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Cell Metabolism
Perspective

The Limits of Exercise Physiology: From Performance to Health

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http://dx.doi.org/10.1016/j.cmet.2017.04.018

Many of the established positive health benefits of exercise have been documented by historical discoveries in the field of exercise physiology. These investigations often assess limits: the limits of performance, or the limits of exercise-induced health benefits. Indeed, several key findings have been informed by studying highly trained athletes, in addition to healthy or unhealthy people. Recent progress has been made in regard to skeletal muscle metabolism and personalized exercise regimes. In this perspective, we review some of the historical milestones of exercise physiology, discuss how these inform contemporary knowledge, and speculate on future questions.

Introduction

Regular exercise training has widespread health benefits by positively affecting nearly all organ systems of the body. The mysteries of human physiology and the adaptive response to acute and chronic exercise training have largely been elucidated through exercise science. The field has a rich history of uncovering some of the limits of exercise performance in both health and disease. Exercise physiologists have studied physiological response to physical activity, exercise, sport, and athletic competition, whereas clinical exercise physiologists use exercise training/prescription in the prevention and rehabilitation of acute and chronic disease. For many, the notion that "exercise works" to improve functional work capacity and metabolic health is self-evident. Exercise training is a clinically proven, cost-effective, primary intervention that can delay, and in many cases prevent, the health burdens associated with metabolic disorders (Booth et al., 2012). Thus, the cynics might question, "Why do we need to know more?" Clearly the same approach could be taken to the study of obesity—we know that restricting food intake prevents obesity, so "why do we need to know more?" The reality is that metabolic diseases such as obesity and type 2 diabetes are increasing, and collectively these diseases pose a great threat to modern society. While current pharmacological treatments to combat metabolic diseases are inadequate, many clinical outcome measures induced by long-term exercise programs are of a similar or greater magnitude to those exerted by drug or insulin therapy (Snowling and Hopkins, 2006). While one can safely say exercise keeps you fit and dieting keeps you slim, there remain many unknowns in our understanding of the complex biology behind diversity in the adaptive response to these regimes among individuals and populations. Delineating the mechanism by which regular exercise training alters human physiology among individuals and differing populations will lead to the identification of molecules, pathways, and ultimately new treatments that confer the benefits of exercise to improve insulin sensitivity, preserve mitochondrial energetics, and attenuate loss of strength and power with aging. With this perspective, we will outline some of the historic discoveries in the field of exercise physiology and how early efforts and key discoveries have shaped present day research questions aimed to understand the limits of human performance. We will also discuss current efforts to elucidate the health-promoting benefits of exercise and how advances in molecular medicine may bring forward more personalized approaches to clinical exercise physiology.

Historical Milestones that Are Instrumental in Framing Contemporary Questions

The field of exercise science has evolved from observational field studies to sophisticated mechanistic studies incorporating physiology, biochemistry, and molecular biology. Some of the most pressing research questions today have their roots in key early discoveries throughout the last hundred years. We will review some of these discovery moments in the field and offer a perspective of how they have framed contemporary questions that are being addressed today. This historical journey is not intended to be comprehensive, but merely a reflection on how the past influences the present and how the field has evolved.

Tests and Measurements

Early innovators in physical education, such as the notable Harvard educator Dudley Allen Sargent, were motivated to understand how exercise prescription could make the weak strong and the strong well, and ensure a course of action that would attack the incipient forms of diseases (Sargent, 1883). While Sargent was not an exercise physiologist, he was a physical educator and conducted seminal observational studies using physical examinations, strength assessments, and anthropometric measurements to assess human performance (Sargent, 1897). His work framed questions related to understanding how individuals varied in size, strength, and development compared to the mean values for the same age and sex. This quest for knowledge was anchored in a desire to learn more about the extent of human variation and the physiological limits

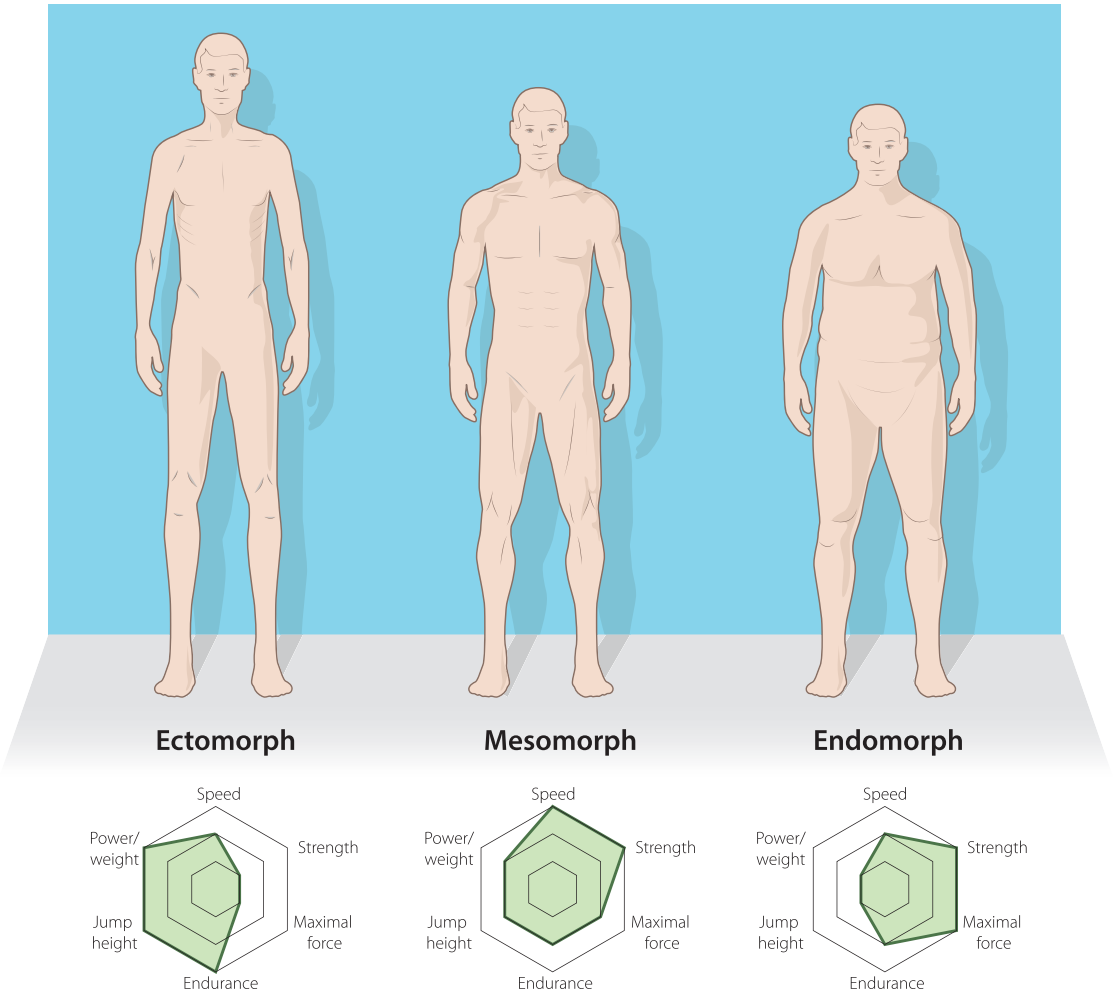

Figure 1. The Concept of Somatotypes
The concept of somatotypes, which describes the diversity of body types ranging from ectomorph to mesomorph to endomorph, is partly influenced by inherited features. Exercise training may have broadly similar beneficial effects on all three somatoypes, but the interplay between inherited factors and environment may result in subtle differences in training response. The diversity of human body characteristics may be advantageous for differing forms of physical activity.

of performance. Simple tests such as the Sargent vertical jump test and the 40-yard dash have been used for decades to assess power, explosive strength, and speed. Clearly, one can appreciate the diversity in body types, or somatotypes, ranging from ectomorph to mesomorph to endomorph (Figure 1). Even within a given body type, exercise training or sedentary living may influence the range of this body type diversity and ultimately impact exercise performance. Specific body types of a fit person may be advantageous for high jumping, sprinting, or wrestling. At the same time, specific body types of an unfit person may be associated with cardiometabolic or cancer risk (Ashwell et al., 2012; Cold et al., 1998). Body fat distribution, arising from changes in hormone levels (e.g., insulin, estrogen, androgens, growth hormone, and cortisol) and metabolic substrates, can influence body type (Björntorp, 1997), but the etiology remains complex.

While meticulous record keeping of anthropometric and performance measures helped to create a picture of the variation in human phenotypes, molecular and physiological mechanisms for this diversity were largely elusive. Many of the modern day exercise physiologists are engaged in questions aimed at understanding the molecular basis for this diversity and how this limits performance. Clearly different body types are more or less conducive to specific forms of physical activity, and this may influence whether or not an individual will excel at a world class or local level. One area of contemporary research deals with the genetics behind different body types and the link to metabolic and cardiovascular disease (Voight et al., 2012). Given the diversity in body types, simple comparisons of one single attribute may not capture the genes controlling diverse phenotypes. For example, a muscular person may weigh as much as an obese person, but disease risk may vary greatly between them. A recent meta-analysis on multiple anthropometric traits identified novel loci for body shape (Ried et al., 2016). These workers calculated the average of six anthropometric traits (body mass index, height, weight, waist and hip circumference, and waist-to-hip ratio), developed a composite index of body shape in its multi-dimensional structure based on data from individuals enrolled in 65 studies of the GIANT consortium (>170,000 individuals), and

identified six genetic loci associated with body shape. Future studies using this approach may provide greater insight into the genetic association between body type and risk for different diseases.

Physiology Meets Biochemistry

Scandinavian physiologists have contributed greatly to the transition from physiological investigations to studies of biochemistry and our modern day knowledge of fuel utilization during exercise. Early physiologists such as August Krogh, who received the Nobel Prize in Physiology or Medicine (1920) ''for his discovery of the capillary motor regulating mechanism'' also addressed questions related to the limits of human performance. His approach was to understand skeletal muscle and whole-body physiology. In elegant work published in 1913 together with Johannes Lindhard, Krogh charted the changes in ventilation, blood flow, pulse rate, respiratory exchange, and alveolar CO_2 tension in man during the first few minutes of light or heavy exercise (Krogh and Lindhard, 1913). Publishing their beautiful hand-drawn graphs depicting the primary data of the research subjects, Krogh and Lindhard described the response of respiratory and circulatory systems to sudden muscular exertions. Krogh went on to perform his paradigm-shifting discovery work to reveal regulatory mechanisms controlling capillary blood flow in resting and active skeletal muscle, for which he was awarded the Nobel Prize (Krogh, 1919a, 1919b, 1919c). This discovery highlighted the importance of oxygen delivery to the working muscle as one of the rate-limiting steps for exercise performance. Krogh and Lindhard also performed careful studies to assess how work performance is influenced by diets rich in carbohydrate or fat and showed that work efficiency under constant-load exercise and performance under intense exercise are greater when carbohydrates are used as a preferential fuel source (Krogh and Lindhard, 1920). This work, and investigations by Christensen and Hansen (Christensen and Hansen, 1939), highlighted the importance of carbohydrate utilization for the working muscle. Later work by Bergström and Hultman studied skeletal muscle biochemistry during exercise (Bergström and Hultman, 1966, 1967). In seminal work examining human skeletal muscle biopsies after acute exercise and during recovery, they reported that acute exercise caused a rapid depletion of glycogen in the working leg. They also determined glycogen resynthesis after an acute bout of exercise and found that glycogen content was restored within 24 hr, suggesting exercise increases glucose uptake and storage. Interestingly, they found that in response to acute exercise, the glycogen storage capacity in skeletal muscle is expanded, suggesting exercise enhances glycogen resynthesis, and finally, they speculate that an ''enhancing factor'' localized to the muscle cells contributed to the changes in muscle biochemistry. Many of the acute effects of exercise are related to improved glucose metabolism and enhanced skeletal muscle insulin sensitivity (for a contemporary review, see Jensen and Richter, 2012). However, early workers also provided evidence that exercise acutely increases turnover and oxidation of free fatty acids in both healthy and trained subjects (Havel et al., 1963, 1964). Thus, adipose tissue is an important fuel sink, with increased mobilization of free fatty acids from adipocytes serving as an important substrate with prolonged exercise. Conversely, in response to long-term vigorous and prolonged exercise training programs, major changes are seen at the level of oxidative metabolism, with increased mitochondrial oxygen uptake and respiratory enzyme activity in skeletal muscle, leading to an increase in aerobic work capacity (Holloszy, 1967). Thus, fundamental early discoveries included the understanding of physiological mechanisms controlling oxygen delivery to the working muscle, as well as biochemical insight into how skeletal muscle metabolized glycogen and oxidized fatty acids to sustain work.

Taking It to the Clinic

The Scandinavian clinical physiologists took advantage of several in vivo methods to assess glucose metabolism in humans during exercise. The goal was to determine substrate exchange by measures of arterial-femoral venous differences for glucose and free fatty acid concentrations and gain insight into fuel utilization. In response to strenuous exercise, blood glucose was shown to be an important substrate for skeletal muscle oxidation, with peripheral glucose utilization increasing during exercise, despite a reduction in circulating insulin levels (Wahren et al., 1971). This suggested that glucose is the main substrate for exercising muscle. They also showed that hepatic glycogenolytic activity was augmented, indicating that the increased glucose output by the liver provides substrate for the working muscle and contributes to the maintenance of blood glucose homeostasis. The rate of skeletal muscle glucose uptake increases as exercise continues during low-intensity exercise for several hours. With prolonged low-intensity exercise, hepatic glucose production fails to keep up with the increased demand for glucose by the working muscle. Concomitantly, as skeletal muscle glycogen depletion increases, the relative contribution of free fatty acids to total skeletal muscle oxidation progressively increases (Ahlborg et al., 1974). Insulin concentrations are depressed and glucagon concentrations are increased during prolonged exercise (Galbo et al., 1975). Thus, in addition to glucose, free fatty acids are important fuel sources for the exercising muscle, particularly during low-intensity exercise of long duration.

Interestingly, the metabolic response to a prolonged exercise bout has been compared to that observed after several days of starvation (Ahlborg et al., 1974), with depleted glycogen stores and a greater reliance of free fatty acids, reflecting a common homeostatic mechanism to minimize disturbances in glucose homeostasis and maintain glucose supply to the brain (Figure 2). This would suggest that training with high or low nutrient availability may influence the adaptive response to exercise training. How different diets alter the adaptive response to exercise at the molecular level is an emerging question in exercise science. Based on the energy requirements during exercise, new paradigms have been developed around the notion of training in states of low glycogen availability to force the muscle to rely on lipid oxidation in an effort to potentially enhance and extend the time course of transcriptional activation of metabolic genes and their target proteins. The ultimate goal would be to boost the enzymatic and transcriptional machinery to enhance mitochondrial biogenesis and improve functional work capacity. For example, in a nutrient periodization study, cyclists performed high-intensity exercise in the evening with high carbohydrate availability, then restricted carbohydrate intake so that they slept with low carbohydrate availability before performing a submaximal exercise bout the next morning in the fasted state

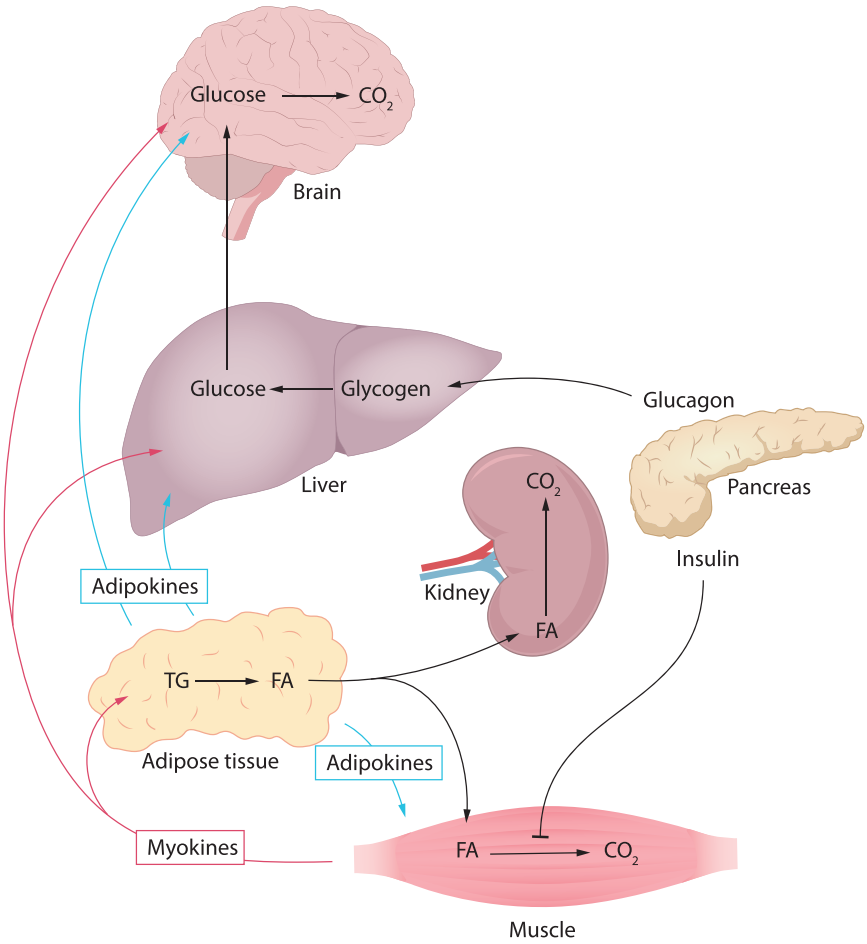

Figure 2. Metabolic Response to Long-Term Aerobic Exercise

Early discoveries in experimental and clinical exercise physiology shaped the way we view the effect of exercise on the body. The metabolic effects of long-term aerobic exercise are similar to those of prolonged starvation. For example, long-term aerobic exercise and fasting both lead to a depletion of glycogen stores in skeletal muscle and mobilization of free fatty acids from adipose tissue. The discovery that adipokines and myokines are released into the circulation and induce signaling effects in other tissues has enhanced the understanding of organ crosstalk.

(Lane et al., 2015). This resulted in a greater upregulation of several exercise-responsive signaling markers with roles in lipid oxidation. Understanding how different diets affect work performance is a long-standing and evolving area of research in the field (Burke, 2015). Insight into individualized diet-exercise interventions is relevant, given the specific energy requirements for the activity to be performed, the training state, muscle fiber type distribution, or genetic background of the participant, and whether medical issues that affect metabolism need to be considered.

Clinicians have long appreciated the fact that exercise reduces blood sugar and enhances insulin sensitivity in people with diabetes (Lawrence, 1926). Using the insulin clamp and hepatic venous catheter techniques, skeletal muscle was identified as the primary tissue responsible for the increase in glucose metabolism following hyperinsulinemia and exercise (DeFronzo et al., 1981). This clinical study revealed that exercise and insulin have a synergistic effect on glucose uptake in skeletal muscle (DeFronzo et al., 1981). Studies in rodents revealed that exercise enhances skeletal muscle glucose uptake by two mechanisms, namely by enhancing insulin sensitivity (Richter et al., 1982) and also by directly increasing glucose uptake via an insulin-independent mechanism (Wallberg-Henriksson and Holloszy, 1984). Using insulin-deficient rodents (Wallberg-Henriksson and Holloszy, 1984) and isolated skeletal muscle preparations (Wallberg-Henriksson and Holloszy, 1985), Wallberg-Henriksson and Holloszy presented direct evidence that muscle contraction increases glucose uptake by an insulin-independent mechanism (Wallberg-Henriksson et al., 1988). These discoveries form the basis of early work to highlight the importance of exercise as a medicine to enhance insulin sensitivity and improve glucose homeostasis in diabetes. Contemporary questions in the field are related to the design and implementation of exercise training protocols to improve glucose homeostasis. A recent meta-analysis designed to ascertain the beneficial effects of exercise training on clinical outcomes and glucose homeostasis in people with type 2 diabetes concludes that exercise at higher intensity may offer superior fitness benefits, while longer program duration could optimize reductions in $HbA1_C$% (Grace et al., 2017).

Molecular Adaptations of Skeletal Muscle in Response to Exercise

Today we appreciate that multiple pathways coordinate the metabolic response and tissue remodeling with exercise training, and much of this biology has been reviewed elsewhere (Cartee et al., 2016; Egan et al., 2016; Egan and Zierath, 2013; Hawley et al., 2014). Acute and long-term exercise training orchestrate a continuum of adaptive changes in skeletal muscle (Egan and Zierath, 2013). Early adaptions to an acute exercise bout include altered mRNA levels of many genes involved in metabolism and muscle function (Yang et al., 2005). Global phosphoproteome studies of human skeletal muscle in response to acute exercise reveal over 900 exercise-regulated phosphorylation sites are changed, indicating that the range of signaling pathways and kinases modulated by exercise is extensive (Hoffman et al., 2015). Upon repeated training, changes in the abundance of proteins involved in diverse metabolic responses occur within days. For example, exercise training increases the level of GLUT4 protein and improves glucose uptake in skeletal muscle (O'Gorman et al., 2006), suggesting that optimizing the modalities to increase the expression of this protein may be one strategy to improve glucose homeostasis in insulin-resistant states. Proteomic analyses reveal

widespread changes in the abundance of a variety of proteins in skeletal muscle after different exercise training regimes (Robinson et al., 2017). In particular, mitochondrial proteins show a robust upregulation in response to both resistance and high-intensity interval training, even in older adults. With longer-term training, mitochondrial concentration, aerobic threshold, and functional work capacity are increased in skeletal muscle, even in aging or type 2 diabetes (Larsen et al., 2014). Contemporary questions to address in the field are related to the identification and validation of exercise-responsive molecules and signaling pathways that control glucose uptake, lipid oxidation, mitochondrial biogenesis, and even functional measures such as strength and power. Rather than taking a reductionist approach, studying genes, proteins, and physiological responses in multiple tissues together may reveal the integrated biology of exercise (Zierath and Wallberg-Henriksson, 2015). Note that many of the early studies in the field were conducted on relatively small cohorts consisting mainly of Caucasian men, and in some cases, the study participants were also the principal investigators themselves. Thus, a greater appreciation of the divergent response to exercise within and between different populations, as well as the influence of genetic background, is an emerging area of interest. Some of this diversity can be ascertained by defining the physiological variables that limit exercise performance.

The Limits of Exercise

Why Do We Study the Limits of Exercise?

As exercise physiology has evolved, investigators now have many powerful tools at their disposal to address physiological, cellular, and molecular adaptations to exercise. However, when delving into the biology of exercise, it is incumbent to assess scientific challenges with a broader scope. Exercise physiology is often the science of studying limits: the limits of elite performance, the limits of the health benefits of exercise, or the limits/barriers to achieving increased population-wide participation in exercise. Of note, many of the novel findings in the field have been informed by studying not just healthy versus unhealthy people, but also by comparing this with data from the highly trained athlete. By assessing the limits of all of these groups, one can obtain a more complete picture of the processes involved in the health benefits of exercise, which can inform translational research. An example of the benefit of studying diverse subject groups in exercise physiology comes from the original ''athlete's paradox.'' Initially described by Goodpaster et al., this paradox comes from the finding that although there is a strong correlation between intra-muscular lipid content and insulin resistance in obese and sedentary people, when trained endurance athletes are included in the analysis, this correlation disappears (Goodpaster et al., 2001). Conversely, trained endurance athletes have high intra-muscular lipid content, but excellent insulin sensitivity, which may be partly explained by increased turnover of intra-muscular lipid pools and protection against reactive oxygen species (Coen and Goodpaster, 2012). This finding has enhanced our understanding of the skeletal muscle effects of endurance training, in addition to elucidating mechanisms for the impaired lipid oxidation, excess muscle lipid accrual, and insulin resistance in type 2 diabetes.

When assessing the health benefits of endurance training, it is important to note that humans have evolved as extremely capable endurance athletes. Indeed, if all land mammals are ranked, humans score highly in many parameters of endurance performance, particularly during running. Long limbs relative to our body size and the ability to rapidly dissipate excess heat through sweating engender humans with superb endurance capabilities. This ability may have been crucial in the evolution of humans, enabling us to broaden the range of habitable environments and perhaps become successful persistence hunters (Bramble and Lieberman, 2004). The necessity of performing regular endurance-type activities may have also fed back into the evolutionary pathway of the modern human. Homo sapiens are of superior intelligence when compared to other species, and emerging evidence suggests that physical activity and brain function are somewhat intertwined in an evolutionary sense (Raichlen and Polk, 2013). Additionally, exercise is long known to enhance learning and memory function, partly through the upregulation of neurotrophic molecules in the brain (Hillman et al., 2008). Signal transduction in the hypothalamus may also be required for some of the beneficial metabolic effects of exercise (Fujikawa et al., 2016). Thus, it may be prescient to consider the apparent evolutionary benefits of human exercise performance when conducting future exercise physiology studies.

The Limits of Exercise Performance

Given the historical study of, and the human aptitude for, endurance exercise, it is interesting to assess the limiting factors of endurance performance (Figure 3). Endurance performance has traditionally been viewed as being chiefly limited by three main physiological factors: maximal oxygen uptake ($\dot{V}O_{2max}$), the ability to sustain work at a high percentage of $\dot{V}O_{2max}$, and the economy of movement (Joyner and Coyle, 2008). These limiting factors are still relevant today and remain in the exercise physiologists' tool kit of assessments. For example, champion endurance athletes have a combination of both a high baseline $\dot{V}O_{2max}$ and a high level of trainability, with the highest relative $\dot{V}O_{2max}$ values often seen in elite cross-country skiers ($\sim$90 $mL \cdot min^{-1} \cdot kg^{-1}$; Sandbakk and Holmberg, 2014). Some of the major physiological adaptations that are responsible for the training-induced increase in $\dot{V}O_{2max}$ are increased cardiac output, blood volume, hemoglobin mass, capillary density, and mitochondrial function in the working muscles (Costill et al., 1976; Joyner and Coyle, 2008; Thomsen et al., 2007). In general terms, cardiac output and the O_2 carrying capacity of the cardiovascular system are regarded as the principal determining factors of $\dot{V}O_{2max}$ at sea level (Blomqvist and Saltin, 1983; Montero et al., 2015). However, although it has been almost a hundred years since Krogh determined oxygen as a rate-limiting parameter during exercise, the details have not been fully elucidated. Recently, debate has focused on whether the diffusion rate of O_2 from microvessels into skeletal muscle is a limiting step in the capacity of $\dot{V}O_{2max}$ (Lundby and Montero, 2015; Wagner, 2015). This debate is partly obscured by methodological limitations of measuring myofiber O_2 extraction in vivo, which may, in the future, prove conclusive. Nevertheless, it appears that matching of O_2 transport and extraction by innervated muscles in a spatially and temporally dependent manner may be a contributory limiting factor of exercise performance. Indeed, trained endurance athletes possess superior matching of blood flow distribution with local $\dot{V}O_2$ (Kalliokoski et al., 2005), and may also have improved O_2 myoglobin-mitochondrial dynamics

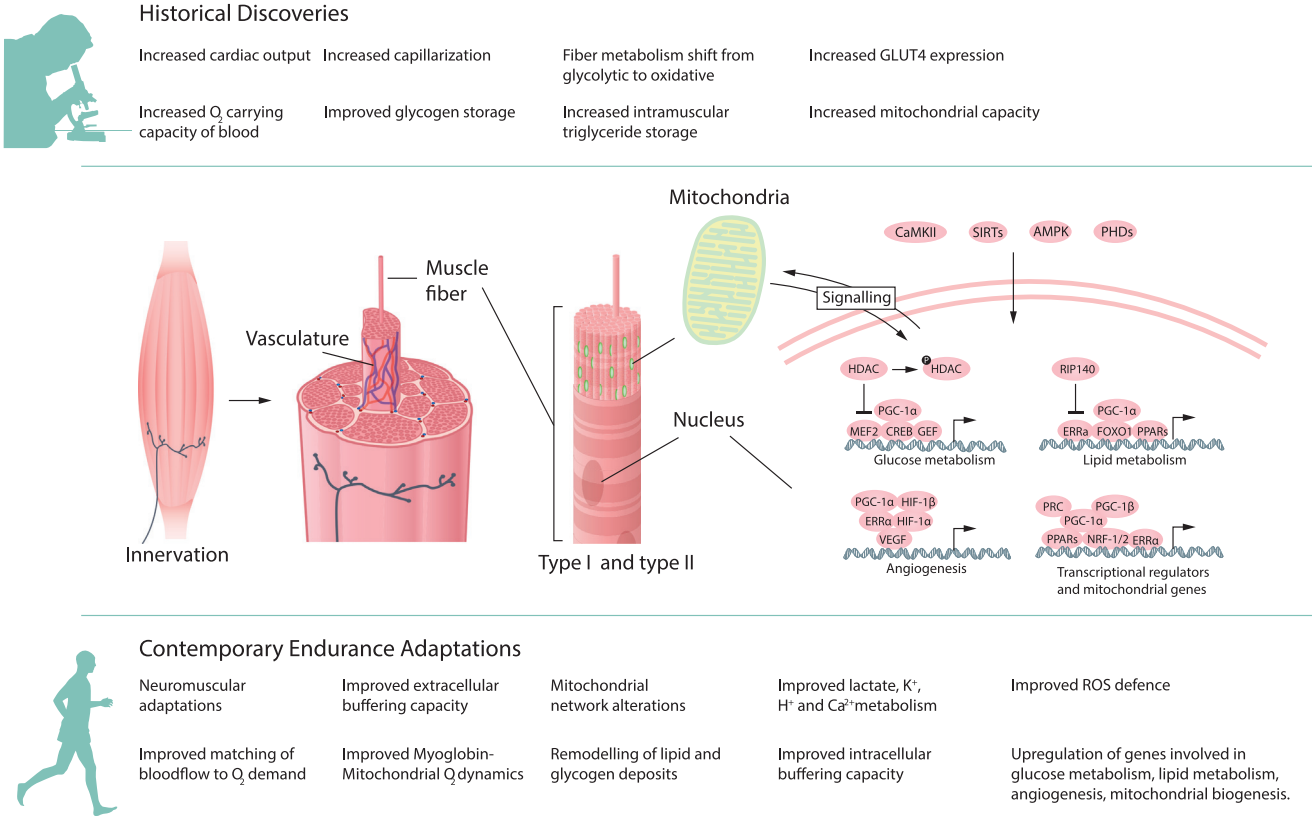

Figure 3. The Limits of Exercise Performance

Endurance athletes have a multitude of adaptations that enable them to sustain work at a high percentage of $\dot{V}O_{2max}$. This figure is not intended to be a comprehensive list of all adaptations. Furthermore, it is worth noting that many of these, or additional, adaptations are induced by several modalities of exercise. Historical milestones include the following discoveries: (1) increased cardiac output, (2) increased O_2 carrying capacity of the blood, (3) increased capillarization, (4) increased glycogen storage, (5) fiber metabolism shift from glycolytic to oxidative, (6) increased intramuscular triglyceride storage, (7) increased GLUT4 abundance, and (8) increased mitochondrial capacity. Contemporary thinking includes (1) neuromuscular adaptations; (2) improved matching of blood flow to O_2 demand; (3) improved extracellular buffering capacity; (4) improved myoglobin-mitochondrial O_2 dynamics; (5) mitochondrial network adaptations; (6) remodeling of lipid and glycogen deposits; (7) improved reactive oxygen species (ROS) defense; (8) improved intracellular buffering; (9) improvements in metabolism of lactate, K^+, H^+, and Ca^{2+}; and (10) transcription factor/co-activator-induced upregulation of exercise-responsive genes involved in glucose metabolism, lipid metabolism, angiogenesis, mitochondrial biogenesis, and other transcriptional regulators. AMPK, AMP-activated protein kinase; CaMK, Ca^{2+}/calmodulin-dependent protein kinase; CREB, cyclic AMP response element-binding protein; ERR, estrogen-related receptor; FOXO, forkhead transcription factor, O-box subfamily; GEF, GLUT4 enhancer factor; HDAC, histone deacetylase; HIF, hypoxia-inducible factor; MEF2, myocyte enhancer factor 2; NRF, nuclear respiratory factor; PGC-1, PPARγ co-activator 1; PHD, prolyl hydroxylase domain; PPAR, peroxisome proliferator-activated receptor; PRC, PGC-1-related coactivator; RIP140, nuclear receptor-interacting protein 1; SIRT, sirtuin; VEGF, vascular endothelial growth factor.

(Takakura et al., 2015). Furthermore, attributing importance to a single rate-limiting step of $\dot{V}O_{2max}$ is possibly a redundant hypothesis. Perhaps, O_2 delivery to, and utilization by, skeletal muscle mitochondria should be viewed as an integrated system, with simultaneous improvements in all aspects necessary to increase capacity.

Factors that limit O_2 delivery to the working muscle may also influence thinking in regard to another classical limit of endurance performance: the ability of an athlete to sustain work at a high percentage of $\dot{V}O_{2max}$ without either the accumulation of exponential levels of blood lactate or skeletal muscle fatigue (signified by the slow $\dot{V}O_2$ component) (Farrell et al., 1979; Keir et al., 2016; Temesi et al., 2017). At present, two metabolic thresholds that infer exercise performance are commonly identified during exercise testing. First, there is the appearance of an exponential release rate of lactate in the blood, and second, an inability to sufficiently buffer the associated acidosis (Mattioni Maturana et al., 2017). The appearance of blood lactate during exercise is a byproduct of an increasing rate of glycolysis, whereby the intensity of exercise exceeds the muscles' capacity to generate energy through mitochondrial oxidation. A high threshold of sustainable work is attained partly by a training-induced increase in the oxidative capacity of the working muscles, which can more than double after training (Holloszy and Coyle, 1984; Holloszy et al., 1977). This increase in oxidative capacity can aid glycogen sparing, which, as Bergström and Hultman demonstrated (Bergström and Hultman, 1966, 1967), is a limiting factor for prolonged exercise bouts (Kim et al., 2015). In addition to oxidative capacity, other mechanisms contribute to skeletal muscles' ability to withstand fatigue in endurance exercise, and enable an athlete to maintain work at a high percentage of $\dot{V}O_{2max}$. Among the many molecular factors, improvements in skeletal muscle ion handling, including lowered interstitial concentrations of K^+ during intense exercise,

improved H^+ regulation, and enhanced Ca^{2+} release function may also play a role in improved resistance to peripheral fatigue and aid glycogen sparing (Hostrup and Bangsbo, 2016; Nielsen et al., 2014). In summary, endurance athletes typically have both a higher $\dot{V}O_{2max}$ and can exercise at a higher relative percentage of $\dot{V}O_{2max}$ than untrained subjects.

Additionally, endurance athletes employ these advantages with greater efficiency than less well-equipped sedentary subjects. Exercise economy is a key player in the traditional triad of limiting endurance factors; this describes the efficiency of transferring skeletal muscle chemical energy into locomotion, rather than heat. This is of particular importance during running, where economy can vary by up to 30%, with >85% of this variance accounted for independently of $\dot{V}O_{2max}$ (Shaw et al., 2015). Exercise economy is an all-encompassing term for a multitude of physiological elements, which differ between activity types. These include metabolic efficiency of local skeletal muscle fibers, biomechanical efficiency, and neuromuscular efficiency. The composition and recruitment profile of skeletal muscle during exercise probably plays a role in metabolic efficiency. The economic recruitment of a large population of mitochondria-dense, O_2 efficient, type I fibers and matching of O_2 delivery systems may be one mechanism by which trained athletes possess increased exercise economy (Coyle et al., 1992; Krustrup et al., 2008). During running, however, this may play less of a role as exercise economy is also determined by a number of other physiological factors including joint stiffness, braking impulse, body weight, and storage and recovery of elastic energy, among others (Lundby et al., 2017; Saunders et al., 2004; Warne and Warrington, 2014).

There are a number of other limiting factors of exercise performance, but it is beyond the scope of this review to fully discuss this topic. For example, the concerted regulation of neuromuscular innervation between central and peripheral mechanisms is incompletely understood, but has been discussed elsewhere (Carroll et al., 2016; Sidhu et al., 2017; Siebenmann and Rasmussen, 2016; Temesi et al., 2014). Furthermore, this review has mainly considered limitations to performance in healthy individuals. Thus, additional considerations should be taken into account when considering exercise prescription for people diagnosed with myopathy, sarcopenia, pulmonary and cardiovascular disease, chronic neurological disease, and bone/joint disorders such as osteoarthritis or osteoporosis, as these conditions, and others, can limit exercise performance.

The Limits of Mitochondria in Exercise Physiology

Mitochondrial function is key in the regulation of all three of the classical physiological factors that limit endurance performance. Mitochondria have been somewhat overlooked in the era of genomic research, but these organelles are experiencing something of a renaissance as their importance as signaling modulators—not just energy producers—becomes clear. Although exercise physiologists have consistently studied mitochondria in respect to their ability to metabolize substrates (Hawley et al., 2015), new data further elucidate the mechanism of energy generation and delivery within skeletal muscles (Glancy et al., 2015). Subsarcolemmal mitochondria and intermyofibrillar mitochondria are heterogeneous sub-populations (Kuznetsov and Margreiter, 2009). This heterogeneity may be partly due to the necessity of subsarcolemmal mitochondria's regulation of sarcolemmal membrane function, whereas intermyofibrillar mitochondria are the primary exercise powerhouses due to their proximity to contracting sarcomeres (Hood, 2001; Lundby and Jacobs, 2016). However, subsarcolemmal and intermyofibrillar mitochondrial populations are, additionally, part of a mitochondrial reticulum that provides a conductive pathway for energy distribution (Glancy et al., 2015). Within this mitochondrial reticulum, proteins associated with mitochondrial proton-motive force production are located preferentially in the cell periphery and proteins that use the proton-motive force for ATP production in the cell interior. Given this recent advancement in the understanding of the mechanism of mitochondrial energy creation and delivery, it is prudent to ask what else is opaque in regard to skeletal muscle metabolism. In particular, it is noteworthy that several key principles of skeletal muscle metabolism have come as a result of perceiving muscle as an integrated network of energy creation and delivery rather than a compartmentalized system. This also holds true when thinking of O_2 kinetics at the cellular level, the peripheral fatigue of endurance exercise, and the economy of energy generation and locomotion. In future, exercise physiologists may look to utilize a growing number of non-invasive techniques to study muscle metabolic functioning, which may answer key questions. For example, how do mitochondrial networks interact with O_2 kinetics? How does exercise remodel mitochondrial networks to enhance efficiency? And lastly, how does training affect the interaction between glycogen/lipid storage locale and mitochondrial networks?

Precision Medicine, Exercise Amount, and Timing

Individual Response to Exercise

In addition to being a limiting factor for endurance exercise, $\dot{V}O_{2max}$ is also an independent predictor of cardiovascular, metabolic, and all-cause mortality (Lee et al., 2010). The response of $\dot{V}O_{2max}$ to exercise differs among subjects and appears to be at least partly inherited. For example, baseline $\dot{V}O_{2max}$ is ~50% dependent on inherited traits (Bouchard et al., 1998); additionally, the trainability of $\dot{V}O_{2max}$ also has an ~50% genetic component (Bouchard, 2012; Bouchard et al., 2000). Generally, increases of 15%–25% $\dot{V}O_{2max}$ are seen after training, although this can vary substantially (Bouchard et al., 2011), and up to 20% of healthy individuals have previously been reported as non-responders (Timmons et al., 2010). This has led to public speculation that trainability is largely due to the chance of inheritance, which may disincentivize certain sections of the sedentary population from exercising. However, recent work demonstrates that increasing exercise dose (intensity × duration) may ameliorate the apparent lack of training response (Montero and Lundby, 2017). Despite still finding large variability in training response, the study of Montero and Lundby demonstrated that all healthy subjects displayed a training-induced increase in cardiorespiratory fitness (W_{max}) when subjected to sufficient exercise dose. Furthermore, notwithstanding heterogeneity, all individuals responded to at least one parameter of exercise performance after either endurance or sprint-interval training in one recent study (Bonafiglia et al., 2016). Consequently, exercise physiologists should be aware of the range in exercise performance parameter response during training interventions. Moreover, various exercise modalities should be considered when designing training interventions, as these may have differing, potentially

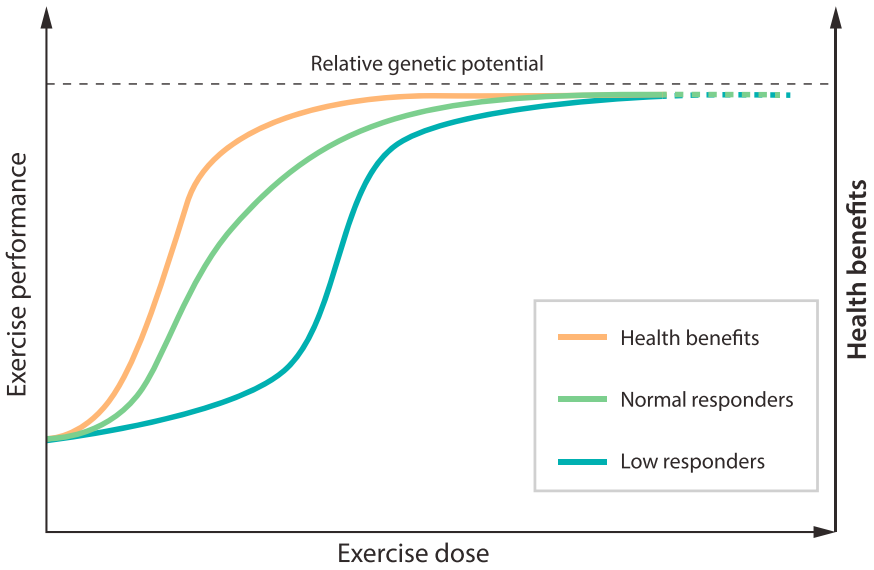

Figure 4. Individual Response to Exercise
Individual exercise-dose response is likely a continuum, rather than a binary outcome. "Low and Normal responders" are plotted against left y axis, while "Health Benefits" is plotted against right y axis; i.e., the acute health benefits of exercise are often apparent before exercise performance is increased, although there may also be heterogeneity in the exercise health response. Nevertheless, exercise interventions are often only a snapshot, where the exercise dose may not meet an individual's threshold for response. Further research in this area may inform clinically relevant personalized exercise interventions.

complementary, physiological outcomes (Bacon et al., 2013; Bishop et al., 2014; Bonafiglia et al., 2016; Churchward-Venne et al., 2015). Thus, although inherited magnitude of dose response to exercise is present and presumably Gaussian in distribution, there are likely no healthy non-responders in terms of cardiorespiratory fitness. Whether exercise-dose response is entirely an inherited phenomenon remains unclear. For example, lack of (or very low) response to exercise may be apparent in pathological conditions. The impact of different disease states on the adaptive response to exercise is an area that warrants investigation. Additionally, prescribed exercise intensity is normally determined via percentage of heart rate (HR), $\dot{V}O_{2max}$, or W_{max}, which often does not standardize the homeostatic energy perturbance induced by endurance training. This should be taken into account when assessing the outcomes of training studies comparing healthy versus unhealthy people. Therefore, one should think of individual exercise-dose response as a continuum, rather than a binary outcome (Figure 4). Furthermore, on a public health level, it is important to note that exercise has many acute benefits that are apparent well before exercise performance is increased. However, after training, health benefit response may not be predicted by heterogeneity in exercise performance markers (Pandey et al., 2015). This leads to the question of whether exercise physiologists can determine effective individualized dose-response training protocols.

Timing the Exercise Bout to Optimize Metabolic Responses

Emerging epidemiological evidence suggests a connection between circadian biology and human health (Reutrakul and Knutson, 2015). Thus, another current dilemma, which may be addressed by more precise training interventions, is the interplay between circadian rhythm and metabolic health. Under homeostatic conditions, circadian clocks are drivers of whole-body metabolism. At the molecular level, cell-autonomous circadian rhythms are generated by a transcriptionally auto-regulatory feedback loop composed of transcriptional activators CLOCK and BMAL1 and their target genes, which forms a repressor complex that interacts with CLOCK and BMAL1 to inhibit transcription (Gerhart-Hines and Lazar, 2015). Disturbed circadian rhythms are associated with metabolic dysfunction, highlighting the critical role of this circuit in metabolic health (Manoogian and Panda, 2016). Subjects with perturbed circadian rhythms and mouse models with genetic central clock disruption are more prone to developing metabolic disturbances or diseases (Coomans et al., 2015). Additionally, skeletal muscle regulation of circadian rhythm is key in metabolic homeostasis (Zhou et al., 2013). For example, *Bmal1*$^{-/-}$ myotubes display reduced anaerobic glycolysis, mitochondrial respiration with glycolytic fuel, and transcription of HIF1α targets. Further, induction of clock and HIF1α target genes, in response to strenuous exercise, varied according to the time of day in wild-type mice (Peek et al., 2017). These results indicate that metabolic and clock pathways interact with exercise, and although it is unknown whether these results would be recapitulated in humans, emerging evidence suggests that human skeletal muscle has metabolic periodicity (van Moorsel et al., 2016). Small "doses" of intense exercise spread throughout the day rapidly improve glucose control (Francois et al., 2014). This raises the notion that timing the exercise bout in conjunction with each meal may lead to better glucose homeostasis throughout the day.

Diet and exercise have a synergistic effect on insulin sensitivity, such that the timing of a meal or distribution of nutrients throughout the day may optimize the exercise response. Recent evidence suggests time-restricted feeding without caloric restriction synchronizes diet-induced rhythms with the central circadian pacemaker, thereby amplifying circadian and metabolic cycles, which may produce long-term effects to prevent obesity and its metabolic consequences (Chaix et al., 2014; Hatori et al., 2012). One could imagine a similar scenario for dosing bouts of exercise training. Optimizing the timing of external cues such as an exercise bout with defined eating patterns could have the potential to sustain a robust circadian clock signal, which may prevent disease and improve prognosis. However, before this nutritional/exercise approach to combat obesity and metabolic disease is accepted into clinical practice, further validation in human cohorts is warranted. While no large-scale study has specifically determined the effects of exercise and time-restricted feeding on metabolic homeostasis in humans, preliminary studies suggest that synchronizing nutrient intake with light/dark circadian rhythms may alter energy homeostasis and

have weight loss benefits. For example, consuming the bulk of calories in the morning, rather than later in the day, promotes better weight loss (Garaulet et al., 2013). High caloric intake at breakfast improves weight loss in obese women and leads to better fasting glucose levels, enhanced insulin sensitivity, and improved lipid profile compared to the high-calorie dinner consumers (Jakubowicz et al., 2013). Thus, there is therapeutic potential to apply chronobiology to explore the impact of diet and exercise intervention strategies to manage metabolic diseases such as obesity and diabetes. One question for the field is whether there is an optimal time of day to induce the greatest metabolic effect of exercise on signal transduction, mitochondrial function, and expression of exercise-responsive genes. Additionally, whether acute or chronic exercise can correct for, or impact, disruptions in circadian biology remains to be determined. However, there appear to be insufficient data on the best time of day to exercise, in regard to health and exercise performance outcomes.

Moving Forward

Exercise improves functional work capacity and metabolic health, but there is growing appreciation that there is a wide continuum of adaptations within any given population. There is a rising interest in defining the limits of performance and also the diverse response to exercise training in terms of improvements in insulin sensitivity, mitochondrial function, oxygen consumption, and muscle function and strength. Just as food restriction has profound effects on body weight and energy homeostasis, exercise training is a clinically proven intervention that can delay, and in many cases prevent, the health burdens associated with metabolic disorders. Future questions for clinical and experimental exercise physiologists include the identification and validation of genes and molecules responsible for the health-promoting effects of diet and exercise and the spectrum of individual responses to exercise training protocols. This endeavor may be aided by perceptions of metabolic systems within tissues as part of dynamic networks, which are often highly plastic. Moving from fundamental concepts developed by early exercise physiologists, the field is embracing personalized exercise prescription to confer the health benefits of regular exercise training. Coordinating nutritional intake with the timing of each daily exercise bout may optimize improvements in whole-body insulin sensitivity and energy homeostasis, and thus mitigate metabolic disease. How this can be achieved in practice will require communication between scientists working from bench to bedside and back again, in addition to public engagement.

ACKNOWLEDGMENTS

The authors are particularly grateful to Professors John A. Hawley, Anna Krook, Henning Wackerhage, and Mr. Petter Alm for critical comments and helpful discussions during the preparation of this Perspective and to Mattias Karlén for preparation of the artwork. The authors are supported by grants from Novo Nordisk Foundation (NNF14OC0011493 and NNF14OC0009941), Wenner-Gren Foundation, Swedish Research Council (2015-00165), European Research Council (233285), and the Strategic Program in Diabetes Research at Karolinska Institutet (2009-1068).

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