaves, 2010). Of note, MEF2 activation is associated with increased muscle oxidative capacity and running endurance (Potthoff et al., 2007). p38 MAPK phosphorylates and activates PGC-1α (Puigserver et al., 2001) and also increases PGC-1α expression by phosphorylating the transcription factor ATF-2, which in turn increases PGC-1α protein abundance by binding to and activating the CREB site on the PGC-1α promoter (Akimoto et al., 2005). The tumor suppressor protein p53, presumably activated by AMPK and/or p38 MAPK, is emerging as another transcription factor involved in exercise-induced mitochondrial biogenesis in skeletal muscle. p53 knockout mice display reduced endurance exercise capacity compared with wild-type mice, along with reduced subsarcolemmal and intermyofibrillar mitochondrial content and PGC-1α expression. p53 may regulate exercise-induced mitochondrial biogenesis through interactions with TFAM in the mitochondria, where it functions to co-ordinate regulation of the mitochondrial genome (Bartlett et al., 2014).

Muscle Hypertrophy and Myogenic Pathways

Strength training increases muscle fiber size (hypertrophy) and maximal tension output. These adaptations are attained by positive muscle protein balance and satellite cell addition to pre-existing fibers. Positive muscle protein balance occurs when the rate of new muscle protein synthesis exceeds that of breakdown. Although resistance exercise and postprandial hyper-aminoacidemia both stimulate muscle protein synthesis, it is through the synergistic effects of these stimuli that a net gain in muscle protein occurs and fiber hypertrophy takes place (Phillips 2014).

Activation of mTOR appears to be important for contraction-induced increases in muscle protein synthesis (Drummond et al., 2009). Once activated, mTOR exists as two distinct complexes, mTOR complex 1 (TORC1) and mTOR complex 2 (TORC2). TORC1 is characterized by the presence of regulatory-associated protein of mTOR (RAPTOR), whereas TORC2 binds rapamycin-insensitive companion of mTOR (RICTOR). These two protein complexes sense diverse signals and produce a multitude of responses, including mRNA translation, ribosomal biogenesis, and nutrient metabolism (Coffey and Hawley 2007; Egerman and Glass, 2014). IGF-1 has long been considered a key upstream regulator of mTOR. Signaling activated by IGF-1 positively regulates skeletal muscle mass via induction of protein synthesis downstream of protein kinase B/Akt and the mTOR pathway (Bodine et al., 2001). IGF-1 transmits signaling along the PI3K/Akt pathway (Figure 4), resulting in the parallel activation of the mTOR pathway, producing a multitude of responses, including mRNA translation, ribosomal biogenesis, and nutrient metabolism (Coffey and Hawley 2007). Growth-factor-independent, mechanosensitive activation of mTOR also contributes to muscle protein synthesis (Philp et al., 2011).

The most well-defined effectors of mTOR signaling are proteins implicated in translational control: ribosomal protein S6 kinase (p70S6K) and eukaryotic initiation factor 4E-binding protein (4E-BP1). Indeed, after activation by Akt, TORC1 controls protein synthesis by phosphorylating p70S6 kinase 1 and the 4E-BP1, and the TORC2 multiprotein complex contributes to the prolonged activation of Akt. Phosphorylation of p70S6K and subsequent activation of ribosomal protein S6 enhances translation of mRNAs, encoding elongation factors and ribosomal proteins and thereby increasing translational capacity. p70S6K plays a fundamental role in skeletal muscle hypertrophy (Liu et al., 2002). A single bout of resistance exercise in humans leads to increases in p70S6K phosphorylation, which is correlated with the chronic increase in muscle mass and strength observed after chronic resistance training. Thus, the acute responses to exercise, including dynamic changes in muscle protein turnover and the early activation of signaling proteins, may act as surrogates of long-term phenotypic changes in muscle mass and strength.

PGC-1α4, a transcript from the PGC-1α gene, is abundantly expressed in skeletal muscle and appears to play a role in the adaptive response to exercise, particularly in the setting of resistance training (Ruas et al., 2012). This protein does not appear to regulate the same set of oxidative genes induced by PGC-1α but, rather, activates the expression of IGF-1 while concomitantly suppressing myostatin (an inhibitor of muscle cell differentiation and growth) pathways. After training consisting of either endurance exercise, resistance exercise, or a combination of both endurance and resistance exercise, increases in PGC-1α4 were confined to resistance-only and combined exercise training programs, with no changes in this transcript after endurance-only training (Ruas et al., 2012). Though these results are notable, the proposal that skeletal muscle hypertrophy following resistance exercise is mediated through PGC-1α4 remains a matter of debate.

Skeletal muscle from endurance- and strength-trained individuals represents diverse adaptive states (Figure 4). Thus, it is hardly surprisingly that simultaneously training for both endurance and strength results in a compromised adaptation compared with training for either exercise modality alone (Hickson 1980), a phenomemon known as the ‘‘interference effect.’’ These observations made more than 30 years ago (Hickson 1980) raised the possibility that the genetic and molecular mechanisms of adaptation induced by endurance and resistance training are distinct, with each mode of exercise activating and/or repressing specific subsets of genes and cellular signaling pathways. Preliminary evidence for selective activation and/or downregulation of the AMPK-PGC-1α or Akt-mTOR signaling pathways was reported in rodent skeletal muscle in response to either low-frequency (to mimic endurance training) or high-frequency (to mimic resistance training) electrical stimulation in vitro (Atherton et al., 2005). However, in well-trained humans, little evidence exists for an AMPK-Akt ‘‘master switch.’’ Using highly trained athletes with a history of either endurance or strength training who performed both an acute bout of exercise in their specialized discipline and then ‘‘crossed over’’ and undertook a bout of unfamiliar exercise, a high degree of ‘‘response plasticity’’ is conserved at opposite ends of the endurance-hypertrophic adaptation continuum (Coffey et al., 2006). Given that genotypes were originally selected to support diverse physical activity patterns obligatory for human survival and that modern day success in many sporting endeavors

requires a high endurance capacity coupled with superior explosive power, the conservation of multiple signaling networks to meet divergent physiological demands seems to make sound evolutionary and biological sense!

Spreading the Message: Skeletal Muscle Crosstalk

More than 50 years ago, Goldstein proposed that skeletal muscle cells possessed a "humoral" factor that contributed to the maintenance of glucose homeostasis during exercise (Goldstein, 1961). During the past decade, skeletal muscle has been confirmed as an endocrine organ. Cytokines and other peptides that are expressed, produced, expressed, and/or released by muscle fibers and exert their autocrine, paracrine, or endocrine effects are now classified as "myokines" (Pedersen et al., 2003). The finding of muscle "crosstalk" with other organs, including adipose tissue, liver, pancreas, bone, and the brain, provides a framework for understanding how exercise mediates many of its beneficial "whole-body" effects. Although some myokines exert their actions on other organs in an endocrine fashion, many operate locally on skeletal muscle and thereby provide a feedback loop for the muscle to regulate its own growth and regeneration for adaptation to exercise training. The first cytokine found to be released into the bloodstream in response to muscle contraction was interleukin 6 (IL-6). Human skeletal muscle is unique in that it can produce IL-6 during exercise independently of tumor necrosis factor, suggesting that muscle-derived IL-6 has a role in metabolism rather than in inflammation. IL-6 increases both muscle and whole-body rates of lipid oxidation (possibly though activation of AMPK) and also contributes to hepatic glucose production during exercise.

Contracting muscle fibers produce many circulating factors. The current list of potential myokines includes but is not limited to IL-8, IL-15, decorin, follistatin-like 1, fibroblast growth factor-21 (FGF21), irisin, chemokine CXC motif ligand-1 (CXCL-1) also known as KC (keratinocyte-derived chemokine), and meteorin-like (Metrnl) (Boström et al., 2012; Rao et al., 2014; Pedersen et al., 2012; Wrann et al., 2013). A small-molecule myokine, β-aminoisobutyric acid (BAIBA), with effects on adipose tissue and liver, has also been described (Roberts et al., 2014). Most recently, exercise-induced alterations in kynurenine metabolism, mediated via increased skeletal muscle PGC-1α1 expression, increase resilience to stress-induced depression in mice (Agudelo et al., 2014). Although the potential therapeutic benefits of muscle-derived molecules for treating obesity and other inactivity-related disorders are appealing, there is little clinical evidence in humans to date.

Can "Exercise Mimetics" Ever Replace Exercise?

Many of the adaptive responses of skeletal muscle to exercise training can be mimicked by genetic manipulation and/or drug treatment, at least in animal models (Narkar et al., 2008). Consequently, given the numerous benefits of exercise on general health, it has been stated that "the identification of genetic and/or orally active agents that mimic or potentiate the effects of endurance exercise is a longstanding albeit elusive medical goal" (Narkar et al., 2008). Recognizing the proven benefits of exercise training on health outcomes and the trend toward increasing inactivity at the population level, efforts are underway to discover orally active compounds that mimic or potentiate the effects of exercise training, so-called "exercise mimetics." Although the concept of taking a pill to obtain the benefits of exercise without actually expending any energy has mass appeal for a large majority of sedentary individuals, such an approach is likely to fail. Exercise training provokes widespread perturbations in numerous cells, tissues, and organ, conferring multiple health-promoting benefits, and it is the multiplicity and complexity of these responses and adaptations that make it highly improbable that any single pharmacological approach could ever mimic such wide-ranging effects. Though a "polypill" containing several agonists aimed at selected exercise-induced targets is a possibility, such an approach is likely to be associated with multiple off-target and potential deleterious side effects. A more achievable goal will be to identify tissue-specific targets through a deeper understanding of the molecular pathways activated by exercise in various organ systems, enabling limited aspects of the exercise response to be pharmacologically mimicked. Although such agents may be useful adjuvants in some settings, exercise itself remains the best "polypill" to improve health and wellbeing (Fiuza-Luces et al., 2013). In our opinion, finding ways to motivate people to adopt and maintain a physically active lifestyle will have a greater impact on individual and population health than searching for potential pharmacological treatments. If finding orally active exercise mimetics is really a "longstanding" medical goal, we believe it will continue to be elusive for reasons evident in this Review.

Beyond the Finish Line: The Next 40 Years

During the last 40 years, with the application of molecular techniques to exercise biology, multiple and apparently redundant molecular pathways engaged in many key acute and chronic responses to exercise have been elucidated in skeletal muscle and other tissues. Although major breakthroughs in the knowledge of how exercise activates numerous cellular, molecular, and biochemical pathways have been witnessed, direct evidence linking such effects to specific health outcomes and understanding how these effects exert their benefits in different populations remains elusive and a challenge for future research. During the past two decades, the long-hypothesized crosstalk between muscle and other organs via the release of substances by the contracting muscles has been confirmed. However, in many cases, normal responses and adaptations to both acute exercise and chronic exercise training can be seen when one or more key pathways are absent, are blocked with drugs, or are otherwise attenuated. This biological redundancy indicates that perhaps the only obligatory response to exercise is the defense of homeostasis itself. Clearly a big challenge for exercise biologists in the next 40 years will be to connect distinct signaling cascades to defined metabolic responses and specific changes in gene expression in skeletal muscle that occur after exercise. This will be complicated because many of these pathways are not linear, but, rather, they constitute a complex network, with a high degree of crosstalk, feedback regulation, and transient activation. The various "omics" technologies and the application of computational and systems biology approaches to problems in exercise biology should facilitate future progress.

Future research in the field of exercise biology requires increasingly sophisticated approaches to understand the critical

nodes of energy homeostasis and how these pathways are disrupted in a number of inactivity-related disorders. Various strains of mice have long been used to examine research questions in exercise biology. Recent studies using the worm (*Caenorhabditis elegans*), fly (*Drosophila melanogaster*), and zebrafish (*Danio rerio*) indicate that these "lower" metazoans also possess unique attributes that could prove valuable in the study of metabolic diseases, including the effects of exercise/muscle contraction. Detailed characterization of the known pathways regulating lower metazoan energy metabolism may aid in identifying and characterizing novel candidate genes for human diseases such as obesity and type 2 diabetes mellitus, and the function of such genes may be more amenable to streamlined characterization in lower organisms. Although there are limitations of each model system that need to be recognized when deploying these organisms for target validation and, ultimately, translation into humans, the ability to perform sophisticated and mechanistic studies indicates that such an approach could yield transformative research outcomes in the coming decades. In the final analysis, it is the organism's phenotype as a whole that interacts with and adapts to the external world. The study of exercise biology shows that the need to integrate observations from genes, molecules, and cells in a physiological context has never been greater.

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