Running Forward
New Frontiers in Endurance Exercise Biology

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Give every individual the right amount of exercise, not too little and not too much.1 —Hippocrates

Physical activity has long been tied to good health. Hippocrates was guided by his theory of balanced humors to advocate that absolutely everyone, young or old, needs exercise, but not too much. A landmark 1953 study noted that drivers of public trolleys in London had twice as many acute coronary syndromes as did conductors of the same trolleys, the only notable difference being that conductors walked as they collected tickets and drivers sat.2,3 In the decades since this seminal epidemiological observation, nearly every aspect of human physiology has been demonstrated to benefit from exercise, ranging from lung and cardiac function to cognition and aging (Figure 1). The same decades, however, have witnessed a dramatic sedentarization of the US population (despite the newfound popularity of recreational exercise). Today, the consequences of sedentary lifestyles, synergizing with dramatic increases in caloric intake, are ubiquitous and devastating.

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content in muscle fibers (Figure 2). How does this process occur? Research done during the last 10 years has begun to answer this question at the molecular level (Figure 3). Much of this work has relied on the study of genetically modified mice (e.g., that depicted in Figure 4). Rodents are natural-born endurance exercisers. Provided with in-cage running wheels, most mice will voluntarily run 5 to 8 km per night. Although this level of exercise does not precisely model the human condition, the ability to model endurance exercise in an organism amenable to genetic manipulation has proven invaluable. Conceptually, the adaptation process can be divided into mechanisms that sense endurance activity and mechanisms that carry out the desired adaptations (Figure 3).

**Figure 1.** The many long-term benefits of regular endurance exercise.

**Figure 2.** Simplified overview of muscle fiber types. Muscle consists of cellular syncytia called fibers, which often span from tendon to tendon. Individual fibers have specific attributes, dictated by their myofibrillar and metabolic makeup and ranging from fast and glycolytic to slow and mostly oxidative. Most human muscles comprise mixtures of fiber types. Exercise and other stimuli can alter fiber-type profiles. Endurance exercise promotes conversion to more oxidative/slow fiber phenotype, a process that requires activation of various programs of gene expression (see Figure 3).

**Figure 3.** Modular signaling pathways that underpin muscular adaptations to endurance exercise. Exercise triggers patterns of innervation and metabolic perturbations that are sensed by skeletal muscle. These inputs activate intermediate signaling mechanisms, including calcium-mediated signaling and metabolic sensing pathways like AMP-activated protein kinase (AMPK) and sirtuin (SIRT). These signals ultimately impinge on groups of transcription factors, each of which controls a broad biological module such as mitochondrial biogenesis or fatty acid transport and metabolism. Adaptations to exercise can thus be regulated in a modular fashion. The box indicates β-oxidation; CaMK, calcium/calcmodulin-modulated phosphatase (calcineurin) and kinase; ERα, estrogen-related receptor-α; FFA, free fatty acid; HDAC, histone deacetylase; MAPK, mitogen-activated protein kinase; MEF2, myocyte enhancer factor-2; mTOR, mammalian target of rapamycin; NFAT, nuclear factor of activated T-cells; NRF1/2, nuclear respiratory factor-1/2; PGC, proliferator-activated receptor-γ coactivator; PKA, protein kinase A; PPAR, peroxisome proliferator-activated receptor-α; and TCA, tricarboxylic acid.

**How Does Muscle Sense Exercise Activity? Nerves and Energy**

Key molecular sensors of exercise include the calcium oscillations and other signaling cascades activated by neural stimulation and the profoundly altered metabolic state of contracting myofibers. Stimulation of muscle by motor nerves generates cytosolic calcium transients that are detected by various intracellular pathways, including those regulated by the calcium/calmodulin-modulated phosphatase (calcineurin) and kinase (CaMK). Continuous low-amplitude transients, typical of endurance activity, activate calcineurin. Transgenic overexpression of calcineurin in skeletal muscle of mice promotes the formation of slow red fibers. Conversely, genetic deletion of calcineurin or treatment with calcineurin inhibitors has the opposing effect, blocking exercise-induced adaptations. CaMK is likely another decoder of calcium transients. CaMKII is the predominant isoform in skeletal muscle, but experiments with transgenic expression of a constitutively active CaMKIV isoform showed that CaMK can promote the formation of slow red fibers. Conversely, mice with decreased CAMKII activity have lower expression of slow genes. Sensing of calcium...
transients thus appears to be a critical transducer of motor nerve activity.

Sympathetic stimulation also contributes to muscle adaptations. β2-Adrenergic receptors predominate in skeletal muscle and transduce via canonical and alternative G-protein signaling to cAMP, protein kinase A, and exchange protein directly activated by cAMP, ultimately affecting numerous cellular pathways. Among these, the p38 mitogen–activated protein kinase system may predominate in muscle adaptations. Genetic deletion of p38 isoforms in mouse skeletal muscle blocks many adaptations to endurance exercise, whereas genetic activation of p38 increases mitochondrial markers. G-protein–mediated sympathetic stimulation cross-talks with the CaMK system in complex ways, and these signals are likely integrated in skeletal muscle. The mitogen-activated protein kinase system also responds to, and likely integrates, endurance exercise–induced increases in reactive oxygen species generated by both the increased electron transport chain activity and activated NADPH oxidase.

Endurance exercise also elicits profound metabolic changes that can be sensed to trigger long-term adaptations. A key sensor is the AMP-activated protein kinase (AMPK). AMPK integrates multiple signals that alert to cellular energetic insufficiency such as elevated AMP/ATP ratios. Once activated, AMPK triggers metabolic pathways that can compensate for this deficiency such as mitochondrial biogenesis while inhibiting anabolic pathways that consume ATP. Mice engineered to lack AMPK activity in skeletal muscle run poorly and lack nearly half their mitochondrial content. Conversely, mice engineered to express an activated form of AMPK have higher mitochondrial content and β-oxidation and are resistant to fatigue. Other metabolic sensors are less conclusively implicated in exercise adaptations but likely also play a role, including the aging-associated sirtuin family of proteins. Sirtuins are deacetylating enzymes that require NAD and sense NAD/NADH ratios, which reflect the cellular redox state. The NAD/NADH ratio and thus likely sirtuin activity increase significantly with exhaustive exercise. Sirtuin activity is also enhanced by AMPK, thus linking these 2 pathways. Exercise-induced hypoxia or ischemia has also long been advocated as a potential mediator of muscle adaptation to endurance exercise. Short-term exercise reduces the partial pressure of oxygen in the vicinity of myoglobin to <4 mm Hg (<1/40th of atmospheric), which is sufficiently low to activate the critical hypoxia sensor hypoxia-inducible factor-1α (HIF-1α). However, it is not clear that this level of hypoxia is achieved in the nucleus, where HIF-1α resides. Moreover, mice genetically modified to lack HIF-1α in muscle have higher, rather than lower, oxidative capacity. Conversely, people living at higher altitudes, where oxygen tension is low, tend to have less oxidative muscle rather than more. It thus remains uncertain whether hypoxia mediates pro-oxidative adaptations to endurance exercise in muscle.

In summary, nerve stimulation, calcium signaling, and metabolic changes that occur in the contracting myofiber are likely the key instigators of adaptations to exercise. The effects of exercise can differ widely, depending on exercise type, dose, intensity, or frequency, and interindividual adaptations to exercise also vary widely. Thus, a key advantage of studying rodent models, that is, their genetic and phenotypic homogeneity, can also be a drawback. Experimental exercise regimens are not fully standardized, and comparing studies can sometimes be difficult. It is also difficult to scale rodent exercise to human exercise. For example, what does 5 km of voluntary running overnight by a 40-g mouse equate to?

Figure 4. Mighty mice. Left, Sample images of skinned mice from control (top) and mice genetically engineered to overexpress proliferator-activated receptor-γ coactivator-1β in skeletal myotubes. High levels of myoglobin and mitochondria render the muscles red (bottom). Myofibers have high oxidative capacity, and the mice are capable of running farther and harder on treadmill endurance tests. Right, Other known genetic mouse models with increased mitochondrial capacity in skeletal muscle. KO indicates total-body knockout of the indicated gene; MAP, mitogen-activated kinase; mKO, myotube-specific deletion (knockout) of the indicated gene; and mTg, myotube-specific overexpression via transgenesis.
in human terms? Nevertheless, the ability to model endurance exercise in an organism that is amenable to genetic manipulation continues to prove invaluable. How the complex inputs of endurance exercise are integrated thus remains incompletely understood. Metabolic signaling in particular is likely to be a prominent focus of research in the forthcoming years.

How Does Muscle Reprogram in Response to Exercise? Plug and Play

The signals discussed above impinge on a number of regulators of gene expression that reprogram myofibers from glycolytic to more oxidative fibers. Two important concepts guide this process. First, these regulating factors typically control broad programs. The transcriptional regulators peroxisome proliferator–activated receptor-γ coactivator (PGC)-1α and PGC-1β, for example, can activate an entire program of mitochondrial biogenesis, including all components of the electron transport chain, the tricarboxylic acid cycle, and fatty acid β-oxidation.44 Simple transgenic expression of PGC-1 in skeletal myofibers dramatically increases mitochondrial content, yielding mice with improved endurance exercise capacity and peak oxygen uptake (Figure 4).13,15 Conversely, deletion of both PGC-1 isoforms in muscle drastically reduces oxidative capacity.45,46 Most signaling pathways described above, including calcineurin, p38 mitogen–activated protein kinase, adrenergic signaling, and sirtuin proteins, impinge on the PGC-1s, and do so in a variety of molecular ways, including protein phosphorylation and deacetylation and induction of gene expression.47 The PGC-1s are thus potent nodal points of gene regulation used by myofibers to coordinate adaptations to exercise. A number of other such regulators exist (see Figure 4).

A second important concept is that these nodal points regulate defined and modular programs (Figure 3). For example, the nuclear receptor peroxisome proliferator–activated receptor-α modulates the use of fatty acid as a fuel by regulating the specific subset of genes encoding rate-limiting enzymes for fatty acid transport and oxidation.48 Similarly, a small set of transcription factors, including nuclear respiratory factor, nuclear respiratory factor-2, and estrogen-related receptor-α, control the complex generation of new mitochondria, including control of the replication and expression of the mitochondrial genome.49 The specific makeup of the myofibrillar apparatus, on the other hand, appears to be regulated primarily by myocyte enhancer factor-2 and nuclear factor of activated T-cell transcription factors, modified in turn by a number of histone deacetylases.21 Any of these independent programs can be coordinately modulated by the PGC-1s or other coordinating agents. Cellular reprogramming can thus occur in a modular fashion, with a relatively simple interface between input and output, analogous to plug and play.

An important implication of this modular regulatory concept is that modules can be activated independently, even if there is often significant cross-talk between them. For example, genetic deletion of both PGC-1α and PGC-1β in murine muscle leaves the myofibers metabolically crippled but has almost no effect on the myofibrillar content of the myofibers.35,46 The myofibrillar and metabolic responses to endurance exercise thus appear genetically dissociable and are likely regulated by exercise differently. In a highly simplified schema, it can be suggested that calcium signaling, occurring via calcineurin and CAMKII, affects predominantly myofibrillar adaptations, whereas metabolic sensing, via AMPK and other sensors, affects predominantly mitochondrial and other metabolic adaptations.7,8,50,51

In summary, nerve activity and metabolic changes are transduced to a cadre of transcriptional regulators that modularly translate this information into specific muscle adaptations. Other forms of modulation are superimposed on these gene regulatory pathways. Recent exciting additions include epigenetic modifications, and microRNAs (miRNAs). Methylation of DNA had long been thought to be a slow and meta-stable way to regulate gene expression, sometimes even allowing transmission of information across generations. It is now clear, however, that even a single bout of exercise can dramatically alter the methylation of certain genes, including PGC-1α.52 This surprising finding unveils entirely new potential mechanisms of exercise adaptations. Similarly, the recent discovery of miRNAs has opened new avenues of potential regulation. miRNAs are short RNAs that potently inhibit gene expression by binding to target mRNAs and causing their degradation or blocking their translation. Skeletal muscle expresses a number of miRNAs, dubbed myomiRs. For example, miRNAs 208b and 499 are coexpressed with slow myosin heavy chain genes and act to inhibit the expression of genes specific to fast myofibers, thus reinforcing the slow program.53 A number of miRNAs are regulated by exercise,54 but their role in exercise adaptations remains unknown, and appropriate mouse models have yet to be generated. Some miRNAs are also secreted into the circulation, raising the enticing notion that they may act as endocrine signals.54 The study of miRNAs as potential mediators of adaptations to exercise remains in its infancy but holds great promise.

Beyond Fiber Types: Other Important Ways That Exercise Changes Skeletal Muscle

Discussions of metabolic adaptations to endurance exercise are often limited to mitochondrial biogenesis, but mitochondrial reprogramming is likely much more complex. Mitochondria are traditionally portrayed as static, oval organelles. Recent work has challenged this view and revealed mitochondria as forming a large and dynamic reticulur network, constantly fusing and breaking apart in a highly coordinated manner.56–58 These fusion/fission events appear imperative for normal mitochondrial function. For example, they allow constant redistribution of both genetic and biochemical components between separate mitochondria, a process that is somehow used to segregate dysfunctional mitochondrial components and to remove them from the network via autophagy (discussed below).59 The regulation of mitochondrial fusion is controlled by mitofusins (Mfn1 and Mfn2) on the outer mitochondrial membrane and optic atrophy type 1 (Opal) on the inner mitochondrial membrane, whereas fission is regulated by dynamin-related protein (Drpl) and fission 1 (Fisl).57 Exercise induces the expression of most of these genes in both human and rodent muscle.60–62 PGC-1α induces the expression both Mfn1 and Mfn2 via activation of estrogen-related receptor-α.63 Deletion of both Mfn1 and Mfn2 in mouse skeletal muscle leads to profound lactic acidosis in response to
exercise. These observations thus suggest that mitochondria in muscle are not static objects and that mitochondrial dynamics play an important role in exercise adaptations. The specifics of that role remain poorly understood.

Autophagy (Greek for self-eating) is a process by which cells selectively move damaged organelles like mitochondria to the lysosome for subsequent degradation. This quality control process is critical for maintaining tissue integrity. The process also appears important for adaptations to exercise. Exercise strongly and rapidly induces autophagy in skeletal muscle and numerous other tissues. Moreover, genetically modified mice that lack stimulus-induced autophagy have drastically reduced treadmill-running capacity, suggesting that autophagy is important for endurance capacity. On the other hand, inappropriate autophagy in muscle can be pathological, as seen after mice were treated with doxorubicin, and in this case, exercise is protective. The interplay between exercise and autophagy in muscle is thus complex. Mitochondria are not the only organelles that respond to exercise. Exercise, for example, also activates the unfolded protein response in the sarcoplasmic reticulum. Mice that lack activating transcription factor 6, a key regulator of the unfolded protein response, exhibit severe exercise intolerance. In sum, burgeoning data with novel mouse models suggest that exercise helps to maintain homeostasis of multiple organelles in skeletal muscle, with likely important implications for the health of the whole organism.

Exercise also promotes neovascularization in skeletal muscle. Exercise-induced angiogenesis is a physiological process, in contrast to most postdevelopmental angiogenesis (eg, neoplasms, retinal diseases). Exercise also remains the most efficacious intervention for peripheral artery disease. Understanding exercise-induced angiogenesis could thus inform novel approaches for the treatment of peripheral artery disease. Until recently, the prevailing paradigm had been that metabolic demands created by exercising muscle cause a supply/demand mismatch, leading to activation of ischemic sensors like AMPK and HIF-1α. Recent data with genetically modified mice, however, do not support this notion. Mice lacking AMPK activity in skeletal muscle have intact exercise-induced angiogenesis, and mice lacking HIF-1α in skeletal muscle have more, rather than fewer, microvessels. On the other hand, mice lacking PGC-1α in skeletal muscle have less, rather than more, angiogenesis. Although, interestingly, not for exercise-induced mitochondrial biogenesis. Conversely, mice transgenically overexpressing PGC-1 in skeletal muscle have dramatic increases in microvascular density and are protected in a hind-limb ischemia model of peripheral artery disease. β-Adrenergic and other signals induce PGC-1α in skeletal muscle, and PGC-1α directly activates the expression of vascular endothelial growth factor, a canonical angiogenic factor, in an HIF-independent fashion. Physiological angiogenesis in muscle is thus likely triggered more preemptively by nerve activity than reactively by ischemia.

Stepping Outside the Muscle: Is Skeletal Muscle an Endocrine Organ?

Experiments over the last decade have made it increasingly clear that, in response to exercise, muscle secretes into the circulation sometimes copious amounts of factors, now known as myokines. In retrospect, this is perhaps not surprising. Muscle is the largest organ in the body and can command up to 80% of cardiac output during exercise. Muscle is thus ideally suited to disseminate blood-borne substances in response to exercise.

Interleukin (IL)-6 was one of the earliest discovered myokines and remains one of the most studied (Figure 5). Muscle contraction and exercise increase the expression of IL-6 in muscle and levels of IL-6 in the circulation by as much as 100-fold. IL-6 increases glucose uptake and fatty acid oxidation in muscle in an autocrine and paracrine fashion, thus facilitating fuel consumption locally. At the same time, in an endocrine fashion, IL-6 stimulates lipolysis in adipose tissue and gluconeogenesis in the liver, both of which increase fuel delivery back to the muscle. Recently, IL-6 secreted by muscle has also been shown to cross-talk with the gut and pancreas to regulate insulin homeostasis. IL-6 stimulates the secretion of glucagon-like peptide-1 (GLP-1) in intestinal L cells and pancreatic α cells. GLP-1, in turn, potentiates insulin secretion from pancreatic β cells. IL-6−/− mice fail to induce GLP-1 during exercise and develop mature-onset obesity and glucose intolerance. Conversely, exogenous IL-6 improves insulin sensitivity, but not in GLP-1−/− mice. This IL-6/GLP-1 axis thus likely mediates the enhanced insulin action seen in the immediate postexercise period. Interestingly, GLP-1 may also contribute to cognitive effects of exercise. GLP-1 knockout mice have learning deficits, whereas GLP-1 agonists improve memory tasks in mice (see below for a discussion of the cognitive effects of exercise). In sum, muscle-derived IL-6 appears to help integrate the entire organism response to the needs of exercising muscle (Figure 5). Other interleukins and traditional inflammatory cytokines, including IL-8 and IL-15, also likely double as exercise-induced myokines, although their functions are less clear.

Irisin is a different potential myokine that has received much attention recently. Irisin is a soluble cleavage product of fibronectin type III domain containing 5, a transmembrane protein found in myocytes. Release of irisin, acting via an
as-yet unknown receptor, “browns” nearby white fat cells that are interdigitated between muscle fibers. Uncoupling protein 1, which is an uncoupler, and other markers of brown fat are induced in these cells, leading to a futile cycle of uncoupled mitochondrial respiration and dissipation of heat. Paracrine release of irisin thus promotes the consumption of fuels. Consistent with this, exogenous administration of irisin ameliorates diet-induced obesity in mice. Circulating levels of irisin correlate with endurance capacity and increase during exercise (although some controversy exists on this point), but the endocrine effects of irisin remain uncertain. It is also unclear, but interesting, why exercise should promote waste-
age of fuel in adjacent brown fat cells when energy efficiency would seem preferable during exercise. One possibility is that irisin is in fact primarily part of a shivering program to produce heat rather than an exercising program. Interestingly, irisin is also induced by myostatin, another molecule secreted from myocytes. Myostatin inhibits muscle growth and has received wide attention as a potential target to preserve muscle mass. Deletion of myostatin in mice leads to secretion of irisin and browning of white fat.

Numerous other myokines exist that can have profound effects on systemic metabolism. Fibroblast growth factor-21 is secreted from muscle in response to mitochondrial dysfunction or anabolic signals and protects against obesity and diabetes mellitus, likely in part by inducing a hypermetabolic state. Myonectin secretion increases in response to numerous metabolic perturbations, including exercise, and modulates systemic handling of fatty acids. However, other myokines like vascular endothelial growth factor and other angiogenic factors (see above) may function primarily as paracrine factors. Exercise, for example, liberates membrane-bound neuroregulin, freeing it to both stabilize neuromuscular junctions and promote glucose uptake in myocytes. Similarly, the neurotrophin brain-derived neurotrophic factor (BDNF) and the interleukin-like leukemia-inhibitory factor are induced by exercise and may promote fatty acid oxidation in muscle. BDNF may additionally contribute to muscle-brain cross-talk in exercise (see below).

Signals emanating from muscle also need not be polypeptides. Exercise significantly alters circulating levels of numerous metabolites, including, for example, lactate. Such metabolites can alter systemic metabolism in important ways. A classic example is the Cori cycle, in which lactate secreted from exercising muscle is recycled by the liver to glucose that is then returned as fuel to muscle. The recent advent of powerful techniques for high-throughput measurements of metabolites (metabolomics) is significantly expanding the list of known metabolites altered by exercise. We are only beginning to understand their role, if any, in organ interdependence. Many metabolites can affect cellular signaling directly, often via ligand-specific G-protein–coupled receptors. Examples include succinate and G-protein–coupled receptor-91 and the recognition of lactate by G-protein–coupled receptor-81 and other G-protein–coupled receptor/metabolite combinations are likely to be discovered. It is likely that at least some of these metabolites serve as myokine-like signaling molecules. This aspect of exercise signaling is in its infancy and especially promising.

In summary, exercise (and other metabolic stimuli) triggers from muscle the secretion of proteins and small molecules that integrate systemic adaptations to the demands of exercise. The study of these factors is still in an early discovery phase. Skeletal muscle may well be a large, underappreciated endocrine organ.

**Exercise and the Heart**

The effects of exercise on the heart have been reviewed recently in *Circulation* and are covered only briefly here. The adult human heart retains considerable plasticity. This is most evident in the maladaptive cardiac remodeling that often follows myocardial infarction, chronic hypertension, and other cardiac insults. Efforts to impede this pathophysiological remodeling, via blockade of the adrenergic and angiotensin systems, form the mainstay of current heart failure management. Despite these treatments, however, the 5-year mortality of patients with heart failure remains >30%. New and qualitatively different therapeutic approaches are clearly needed.

Endurance exercise can induce cardiac mass by as much as 20%. This physiological remodeling differs significantly from the pathophysiological remodeling noted above. In model organisms, exercise similarly induces cardiac hypertrophy and improves outcomes after experimental myocardial infarct and other cardiac insults. Understanding these physiological remodeling pathways may thus afford therapeutic opportunities that differ from the existing paradigm of blocking neurohormonal activation. Many effects of exercise on the heart are indirect, including reductions in body mass index and improvements in insulin sensitivity. Other effects, however, are clearly direct. Insulin-like growth factor-1 engages the insulin-like growth factor-1 receptor on cardiomyocytes and activates the intracellular PI3K/Akt pathway. This leads to inhibition of apoptosis, improvements in metabolism and calcium handling, and activation of the mammalian target or rapamycin–dependent hypertrophic pathway. As with skeletal muscle, concomitant adrenergic input is likely also critical for exercise-induced cardiac adaptations, in this case, via β3 receptors and increased nitric oxide bioavailability.

Other prophysiological hypertrophy mechanisms likely exist.

One of the exciting developments in cardiac research in the last decade has been the realization that the adult heart harbors at least the potential for endogenous regeneration. Adult newts and zebrafish and newborn mice can regenerate seemingly normal hearts after apical resection. In humans, calculations based on the incorporation of ambient radioactivity generated in the 1950s by above-ground testing of nuclear bombs have conclusively demonstrated that human cardiomyocytes can turn over, albeit slowly. It remains controversial whether this turnover stems mostly from the replication of existing cardiomyocytes or from resident or circulating stem cells. In either case, data are emerging to suggest that exercise may activate this process. Endurance exercise in rodents induces measurable replication of cardiomyocytes.

Transcriptional profiling of exercising rodent hearts revealed that endurance exercise represses the expression of the transcription factor CCAT-enhancer binding protein. Haploinsufficiency of CCAT-enhancer binding protein in mice led to physiological cardiac hypertrophy and cardiomyocyte proliferation, thus...
mimicking some effects of exercise on the heart. Akt inhibits CCAT-enhancer binding protein and may thus promote cardiomyocyte proliferation. Other Akt-dependent pathways likely exist. The mechanisms underlying these observations are being studied intensively.

A long-standing and often controversial debate exists over the ideal amount of exercise needed for cardiac protection and the possibility that too much exercise may have ill effects. A link between strenuous exercise and sudden death is well established but can be explained only partly by the well-known high prevalence of idiopathic hypertrophic cardiomyopathy in this population. Strenuous exercise may cause disproportionate adaptations in the right ventricle, which have been postulated to predispose to arrhythmias, most commonly atrial fibrillation. Twelve weeks of aggressive, exhaustive exercise training in rats led to right ventricular enlargement, diastolic dysfunction, fibrosis, and increased susceptibility to triggered VT. The changes were likely caused by pathological angiotensin II activation because angiotensin-converting enzymes inhibitors reversed the phenotype, but the mechanisms of cardiotoxicity by strenuous exercise remain unclear. These studies highlight a number of important issues: (1) The effects of exercise differ, depending on exercise type, intensity, or frequency; (2) the appropriate “dose” of endurance exercise in humans is likely variable because interindividual responses to exercise vary widely; (3) the dose that maximally confers cardiovascular protection likely differs from that which maximally confers cardiovascular fitness; (4) rodents provide powerful tools with which to probe the molecular mechanisms of exercise adaptations, but they are poor models to ascertain the optimal dose of exercise in humans because specific rodent and human exercise regimens are difficult to compare (ie, poor scalability); and (5) the Hippocratic instruction of tempered exercise likely holds true today as it did 2500 years ago.

**Exercise and the Brain: Active Body and Mind**

There is little doubt that exercise improves mental health. Physical activity correlates well with mental well-being, especially in old age. Exercise significantly counteracts at least moderate depression and can prevent loss of memory. Indeed, mental well-being after myocardial infarction is a strong predictor of outcomes, and improvements in mental health may well explain a significant part of why physical activity is so beneficial to cardiovascular health.

Early and exciting inroads are being made into understanding the molecular mechanisms that underlie these neuronal benefits of exercise. One focus has been on a small roster of secreted neurotrophic factors, including BDNF and VGF nerve growth inducible. BDNF is a well-studied neurotrophin thought to be critical for learning and memory. Exercise stimulates BDNF expression in the hippocampus, the seat of learning, and produces antidepressant effects. Interestingly, transcriptional profiles of rodent hippocampus after exercise and after treatment with serotonergic antidepressants yield similar results, suggesting similar mechanisms of action. The presence of the peptide VGF increases in the hippocampus during exercise, and intracerebroventricular infusions of VGF produce antidepressant-like effects in mouse and rat behavioral models. Conversely, antidepressant responsiveness to exercise in the same models was blocked in heterozygous VGF−/− mice. VGF is regulated by BDNF, the expression of which is also low in depression. Like BDNF, VGF likely modulates synaptic plasticity in response to exercise. Other factors exist to mediate the antidepressant effects of exercise. The macrophage migration inhibitory factor (MIF), for example, likely contributes to activation of BDNF during exercise, and MIF−/− mice have a blunted antidepressant response to exercise. Together, these small neurotrophic factors thus appear to mediate important beneficial neurological changes in response to exercise.

The notion that circulating factors can affect neurogenesis and cognitive function was also recently demonstrated elegantly in mice by the use of heterochronic parabiosis, that is, the surgical creation of adult artificially conjoined twins that share blood-borne factors. Young-old parabiotic pairs were generated, and the young animals in these pairs displayed impaired learning and memory and decreased synaptic plasticity, suggesting the existence of blood-borne factors derived from the older animal in these pairs that impair cognitive function. The authors identify 1 such factor, eotaxin (also known as chemokine, cc motif ligand 11), and show that artificially increasing peripheral eotaxin levels in young mice impairs learning and memory. Interestingly, eotaxin levels are decreased by exercise.

The neurological benefits of exercise are not limited to depression and memory. Exercise also benefits neurodegenerative illnesses like Parkinson disease and Alzheimer disease, and this can be recapitated in rodent models. Mice lacking 1 copy of BDNF fail to benefit from exercise, again implicating this factor in exercise-induced neuroplasticity. A genetic model of spinocerebellar ataxia, caused by a polyglutamine expansion in the ataxin-1 protein, is also markedly improved by exercise. Again, a neurotrophic factor induced by exercise is implicated: epidermal growth factor. A downstream effector of epidermal growth factor, capicua (Cic), interacts directly with ataxin and is strongly inhibited by exercise. Genetic reduction of Cic in Cic−/− heterozygote mice rescues multiple abnormal phenotypes in the spinocerebellar ataxia mouse model, thus mimicking the effects of exercise. Finally, although great inroads have been made in recent years in the understanding of neural networks that regulate appetite for food, much less has been done to ask the same...
question of exercise: What regulates appetite for exercise? Different rodent strains have different appetites for voluntary exercise, and rodents can be selectively bred for high versus low voluntary physical activity, indicating the existence of a strong genetic component.\textsuperscript{134,135} Genetic quantitative trait loci that track with these differences can in fact be mapped,\textsuperscript{136} but their function remains unknown. Importantly, subsequent studies reveal few differences in skeletal muscle composition between these selected mouse cohorts,\textsuperscript{137,138} indicating that central networks likely regulate motivation for exercise. Initial experiments, mostly pharmacological, implicate the dopaminergic system,\textsuperscript{139} underscoring links to movement disorders like Parkinson disease. Definitive genetic experiments in mice, akin to those performed to evaluate the neurobiology of food satiety, have not yet been reported.

Spontaneous physical activity (eg, fidgeting, pacing, and activities of daily living) differs from voluntary exercise (eg, sports) but may have equal health benefits. The hypothalamic neuropeptide orexin has been strongly implicated in the regulation of spontaneous physical activity.\textsuperscript{140} Orexinogenic neurons impinge on dopaminergic neurons, and the injection of orexin into the lateral hypothalamus of murine brains stimulates spontaneous activity. Conversely, ablation of orexinergic regions of the thalamus, or genetic deletion of orexin, reduces spontaneous physical activity.\textsuperscript{141} Orexin is also implicated in the regulation of food satiety, underscoring the strong overlap between the appetites for food and exercise.\textsuperscript{140} Complex interplay also exists with mood and reward circuitry and with circadian rhythms. This exciting avenue of research is still in its early phase.

In summary, complex mechanisms underpin the dramatic cognitive and neural benefits of exercise. A number of exercise-induced neurotrophic factors are providing the initial molecular clues to decipher these mechanisms.

**Exercise and Aging**

Exercise capacity predicts longevity,\textsuperscript{142} and physical fitness is one of the best predictors of health in elderly individuals.\textsuperscript{143} For instance, a study of 538 aged runners and 423 age-matched control subjects found that only 15% of the runners died over 21 years compared with 34% of the control group.\textsuperscript{144} Controlled experiments to prove causality are of course difficult in humans, but animal experiments also have strongly supported the conclusion that exercise prolongs life. Forcing rodents to exercise prolongs their average longevity and improves their aged health status.\textsuperscript{145,146} Evolution-in-the-laboratory experiments, in which rodents with higher exercise capacity were selected and interbred over numerous generations, revealed that longevity was coselected with exercise capacity.\textsuperscript{134} Exercise is thus linked to long life in both animal models and humans.

How does exercise prolong life? Does it do so by individually averting each of the illnesses of aging, or does it have a more general and fundamental antiaging power? This question is difficult to answer in part because the process of aging remains poorly understood. A number of theories of aging exist. The mitochondrial theory of aging, borne of the earlier free radical theory of aging proposed in 1956 by Denham Harman,\textsuperscript{147} posits that unstable free radicals produced by mitochondria are an obligatory byproduct of aerobic life. These radicals damage biomolecules, especially mtDNA, leading to progressive mitochondrial dysfunction, worsening free radical generation, and thus a vicious cycle of oxidative damage that ultimately limits mammalian life span.\textsuperscript{148–153} Accumulation of oxidative damage to DNA, proteins, and membranes can be seen in aged tissues across phylogeny, including humans. Recent experiments in genetically modified mice have supported the mitochondrial/free radical theory of aging. Transgenic overexpression of antioxidant enzymes increases the life span in mice.\textsuperscript{154–156} Conversely, mice that harbor an error-prone mitochondrial DNA polymerase rapidly accumulate mtDNA mutations and demonstrate multiple aspects of aging, including graying fur, kyphosis, degenerative diseases, and premature death.\textsuperscript{157,158}

Exercise appears to favorably affect these processes, but the details of how this occurs remain murky. Exercise reduces oxidative damage. Strikingly, endurance exercise reverses nearly all of the aging phenotypes in the “mutator” mice described above, including their multisystem pathology and premature mortality; the mice are nearly indistinguishable from control mice.\textsuperscript{159} Even more strikingly, exercise prevents the accumulation of mtDNA mutations in these mutant mice.\textsuperscript{129} Exercise thus somehow activates systemic mechanisms of mitochondrial quality control, even outside skeletal muscle. There is indeed growing appreciation that exercise induces mitochondrial adaptations in tissues beyond skeletal muscle,\textsuperscript{145–149,160–163} and as noted above, exercise also systemically activates autophagy/mitophagy.

PGC-1α and its powerful regulation by exercise may again play a central role in these processes. PGC-1α simultaneously induces mitochondrial biogenesis and anti–reactive oxygen species programs.\textsuperscript{164} Mice that lack PGC-1α accumulate reactive oxygen species–mediated damage and display dramatically accelerated neurodegeneration.\textsuperscript{164} Strikingly, transgenic mice with boosted PGC-1α expression in skeletal muscle have increased longevity and are healthier in old age.\textsuperscript{165} As noted above, these transgenic mice recapitulate many aspects of endurance exercise adaptations, but they are not more active at baseline. Boosting PGC-1α only in skeletal muscle is thus sufficient to prolong life in rodents. The same approach does not rescue premature aging in mtDNA mutator mice, which suggests either a threshold effect or more unappreciated complexity.\textsuperscript{165} Overall, these observations again point to the powerful influence of muscle on the rest of the body and suggest the existence of organ cross-talk via myokines. The existence of circulating “rejuvenating” factors has been supported by parabiosis experiments (see above for a description of the approach). Old animals in young-old heterochronic parabiotic pairs reacquire numerous attributes of youth, including tissue and stem cell regenerative potential.\textsuperscript{157,166}

Telomeres are nucleoprotein complexes at chromosome ends that preserve chromosomal integrity.\textsuperscript{169,170} The telomere theory of aging holds that cell divisions cause progressive telomere attrition, culminating in activation of p53, the “guardian of the genome” and leading to cellular apoptosis and senescence.\textsuperscript{171} Stem cells, which are hyperproliferative, are especially prone to telomere erosion. Loss of resident stem cells impairs turnover and the repair capacity of tissues and...
slowly leads to tissue degeneration. Short telomeres in human peripheral blood leukocytes correlate with higher mortality rates in individuals >60 years of age, and telomere length predicts good health and longevity in centenarians and their offspring. Telomeres are kept from eroding by the enzyme telomerase. Mice with dysfunctional telomerase develop numerous accelerated aging phenotypes, supporting the telomere theory of aging. Similarly, mice engineered with hyperactive p53 alleles also display premature aging. Exercise appears to favorably affect these processes as well, but again the mechanisms are not clear. Exercise activates telomerase and inhibits telomere erosion in circulating cells in mice and humans. Recent data have also indicated that telomere shortening and p53 activation lead to repression of PGC-1α and mitochondrial capacity. This observation links theories of aging and suggests that activation of PGC-1α by exercise may thus interfere with aging at this level also.

In summary, exercise undeniably attenuates the aging process. Early indications are that common, central mechanisms underlie aging in differing tissues and that exercise may target these key pathways. Longevity and physical inactivity are both on the rise in the industrial world. Studies probing into the mechanisms by which exercise influences aging thus represent one of the most exciting frontiers of exercise research.

Exercise in a Pill: Is It a Dream?

Finding a “gymnomimetic” pill that fully recapitulates exercise is likely an impossible dream. The sheer complexity and pleiotropic effects of exercise almost certainly preclude such a simple approach. Nevertheless, understanding the numerous molecular pathways modulated by exercise may enlighten approaches to mimic individual effects of exercise. Pharmacological activation of AMPK or sirtuin in mice, for example, recapitulates certain aspects of exercise, including increases in exercise capacity. Activating individual effects of exercise could, for example, serve as adjunct treatment to exercise programs.

The challenges to find even partial exercise mimetics, however, are many. First, the number of potential targets identified so far remains small. Thus, efforts to understand the molecular underpinnings of exercise should continue. Second, most of these targets such as transcription factors or coactivators are not easily “druggable.” In this light, recent developments with myokines are particularly exciting because circulating and extracellular factors provide more readily accessible pharmacological targets. Early developments with miRNAs also hold promise in this context because miRNAs are proving to be readily targeted in humans with antagonir and miRNA analogs. Third, because gymnomimetics would likely be used for prevention or long-term treatment, the safety profiles would need to be nearly perfect. This is a serious problem because exercise by its nature has pleiotropic effects, which are generally not ideal for a pharmaceutical agent. In this last context, initial proof-of-concept therapeutic efforts may have more success by targeting patient populations with few other options because such patients would more likely be willing to accept adverse side effects. For example, genetic induction of PGC-1α in muscle in mice reduces degeneration in a murine model of Duchenne muscle dystrophy. Finally, the gymnomimetic field is also muddled by contrasting goals. Whereas many look to exercise for mechanistic insight into disease prevention, many in the sporting arena seek performance enhancement.

In short, although the physiology of exercise has been investigated for centuries, the molecular biology of exercise remains a young science. The translation of molecular findings to therapeutics so far remains more of a promise than a reality.

Conclusions

Interest in exercise predates written history. This interest has been brought to the forefront of modern medical attention by rising physical inactivity and the attendant ill effects on population health. Over the past 10 years, new molecular and genetic tools have allowed probing into the molecular mechanisms of responses to physical activity. A large part of this work has relied on access to genetically modified mice for 2 reasons: Genetic modifications provide precise experimental scalpels with which to probe complex pathways, and rodents provide a tractable and at least partly faithful model of the complexities of exercise in humans. A rising theme from this body of work has been that exercise activates systemic intercellular and interorgan communication via the secretion of factors from skeletal muscle and elsewhere. Much of the health benefits of exercise likely depend on these messengers. The potential good news is that circulating factors are often tractable therapeutic targets.

These advances, however, represent only the first steps of the marathon. Numerous critical questions remain unanswered, including the precise molecular causes of fatigue, the detailed mechanisms of muscle adaptations, how the will to exercise is conjured, and the identity and function of the panoply of myokines and agents of organ cross-talk. Exercise is perhaps the most potent intervention in our arsenal to prevent cardiovascular disease. We are running ahead in the race to understand the complexity of exercise. Using that knowledge to derive novel interventions for cardiovascular disease would represent a formidable finish line.

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Disclosures

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The free radical theory of aging.


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