Exercise: it's the real thing!

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The epidemic emergence of modern chronic diseases largely stems from the adoption of a sedentary lifestyle and excess energy intake. However, it has long been known that regular physical activity induces multiple adaptations within skeletal muscle and other organs and these adaptations have positive outcomes for the prevention and treatment of many metabolic disorders. In recognition of such benefits, a recent goal of industry-funded research is to discover orally active compounds that mimic the effects of exercise training, so-called "exercise pills". This article provides an overview of the role of skeletal muscle in health and disease and discusses whether "exercise mimetics" have any potential to combat metabolic diseases.

INTRODUCTION

There has been a recent explosion in the prevalence of a number of chronic metabolic disorders including obesity, type 2 diabetes, and cardiovascular disease. The proliferation in the rate of diagnosis of these and other diseases stems from the readiness of industrialized nations to adopt a sedentary lifestyle in the face of excess energy intake. While more than a half century of evidence from epidemiological, experimental, and clinical trials pinpoints a positive correlation between dietary intake (i.e., increased fat consumption) and disease risk, only recently has it become recognized that a physically inactive lifestyle initiates maladaptations that cause chronic disease.[1–3] In the United States at least 250,000 deaths each year are premature due to physical inactivity, while epidemiological data have established that a lack of sufficient physical activity increases the incidence of at least 17 unhealthy conditions, almost all of which are chronic diseases or are considered risk factors for chronic diseases.[4] The Centers for Disease Control states that physical inactivity is an actual cause of many chronic diseases.[5] Indeed, the extrapolation of the increased prevalence of chronic diseases by physical inactivity to approximate the number of deaths provides the estimate that 13% of all deaths in the United States are premature due to physical inactivity.[2]

While physical inactivity has emerged as a major risk factor implicated in many "lifestyle" disorders, it has long been known that regular physical activity (i.e., exercise training), in addition to preventing obesity, induces a multitude of favorable adaptations within skeletal muscle and the cardio-respiratory system, which have positive outcomes for both the prevention and treatment of almost all metabolic disease states.[6–8] However, between 50% and 70% of American adults do not get enough physical activity to provide health benefits and 25% of adults are not active at all in their leisure time.[5] Indeed, most individuals in industrialized nations have chosen to ignore the minimum physical activity guidelines recommended by health organizations such as the American College of Sports Medicine[9] and will suffer the consequences.

Recognizing the proven benefits of exercise training on health outcomes and the trend towards increasing inactivity at the population level, several pharmaceutical companies have funded research in an effort to discover orally active compounds that mimic or potentiate the effects of exercise training, so-called "exercise pills". 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; it is equally attractive for big pharmaceutical companies that view a

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potentially huge market and profit numbers. This commentary provides a brief overview of the role of skeletal muscle in health and disease and evaluates whether so-called "exercise mimetics" have any potential to combat metabolic diseases.

SKELETAL MUSCLE IN HEALTH AND DISEASE

Skeletal muscle comprises about 55% of body mass and plays a fundamental role in whole-body energy metabolism and substrate turnover. In healthy individuals, skeletal muscle accounts for approximately 80% of whole-body insulin-stimulated glucose uptake, but in normal-weight subjects with insulin resistance, total body glucose metabolism is reduced by approximately 40%.[10] These results highlight the importance of the peripheral tissues in the disposal of glucose and indicate that skeletal muscle is the most important site of the insulin resistance observed in states such as obesity and type 2 diabetes. Skeletal muscle demonstrates a remarkable plasticity, adapting to a variety of external stressors such as habitual level of contractile activity and substrate availability. Thus, knowledge of the molecular and cellular events that regulate skeletal muscle plasticity can define the potential for adaptation in metabolism and may lead to the discovery of novel pathways in common clinical disease states.

The application of surgical techniques to exercise biochemistry in the late 1960s[11] made it possible to obtain biopsy samples (~150 mg) of human skeletal muscle and by means of histological and biochemical analyses, specific morphological, contractile, and metabolic properties of this tissue were identified. Skeletal muscle was found to contain multiple myofibers differing in their metabolic and contractile properties. These muscle fibers were broadly classified as slow-twitch (ST) and fast-twitch (FT). In humans, a further subdivision of the FT fibers is made, whereby the more aerobic (or oxidative) FT fiber is designated FT_a and the more anaerobic (glycolytic) fiber is termed FT_b. In sedentary individuals, the proportion of ST fibers in the *vastus lateralis* muscle (the largest of the quadriceps muscles and the most commonly studied muscle in humans) is typically around 55%, with FT_a fibers being twice as common as FT_b fibers.[12] While marked differences in the metabolic potentials between FT_a and FT_b fibers are observed in untrained humans, the absolute level for the activities of oxidative and glycolytic enzymes in all fiber types is large enough to accommodate substantial aerobic and anaerobic metabolism.[12] Comprehensive reviews of the morphological and metabolic profile of human skeletal muscle can be found elsewhere.[12,13]

There is a close coupling between muscle fiber type and associated morphological, metabolic, and functional properties. For example, the muscles of endurance athletes possess an abundance of ST oxidative fibers, whereas the muscles of sprinters contain a greater proportion of FT glycolytic fibers.[14] Whole-body insulin sensitivity also correlates with the proportion of ST oxidative fibers.[15] Specifically, insulin-stimulated glucose transport is greater in skeletal muscle enriched with ST fibers,[16–18] thus priming ST muscle for accelerated glucose uptake and oxidative metabolism. A shift in fiber distribution from FT_b glycolytic to FT_a oxidative fibers gives rise to altered activities of key oxidative and glycolytic enzymes.[19] Indeed, the ratio between glycolytic and oxidative enzyme activities in the skeletal muscle of non-insulin-dependent diabetic or obese individuals is related to insulin resistance.[20,21] Similarly, with ageing and physical inactivity, two other conditions associated with ST-to-FT fiber-type transformation, oxidative capacity and insulin sensitivity are diminished.[22]

EXERCISE INDUCES AN INCREASE IN MUSCLE MITOCHONDRIA

Endurance exercise induces an increase in muscle mitochondria.[23,24] A single bout of exercise stimulates mitochondrial biogenesis, as evidenced by increases in the expression of mitochondrial proteins.[25–27] Repeated bouts of exercise (i.e., training), maintain this effect. Training improves exercise capacity and endurance, making it possible to exercise at higher intensities for longer time periods. This increase in the exercise stimulus results in a greater increase in mitochondrial biogenesis. As a result, a progressive exercise training program results in a progressive increase in muscle mitochondria up to the point where a further increase in training stimulus causes no further increase in mitochondria. The mechanism by which an increase in muscle mitochondria increases exercise capacity and endurance is by reducing the disturbance in metabolic homeostasis during submaximal exercise. This is evidenced by smaller decreases in ATP, creatine phosphate, and glycogen, and smaller increases in AMP, inorganic phosphate, and lactate at a given submaximal exercise intensity.[28]

In contrast to the well-documented effect of exercise training on mitochondrial biogenesis, impaired mitochondrial function has been linked to several metabolic disorders including skeletal muscle insulin resistance and type 2 diabetes.[29,30] One hypothesis is that "mitochondrial dysfunction" leads to a reduction in the volume of lipids targeted for oxidation, thereby promoting the accumulation of fatty acids and their metabolites in skeletal muscle.[29] Kelley et al.[31] were the first to report that the muscles of type 2 diabetic patients contain less mitochondria than those of age-matched insulin-sensitive individuals, findings that have since been extended to obese insulin-resistant individuals and to insulin-resistant off-

spring of diabetic patients.[32,33] However, whilst insulin-resistant individuals typically have 30% less mitochondria in their muscle than normal, their capacity for aerobic metabolism is well within the normal range.[34] The notion that skeletal muscle "mitochondrial deficiency" does not mediate insulin resistance has been discussed elsewhere.[35,36]

MOLECULAR MECHANISMS OF SKELETAL MUSCLE MITOCHONDRIAL BIOGENESIS

Transcription factors regulating expression of mitochondrial proteins

Recent advances in molecular biology and in understanding of the mechanisms that regulate mitochondrial biogenesis have made it possible to elucidate how exercise stimulates mitochondrial biogenesis. The initial breakthrough in elucidating how mitochondrial biogenesis is regulated was the discovery of the transcription factors that regulate expression of the nuclear genes that encode mitochondrial proteins.[37] These include nuclear-respiratory factor 1 (NRF-1) and nuclear-respiratory factor 2 (NRF-2), which bind to the promoters and activate transcription of the genes that encode mitochondrial respiratory chain proteins.[38] NRF-1 also activates expression of the nuclear gene that encodes mitochondrial transcription factor A (TFAM), which moves to the mitochondria where it regulates transcription of the mitochondrial DNA, i.e., the mitochondrial genome. Other transcription factors involved in regulating expression of mitochondrial proteins include estrogen receptor-related receptors α and δ, and the peroxisome proliferator-activated receptors (PPARs), which regulate expression of the mitochondrial fatty acid oxidative enzymes.[37,38]

p38 Mitogen-activated protein kinase activation of peroxisome proliferator-activated receptor-gamma coactivator

The second major breakthrough was the discovery of an inducible coactivator, the peroxisome proliferator-activated receptor-gamma coactivator (PGC-1α), which docks on and activates these transcription factors and, thus, activates and regulates the coordinated expression of mitochondrial proteins encoded in the nuclear and mitochondrial genomes.[39] Overexpression of PGC-1α in muscle results in a large increase in functional mitochondria.[40,41] A single bout of exercise induces a rapid increase in PGC-1α in skeletal muscle.[25,42,43] The initial phase of the increase in mitochondrial biogenesis induced by exercise appears to be mediated by activation of PGC-1α, while the second phase is mediated by the increase in PGC-1α protein.[27]

The p38 mitogen-activated protein kinase (p38 MAPK) phosphorylates and activates PGC-1α.[44,45] p38 MAPK also increases PGC-1α expression by phosphorylating the transcription factor ATF-2, which increases PGC-1 protein expression by binding to and activating the CREB site on the PGC-1α promoter.[46,47] Exercise results in rapid activation of p38 MAPK, which mediates both the activation and increased expression of PGC-1α.[46]

Exercise-induced signaling pathways in muscle mitochondrial biogenesis

Calcium and calcium calmodulin dependent protein kinase II. Exercise causes numerous disturbances of cellular homeostasis in muscle,[48,49] making it impossible to use contracting muscles to determine which of the signals generated in muscle during exercise causes the increase in mitochondria. Models that have been used to study individual signals and signaling pathways that lead to increased mitochondrial biogenesis are myotubes in culture, the very thin rat epitrochlearis muscle in vitro, and "exercise mimetics" in rodents. Two signals have been identified. One is the increase in cytosolic Ca^{2+} that occurs when Ca^{2+} is released from the sarcoplasmic reticulum during excitation-contraction coupling. Raising Ca^{2+} in myotubes in culture by exposing them to Ca^{2+} ionophores or caffeine induces an increase in mitochondrial biogenesis that is mediated by an increase in PGC-1α.[50,51] In studies on rat epitrochlearis muscles *in vitro*, it was possible to raise cytosolic Ca^{2+} sufficiently to stimulate mitochondrial biogenesis, but too low to cause contraction. Using this model, it was found that Ca^{2+} activates calcium calmodulin dependent protein kinase II "(CAMKII)", which is the first step in a pathway leading to p38 MAPK activation. Phosphorylation of ATF-2 by p38 MAPK induces an increase in PGC-1α expression, resulting in increased mitochondrial biogenesis.[52]

Adenosine monophosphate (AMP) and AMP-activated protein kinase. The other exercise-induced signal that leads to increased mitochondrial biogenesis is the increase in AMP concentration in muscle during exercise, which results in activation of the enzyme AMP-activated protein kinase (AMPK). AMPK functions as a metabolic "fuel gauge" in skeletal muscle because when it becomes activated in response to decreased energy levels (i.e., muscle contraction), it inhibits ATP-consuming pathways and activates pathways involved in carbohydrate and fatty acid catabolism to restore ATP levels.[53] AMPK promotes FA oxidation in skeletal muscle during exercise by inhibiting acetyl-CoA carboxylase (ACC-β) and activation of malonyl-CoA, thus removing inhibition of mitochondrial fatty acyl-CoA translocation by carnitine palmitoyltransferase-1 (CPT-1). Numerous studies have

reported that these exercise-induced effects on ACC-β and malonyl-CoA are closely paralleled by activation of AMPK.[54,55]

EXERCISE MIMETICS AND MITOCHONDRIAL BIOGENESIS

5-Aminoimidazole-4-carboxamide-1-beta-D-ribofuranoside

It is possible to mimic an exercise effect by exposing muscles to the chemical, 5-aminoimidazole-4-carboxamide-1-beta-D-ribofuranoside (AICAR), which is taken up by the muscle cells and converted to the AMP analog 5-aminoimidazole-4-carboxamide ribonucleotide. Almost a decade ago it was shown that incubation of rodent muscle with AICAR for 18 h resulted in significant increases in GLUT-4 and hexokinase activity.[56] Injecting rats with AICAR for five successive days also resulted in significant increases in GLUT-4 protein, hexokinase activity and resting glycogen content: these adaptations were similar to those observed after a few days of endurance exercise training.[57] It was also shown that injecting rats with AICAR for one month results in an increase in muscle mitochondria.[58] Jorgensen et al.[59] found that this effect is mediated by a subunit of AMPK, the α2 isoform. The mechanism by which activation of AMPK induces increased mitochondrial biogenesis is explained by the recent finding that AMPK directly phosphorylates and activates PGC-1α.[60]

Peroxisome proliferator activator δ

Activation or overexpression of the transcription factor peroxisome proliferator activator δ (PPARd) in muscle also results in an increase in mitochondrial biogenesis.[61] This increase was attributed to a direct effect of PPARd, because activation or overexpession of PPARd did not result in an increase in PGC-1α mRNA.[61] It does not seem possible that PPARd could directly induce mitochondrial biogenesis because it regulates expression of only a subset of mitochondrial proteins, including those involved in fatty acid oxidation. Biogenesis of functional mitochondria requires the coordinated expression of genes encoded in both the nuclear and mitochondrial genomes. This process requires activation and/or increased expression of a number of transcription factors in addition to PPARd, including NRF-1, NRF-2, the estrogen receptor-related receptors, and mitochondrial transcription factor A.[39,62] The coordinated increase in transcription of the genes encoding mitochondrial proteins is mediated by the transcription co-activator PGC-1α, which activates and/or brings about increased expression of these transcription factors. It was recently shown that activation or increased expression of PPARd induces an increase in muscle mitochondria by increasing the expression of PGC-1α protein by a post-transcriptional mechanism.[63] This increase in PGC-1α was overlooked in previous studies because of the common practice of measuring mRNA levels alone and referring to gene transcription as gene expression. This approach is incorrect, because increased gene expression and its associated phenotypic/functional manifestations does not take place until there is an increase in the concentration of the protein encoded by the gene. As gene expression can be regulated at translational and post-translational steps, the extent to which a protein will increase in response to an adaptive stimulus cannot be predicted from the increase in mRNA. This makes the measurement of protein concentrations critical when studying the adaptive responses to exercise or other stimuli.[25]

CONCLUSION

Regular, vigorous exercise has been necessary for survival throughout evolution.[1] It is only during the past 50 years that it has become possible for people to go through life with minimal physical activity. We are not genetically adapted for the sedentary lifestyle that has become so prevalent in developed nations. Lack of exercise is, therefore, abnormal and also unhealthy, leading to obesity, insulin resistance, type 2 diabetes, and increased risk of developing atherosclerosis and cancer.[1,4] 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.[64]

Because a large proportion of the population is not motivated to exercise, there is now considerable interest in identifying/developing "exercise mimetics". To quote from a recent paper by Narker et al.:[65] "Given the numerous benefits of exercise on general health, identification of orally active agents that mimic or potentiate the genetic effects of endurance exercise is a long-standing, albeit elusive, medical goal." The laboratory of the senior author of that paper had previously reported that overexpression of PPARd or activation of PPARd with an agonist (a compound named GW1516), induces an increase in mitochondria in muscle. They, therefore, evaluated GW1516 and AICAR as potential exercise mimetics in skeletal muscle in mice. In accordance with results from previous studies,[58,59] they found that treating mice with AICAR resulted in an increase in muscle mitochondria. This was associated with a 44% increase in running endurance.[65] Narker et al.[65] administered 250 mg/kg or 500 mg/kg AICAR daily by intraperitoneal injection and, as with the

previous findings from their laboratory,[61] they again found that administration of the PPARd activator GW1516 to mice resulted in increases in mitochondrial marker proteins in muscle.[65] It should be noted that Narker et al.[65] stated that AICAR is orally active. We know of no evidence for this. AICAR has a short half-life after intravenous administration and poor bioavailability after oral ingestion; in addition, it is typically accompanied by an increase in blood levels of lactic acid and uric acid, making it a poor candidate for long-term use.[66]

In their concluding remarks, Narker et al.[65] state the following: "We believe that the strategy of reorganizing the preset genetic imprint of muscle (as well as other tissues) with exercise mimetic drugs has therapeutic potential in treating certain muscle diseases such as wasting and frailty as well as obesity where exercise is known to be beneficial." These conclusions are clearly incorrect. Rather than improving muscle wasting, chronic treatment with AICAR would potentiate muscle wasting and make it worse, because AMPK activation is catabolic and results in inhibition of muscle protein synthesis.[53,67,68] Moreover, development of obesity depends on a chronic positive energy balance. Energy intake in excess of energy expenditure leads to weight gain and, eventually, obesity. Exercise prevents obesity by means of increased energy expenditure. An increase in muscle mitochondria enhances exercise capacity and endurance, making it possible to expend more total energy, or the same amount of energy in a shorter time. So, an increase in mitochondria enhances the ability to expend energy by means of exercise, and facilitates protection against obesity by means of exercise. However, an increase in mitochondria has no independent effect, i.e., in the absence of exercise, on energy expenditure. The rate of substrate oxidation in resting muscle is determined by "housekeeping" functions, such as protein synthesis, and maintenance of transmembrane potentials; it is also very low relative to the maximal potential of muscle for substrate oxidation, even in very sedentary individuals. As a result, there is no difference in resting energy utilization between the endurance-trained and -untrained states.

Direct evidence refuting a "health benefit" of an increase in muscle mitochondria independent of exercise is provided by the finding that feeding rats a high-fat diet results in the development of visceral obesity and muscle insulin resistance despite large increases in muscle mitochondria and in the ability to oxidize fat.[63,69] This increase in mitochondrial biogenesis induced by a high-fat diet is mediated by an increase in free fatty acids which, like GW1516, activate PPARd.[63,70] In order for an "exercise mimetic" to mimic the effect of exercise on obesity, it would have to result in an increase in energy expenditure. Such an effect occurs when mitochondria are uncoupled by excess thyroid hormone or by dinitrophenol. However, these "exercise mimetics" would certainly not have health benefits. Prior to reading the paper by Narker et al.,[65] we were not aware that ". . . identification of orally active agents . . . that mimic the effects of endurance exercise" is a "long-standing, albeit elusive, medical goal". We thought the goal was to find ways to motivate people to exercise and adopt healthy lifestyle choices. However, if finding orally active exercise mimetics is really a longstanding medical goal, we believe it will continue to be elusive for reasons we hope are evident from this critique.

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