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Authors

Neufer, P Darrell
Bamman, Marcas M
Muoio, Deborah M
et al.

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Understanding the Cellular and Molecular Mechanisms of Physical Activity-Induced Health Benefits

P. Darrell Neufer,[1,*] Marcas M. Bamman,[2] Deborah M. Muoio,[3] Claude Bouchard,[4] Dan M. Cooper,[5] Bret H. Goodpaster,[6] Frank W. Booth,[7] Wendy M. Kohrt,[8] Robert E. Gerszten,[9] Mark P. Mattson,[10] Russell T. Hepple,[11] William E. Kraus,[3] Michael B. Reid,[12] Sue C. Bodine,[13] John M. Jakicic,[14] Jerome L. Fleg,[15] John P. Williams,[16] Lyndon Joseph,[16] Mary Evans,[17] Padma Maruvada,[17] Mary Rodgers,[18] Mary Roary,[19] Amanda T. Boyce,[20] Jonelle K. Drugan,[20] James I. Koenig,[21] Richard H. Ingraham,[22] Danuta Krotoski,[23] Mary Garcia-Cazarin,[24] Joan A. McGowan,[20] and Maren R. Laughlin[17]

[1]East Carolina Diabetes and Obesity Institute, Departments of Physiology and Kinesiology, Brody School of Medicine, East Carolina University, Greenville, NC 27834, USA
[2]UAB Center for Exercise Medicine and Department of Cell, Developmental, and Integrative Biology, University of Alabama at Birmingham, Birmingham, AL 35294, USA
[3]Duke Molecular Physiology Institute, Duke University School of Medicine, Durham, NC 27701, USA
[4]Human Genomics Laboratory, Pennington Biomedical Research Center, Louisiana State University, Baton Rouge, LA 70808, USA
[5]UC Irvine Institute for Clinical and Translational Science and Department of Pediatrics, University of California, Irvine, CA 92697, USA
[6]Translational Research Institute for Metabolism and Diabetes, Florida Hospital – Sanford-Burnham Medical Research Institute, Orlando, FL 32804, USA
[7]Departments of Biomedical Sciences, Medical Pharmacology and Physiology, and Nutrition and Exercise Physiology, University of Missouri, Columbia, MO 65211, USA
[8]Division of Geriatric Medicine, School of Medicine, University of Colorado Anschutz Medical Campus, Aurora, CO 80045, USA
[9]Department of Medicine, Harvard Medical School, Boston, MA Cardiovascular Research Center, Massachusetts General Hospital, Boston, MA Cardiology Division, Massachusetts General Hospital, Boston, MA 02114, USA
[10]Laboratory of Neurosciences, National Institute on Aging Intramural Research Program, Baltimore, MD 21224, USA
[11]Department of Kinesiology, McGill University Health Center, McGill University, Montreal, QC H2W 1S4, Canada
[12]Department of Applied Physiology and Kinesiology, University of Florida, Gainesville, FL 32611, USA
[13]Department of Neurobiology, Physiology and Behavior, University of California, Davis, Davis, CA 95616, USA
[14]Department of Health and Physical Activity, Physical Activity and Weight Management Research Center, University of Pittsburgh, PA 15261, USA
[15]National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD 20892, USA
[16]National Institute on Aging, National Institutes of Health, Bethesda, MD 20892, USA
[17]National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892, USA
[18]National Institute of Biomedical Imaging and Bioengineering, National Institutes of Health, Bethesda, MD 20892, USA
[19]National Institute of Nursing Research, National Institutes of Health, Bethesda, MD 20892, USA
[20]National Institute of Arthritis and Musculoskeletal and Skin Diseases, National Institutes of Health, Bethesda, MD 20892, USA
[21]National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, MD 20892, USA
[22]Center for Scientific Review, National Institutes of Health, Bethesda, MD 20892, USA
[23]Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD 20892, USA
[24]Office of Disease Prevention, National Institutes of Health, Bethesda, MD 20892, USA
*Correspondence: neuferp@ecu.edu
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The beneficial effects of physical activity (PA) are well documented, yet the mechanisms by which PA prevents disease and improves health outcomes are poorly understood. To identify major gaps in knowledge and potential strategies for catalyzing progress in the field, the NIH convened a workshop in late October 2014 entitled "Understanding the Cellular and Molecular Mechanisms of Physical Activity-Induced Health Benefits." Presentations and discussions emphasized the challenges imposed by the integrative and intermittent nature of PA, the tremendous discovery potential of applying "-omics" technologies to understand interorgan crosstalk and biological networking systems during PA, and the need to establish an infrastructure of clinical trial sites with sufficient expertise to incorporate mechanistic outcome measures into adequately sized human PA trials. Identification of the mechanisms that underlie the link between PA and improved health holds extraordinary promise for discovery of novel therapeutic targets and development of personalized exercise medicine.

Introduction

Physical activity (PA), defined as bodily movement produced by skeletal muscles that requires energy expenditure, is an integral part of human life that influences development and overall health across the lifespan (Bamman et al., 2014; Bouchard et al., 1995; Colpani et al., 2013; Juonala et al., 2013; Whellan et al., 2007). Evolutionarily, the ability of man to perform PA was essential to survival, and therefore adaptive biological responses to both acute and repeated episodes of PA have played a critical role in shaping and defining "normal" human physiology. Whereas physical work associated with gathering food, building shelter, and evading predators was an absolute requirement of daily life for our ancestors, the advent of modern technology has now relegated PA from a necessity of human existence to a

choice of human lifestyle. As a result, physical inactivity has been identified as the fourth leading risk factor for global mortality (WHO, 2009).

Considering that the human species evolved to perform and endure habitual PA, it is not surprising that its absence can lead to devastating physiological and clinical consequences. Conversely, the impact of PA on human health is profound and unequivocal. PA helps to build muscle mass during development and preserve musculoskeletal function during aging (Christianson and Shen, 2013). PA promotes cardiometabolic wellness, improves cognitive performance, and effectively aids in the prevention and treatment of a variety of health conditions, including cardiovascular disease, diabetes and other disorders of metabolism, neurological diseases, sarcopenia, osteoporosis, and cancer (U.S. Department of Health and Human Services, 2008; Bouchard et al., 1990; Garber et al., 2011; Pate et al., 1995; Stranahan and Mattson, 2012). Additionally, tailored exercise programs, while all too often ignored, are essential for optimizing health in people with a wide variety of disabilities (Peterson et al., 2012).

Despite indisputable evidence of the myriad physiological benefits conferred by regular PA, the exact molecular mechanisms by which PA promotes human health remain poorly understood. In fact, epidemiological evidence suggests that the protective effects of PA on cardiovascular disease are nearly double that which would be predicted based on changes in traditional risk factors (Joyner and Green, 2009). In other words, ~50% of the protection afforded by PA remains unexplained. This knowledge gap was the focus of a recent NIH workshop "Understanding the Cellular and Molecular Mechanisms of PA-induced Health Benefits" on October 30–31, 2014 in Bethesda, MD. Attendees were asked to identify the major gaps in current knowledge regarding the molecular mechanisms underlying the health benefits of PA, obstacles to obtaining that knowledge, and possible solutions that would potentiate major progress in the field. Five working groups prepared sessions on (1) tools to facilitate clinical research to elucidate the mechanisms of PA, (2) integrative physiological mechanisms by which PA benefits multiple tissues and organ systems, (3) role of tissue stress in the benefits of PA, (4) role of mitochondria in the mechanisms underlying the benefits of PA, and (5) discovery tools to identify circulating and tissue signals that mediate the effects of PA. Progress toward a clearer mechanistic understanding of the extraordinary link between PA and health outcomes could lead to transformative biomedical discoveries that (1) reveal potential novel molecular and cellular therapeutic targets for disease prevention/treatment and (2) support development of personalized approaches for optimizing health outcomes in response to specific interventions, including the best combination of therapies (i.e., PA alone or plus drug, nutrient, diet, etc.). In response to the recommendations emanating from this workshop, the NIH will initiate a program through the Common Fund to catalog molecular transducers of physical activity in humans and to begin to explore their functions.

Gaps in Knowledge Regarding the Health Benefits of PA

Constructing a Network Model that Guides and Informs PA Research

The sophistication and capacity of modern technology has shifted the landscape of basic life sciences research from that of traditional biological reductionism to a much more integrative, holistic systems approach. Rapid technical progress has led to the growing recognition that living organisms are not merely the sum of their parts, but rather that interactions among cellular components and their environment are ultimately responsible for organismal form, function, and phenotype. Implicit in this philosophy is that the failure of biological networks to maintain homeostasis gives rise to pathophysiology and the development of complex diseases, whereas biological adaptations that enhance network flexibility and build functional reserve confer stress resistance and promote health.

As an energetic and physical challenge that broadly impacts the complex physiologic and metabolic networks of a multi-system organism, PA provides a paradigm through which a deeper and more sophisticated understanding of those networks can be developed. Because PA affects all cells and tissues in the body in numerous ways that vary with the type and intensity of activity, as well as the fitness, developmental, and disease state(s) of the individual, a two-tiered conceptual framework is proposed to capture the integrative and hierarchical nature of network control in response to PA (Figure 1) (Walz, 2005). The first tier encompasses the various vertical levels at which hierarchical control is exerted, as well as the molecular mechanisms that mediate crosstalk between systems to maintain homeostasis in the healthy state. A comprehensive understanding of the integrated regulatory mechanisms that operate within (i.e., horizontal) and between (i.e., vertical) levels informs a model from which hypotheses can be formed and experimentally tested. The second tier considers factors contributing to inherent (genetic, sex, height, etc.) and acquired (age, environment, fitness level, disease state, etc.) variability among individuals that in turn influence network dynamics in the first tier. Construction and application of this model serves the ultimate goal of biomedical science, which is to integrate knowledge of innate regulatory architecture with that of well-defined adaptive, homeostatic mechanisms to effectively forestall, predict, treat, and manage human disease on an individualized basis.

PA challenges homeostasis in virtually every organ system and activates acute and long-term compensatory mechanisms to preserve and/or re-establish homeostasis (see the recent review by Hawley et al., 2014). With a two-tiered approach in mind, the following sections raise several outstanding questions and pinpoint key knowledge gaps regarding the mechanisms by which hierarchical control is integrated within and between levels in response to PA, and the inherent/acquired mitigating factors that influence the response to PA and thereby determine the resulting health benefits.

How Do All Cells/Tissues Respond to Exercise?

Deciphering the molecular mechanisms underlying PA-induced health benefits begins with defining the extent and magnitude to which PA disrupts homeostasis in different cell types, and how various cells react to meet those challenges. Still unknown is whether PA-induced alterations in homeostasis are common or unique among different cell types, and to what degree the mode of exercise influences the responses. For example, does endurance exercise increase energy turnover rate in cell types other than skeletal muscle and heart? How do physical forces (strain, concentric, eccentric, gravitational, etc.) influence metabolism and energetics in cells that do not perform contractile

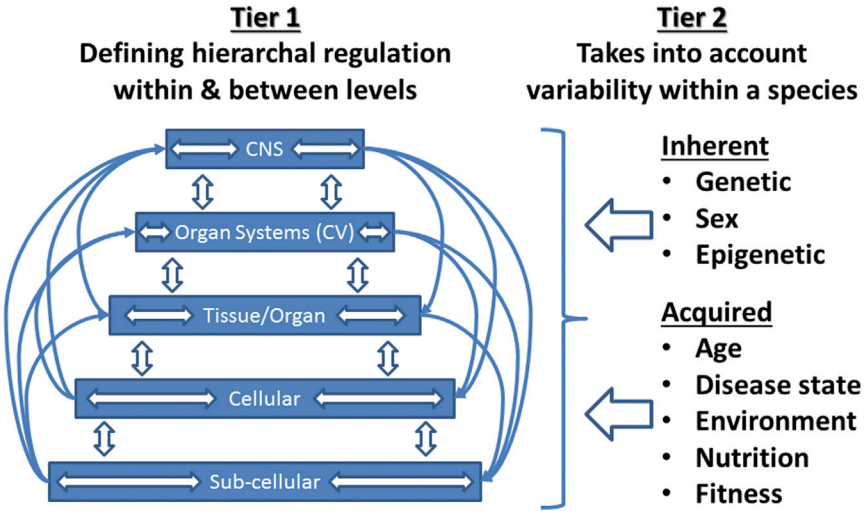

Figure 1. Two-Tiered Model of Systems Control in Response to Physical Activity
Proposed two-tiered conceptual model of the integrative and hierarchical control defining the response to physical activity (PA). Tier 1 illustrates the physiological systems activated in response to PA as well as the crosstalk between systems. Identifying the molecular mechanisms by which hierarchical control is regulated within (horizontal) and between (vertical) levels to maintain homeostasis in the healthy state provides the framework from which hypotheses can be experimentally tested. Tier 2 takes into account the inherent and acquired factors among individuals that influence systems control in Tier 1. Developing a comprehensive understanding of the network dynamics and systems control responses activated by PA will provide the foundation upon which compensatory mechanisms can be predicted and targeted to more effectively prevent and treat disease.

function? How are homeostatic disturbances communicated within (horizontal control) and between (vertical control) different cell types via intracellular and neuroendocrine signaling pathways?

Identifying precisely how, when, and to what extent different regimens of PA alter homeostasis in all affected cell types is a necessary, but clearly formidable, challenge. Whereas some of these questions have been studied in tissues that have obvious biomechanical and metabolic roles during physical work (e.g., skeletal muscle, heart, adipose, liver, vascular, and endocrine), much less is known about acute and chronic adaptations to PA in cell and tissues that are not typically viewed as primary responders during exercise (e.g., brain, kidney, colon, lung, pancreas, immune cells, leukocytes, etc.) (Farrell et al., 2012).

How Are the Responses to Exercise Communicated and Coordinated among Tissues?

Any increase in PA requires a coordinated physiological response appropriately matched to the demand of the activity. An insufficient response will limit the ability of the system to accomplish and/or sustain the task, whereas an excessive response might compromise efficiency and limit sustainability. Evolutionary pressures favoring mechanisms of survival have produced mammalian organisms that are remarkably adept at coping with periods of increased physical demand, often for prolonged periods of time. This observation underscores two core concepts of exercise physiology. First, the physiological network as a whole, as well as its constituent cellular components, harnesses enormous reserve capacity. Second, systemic homeostasis does not depend on a static context, but instead operates on a dynamic continuum as a product of remarkable network flexibility.

Many of the centrally-driven responses to an exercise challenge have been well-characterized, including those that engage sympathetic neural circuitry and the neuroendocrine systems to coordinate adjustments in respiration, blood flow, fuel supply and selection, and thermoregulation (Loucks, 2012). However, much less is known about the autocrine, paracrine, and endocrine factors operating within and between tissues to facilitate crosstalk between both neighboring and distant cells during PA. For example, there is growing evidence that exercise training activates mobilization of endothelial progenitor cells from bone marrow to facilitate endothelial cell regeneration (Moebius-Winkler et al., 2011), neural progenitor cells to promote recovery of neurological function from ischemic stroke (Zheng et al., 2014), and cardiac progenitor cells to induce physiological hypertrophy (Xiao et al., 2014). Skeletal muscles and adipose tissue can function as bona fide "endocrine" organs, producing and releasing proteins into the circulation that modulate physiological responses during and after exercise (Catoire et al., 2014; Pedersen and Febbraio, 2008; You and Nicklas, 2008). In addition to peptide endocrine factors, the exercise-responsive muscle secretome includes numerous metabolic intermediates with known or suspected biological activities. PA can also promote muscle disposal of potentially deleterious molecules. For example, a recent study found that PA increases skeletal muscle expression of kynurenine aminotransferase, accelerating uptake and conversion of circulating kynurenine to kynurenic acid, which in turn protects against stress-induced changes in the brain associated with depression (Agudelo et al., 2014). Moreover, genetically engineered mouse models provide evidence that simply manipulating flux through specific biochemical reactions in skeletal muscle can profoundly influence PA behavior (Hakimi et al., 2007; Tsao et al., 2001), implying a direct communication loop between skeletal muscle and the CNS.

Because neurons and leukocytes infiltrate every tissue in the body, the nervous and immune systems may play particularly important roles in coordinating response to and health benefits of PA. Neuronal networks control the initiation, intensity, duration, and termination of PA, as well as responses of all major organ systems to exercise. However, the mechanisms by which the nervous system may enhance the functionality and disease resistance of different organ systems are largely unknown. Further studies are also needed to delineate the role of PA in regulating inflammation and enhancing certain aspects of immune function. Because disorders of the nervous (Alzheimer's disease, depression, and stroke) and immune (chronic inflammatory diseases) systems can be mitigated by PA (Mattson,

2012; Walsh et al., 2011), an understanding of how PA bolsters the "plasticity" of neural and immune cells may reveal novel approaches for reducing the burden of these disorders.

How do Acute Responses to Exercise Translate over Time to Training Adaptations and Health Benefits?

Most long-term health benefits conferred by PA are thought to arise from adaptive changes in the activity and/or abundance of proteins involved in specific metabolic, physiological, and biomechanical processes (e.g., mitochondrial respiratory function, calcium cycling, contractile function/efficiency, and fuel use). This is accomplished in large part via shifts in gene transcription and protein translation as well as post-translational modifications. Because the energetic and mechanical challenges imposed by exercise are transient in nature, so too are the ensuing adaptive cellular responses, which occur primarily during the hours following exercise (Booth and Neufer, 2012).

The adaptive increase in any protein associated with repeated bouts of PA will be a function of the half-life of the protein, the transient increase in expression that occurs during recovery from each exercise session, and the potential decrease in expression that occurs between exercise sessions. Proteins with a fast turnover rate (i.e., on the order of minutes to hours) tend to be expressed at low levels normally, increase sharply in response to exercise, but, once the stimulus exposure is gone, return to baseline expression levels before the next exercise session. Conversely, proteins with a slower turnover rate (i.e., on the order of days) tend to be expressed at fairly significant levels at baseline, show only a small increase in response to exercise, but, because of their longer half-life, retain most of that increase in expression by the time the next exercise session occurs (Booth and Neufer, 2012). Thus, only those proteins with a long enough half-life relative to the interval of PA will accumulate over time.

These basic principles of first-order protein turnover kinetics have several important implications in terms of understanding the mechanisms by which PA is beneficial. First, the proteins that undergo cumulative responses to regular PA are in a constant state of flux, the concentration of which reflects the balance between synthesis and degradation at any given time. Second, those proteins with fast turnover rates that show rapid, but unsustained, changes in expression in response to exercise (e.g., transcription factors, activating co-factors, etc.) may be critical to triggering induction of genes encoding for proteins with longer half-lives that undergo cumulative increases with training. Third, for studies involving exercise training, the common practice of waiting 48–96 hr after the last exercise session to obtain the "true training effect" will significantly underestimate the cumulative training adaptations regulated by proteins with half-lives < 5 days.

What Are the Dose/Response Relationships that Maximize Specific Health Benefits?

The first-order nature of how cells adapt to an intermittent stimulus raises a number of obvious questions pertinent to defining the molecular and cellular mechanisms of PA-induced health benefits. (1) What are the dose-response parameters (intensity, duration, frequency) that maximize responses over time? (2) How do dose-response relationships change over time? (3) What are the optimal dose-response parameters to sustain PA-induced health benefits? While progress has been made (Bateman et al., 2011; Slentz et al., 2011; Slentz et al., 2007), these questions remain a significant challenge to address in humans.

The goal of any PA program is to maximize the dose/response specific to the long-term objective(s) (e.g., reestablishing energy balance, improving cardiorespiratory capacity, increasing muscle and/or bone mass/strength, improving cholesterol/lipoprotein profiles, etc.). This presents three major challenges: (1) knowing exactly what to measure, (2) knowing when to make the measurement, and (3) connecting that measurement to a well-defined health outcome. Identifying a specific protein(s) representative of the physiological process of interest and having the technical means to quantify the content and/or activity of that protein are pre-requisites to the first challenge. Having some indication as to whether that protein is being regulated during or after exercise is a pre-requisite to the second challenge. Designing and implementing studies to translate or "connect the dots" to a physiological outcome/health benefit is a pre-requisite to the third and most difficult challenge.

A number of studies have focused on pre-translational approaches and documented transient post-exercise increases in transcription rate and/or mRNA content of genes that encode for transcription factors, metabolic transport and control proteins, and enzymes associated with oxidative metabolism (Louis et al., 2007; Mahoney et al., 2005; Perry et al., 2010; Pilegaard et al., 2000, 2003). While this reductionist approach has revealed important aspects of how cells acutely respond to exercise, very little is known as to how these molecular responses ultimately translate to health benefits, which in turn limits the ability to determine the optimal dose required to maximize the acute response for specific long-term desired outcomes.

What Biological and Environmental Factors Likely Mitigate the Acute and Adaptive Responses to PA?

The preceding sections provide the basic framework that will be needed to develop an integrated understanding of how the network responds and adapts to PA to affect improvements in overall health; that is, identifying the various vertical levels within the human body at which control is exerted and the molecular mechanisms that regulate hierarchical control within and between levels to maintain systemic homeostasis (i.e., Tier 1, Figure 1). This establishes the baseline upon which the influence of all potential inherent and acquired mitigating factors within an individual must be determined (Tier 2). For example, it is known that certain acute and adaptive responses to PA diminish with advancing age (Durham et al., 2010; Rivas et al., 2012, 2014). Other baseline characteristics that may influence the responses to PA include the initial level of fitness, age (developmental stage), sex, genetic/epigenetic factors, nutritional state, the microbiome, etc. Within an individual, changes in health status due to pregnancy, prescription drugs, and/or various disease states (cancer, diabetes, cardiovascular disease, COPD, etc.) are likely to impact the molecular and cellular mechanisms regulating the acute and adaptive responses to PA, and thus ultimately the health benefits.

Mitochondrial Response to PA as an Example

Dating back to the seminal work of Holloszy, who first observed that skeletal muscle from treadmill-trained rats express higher levels of mitochondrial proteins (Holloszy, 1967), molecular and functional remodeling of muscle mitochondria has remained a focal point of exercise research. Exercise training activates

mitochondrial biogenesis in skeletal muscle, augmenting overall mitochondrial density and oxidative phosphorylation capacity by as much as 2-fold (Hood et al., 2011). Moreover, PA affects mitochondrial quality as well as quantity, and recent studies suggest that the functional properties of these organelles are much more heterogeneous and dynamic in nature than previously appreciated (Jacobs and Lundby, 2013). Interestingly, PA-induced mitochondrial biogenesis also occurs in tissues other than skeletal muscle, including brain (E et al., 2013; Steiner et al., 2011), liver (Boveris and Navarro, 2008; E et al., 2013; Navarro et al., 2004), adipose tissue (Laye et al., 2009; Sutherland et al., 2009), and kidney (Navarro et al., 2004), providing evidence that exercise also increases metabolic demand in these tissues and/or stimulates inter-organ crosstalk. At the cellular level, alterations in energy charge (ATP/ADP ratio), intracellular Ca^{2+}, reactive oxygen species, and redox state have all been implicated as retrograde signals coordinating the induction of nuclear- and mitochondrial-encoded genes needed for mitochondrial biogenesis (Hood et al., 2011). Over time, the increase in mitochondrial density, coupled with increased capacity of the heart and augmented blood flow, lessen the metabolic disturbance to homeostasis induced by each bout of PA, increasing the efficiency of energy utilization and enhancing the overall endurance capacity. Mitochondrial density is an important regulator of overall cellular function (Picard et al., 2014) and, at the whole-body level, contributes to cardiorespiratory fitness (i.e., measured as maximal oxygen consumption; VO_2 max), which is among the most powerful predictors of morbidity and mortality (Kaminsky et al., 2013). Given that reduced mitochondrial function has been identified as a common feature of many chronic diseases, PA-induced mitochondrial biogenesis is widely viewed as a likely contributor to improved overall health and reduced mortality in active populations. Importantly, however, although a large body of correlational data supports a strong link between oxidative potential and health outcomes, direct experimental evidence of cause and effect remains sparse.

Resources and Research Needed to Potentiate the Discovery of the Mechanisms for the Health Benefits of PA

Controlled Clinical Trials with Standardized PA Interventions and Measures

Large Multi-site Clinical Trials. The field has gained valuable insight from a few important multi-center exercise/lifestyle intervention trials (e.g., HERITAGE Family Study [Bouchard et al., 1995], ACT, HF-ACTION [Whellan et al., 2007], DPP [The Diabetes Prevention Program, 1999], Look AHEAD [Ryan et al., 2003], and LIFE [Fielding et al., 2011]). Results of HF-ACTION, for example, were sufficiently compelling to change clinical practice, as heart failure patients are now eligible for insurance-sponsored cardiac rehabilitation. This emphasis on improving clinical care and public health is invaluable, but large trials adequately sized for clinically relevant outcomes have not collected sufficient functional and molecular data to further understanding of the mechanisms responsible for the effectiveness of PA. Such analyses typically require more sophisticated equipment and expertise and thus present significant logistical challenges for large-scale implementation. Nevertheless, some inroads have been made as a recent study suggests that the biological response to exercise is at least in part determined by multiple genetic alleles with small effects (Bouchard et al., 2011). For these reasons, large, long-duration exercise intervention trials are needed for studies of gene-PA interaction. In addition, large multi-centered controlled intervention studies with creative study designs and flexible approaches are needed to better define the optimal combinations of exercise modes, intensity, and duration for specific health outcomes. Finally, large studies are needed that employ extensive phenotyping, behavioral monitoring, and well-annotated appropriate biological specimen collection for multi-omics studies to systematically interrogate novel molecular mediators of the systemic responses to exercise, especially in the context of various biological conditions. Knowledge of molecules and pathways that transmit the benefits of PA will enable development of potential novel therapeutic approaches.

The incorporation of mechanistic approaches into PA clinical trials will require (1) establishing an infrastructure of clinical trial sites with expertise in exercise physiology, sufficient specialized exercise equipment, and standardization of protocols and (2) populating subsets of those sites with the specialized analytic equipment, technical expertise, and quality control standards needed to incorporate mechanistic outcome measures into adequately sized clinical trials. Regarding the first challenge, the new NIH National Center for Advancing Clinical Trials has a variety of activities through the Clinical Translational Science Award centers designed to improve multisite and multidisciplinary clinical trials. In addition, the Patient Centered Outcomes Research Institute (PCORI) is building a number of national networks specifically designed to facilitate and accelerate health benefits from large-population discovery trials. Finally, the National Exercise Clinical Trials Network (NExTNet) program was recently established at the University of Alabama and currently includes 57 institutions (and growing) from across the country (http://www.uab.edu/medicine/exercise/nextnet). Regarding the second challenge, the ability to conduct mechanistic studies, if present at all, has been restricted to those few individualized sites with specialized capabilities and thus has been underpowered in terms of linking outcome measures to health benefits.

Standards to Enhance the Utility and Comparability of Single-Site PA Studies. While a few large datasets from observational and intervention cohort studies exist, data, protocol, and terminology harmonization and standardization across studies have been lacking, therefore making it extremely difficult, if not impossible, to combine existing datasets. Further, most of the human investigations to date have been in isolation (single-site trials) with no systematic plan for integrating multiple single-site studies for broader applicability. A concerted effort to standardize (1) common protocols and methods of PA and fitness assessment in different patient populations (e.g., healthy young to middle age, pediatric, elderly, specific disease, etc.) and (2) collection and processing of biological samples would foster collaborative research and enable the combining of datasets across sites and studies. Clinical research would also be strengthened by more private/public partnerships to enhance development and accelerate access to the most cutting-edge PA assessment tools, such as wearable unobtrusive accelerometers, GPS trackers, HR monitors, and other technologies. Finally, continued development of non- and/or minimally invasive technologies capable

of providing more mechanistic outcome measures that can be scaled to multi-site trials is needed to facilitate efforts aimed at identifying the molecular and cellular mechanisms responsible for PA-induced health benefits.

Exercise-Drug/Device Interactions. Exercise can substantially influence pharmacokinetics (Lenz, 2011), and pharmacotherapy can influence adaptations to PA (Malin et al., 2012, 2013; Mikus et al., 2013), but the available data are limited. Thus, there is a need for well-designed and appropriately powered studies to understand the interactions between common drugs and PA, including studies of drug metabolism and determinants of synergism or antagonism. Even for some of the most commonly prescribed medications, potential exercise-drug interactions that are either favorable (additive or synergistic) or unfavorable have not been explored. Studies to evaluate these interactions and identify the molecular predictors of responses are critical for the field and would provide a basis for more strategic and targeted therapies for patients. Integral to this effort should be research on drug efficacy in physically active versus inactive individuals, as well as potential differences in pharmacologic effects between those expressing high versus low fitness levels. Similar approaches are needed to better understand the interactions of exercise and PA behaviors with medical devices. In general, clinical trials designed to test pharmaceuticals and devices should, at a minimum, measure physical activity or preferably employ a PA intervention arm. The potential for scientific advancement is profound and nearly untapped.

Exercise Psychology/Behavioral Medicine. A major obstacle to the widespread clinical application of exercise and PA as effective disease-prevention or treatment measures is the low rate of adherence to prescribed exercise or habitual PA behaviors. Although a large investment has been made in behavioral research to improve compliance, the underlying mechanisms of low adherence and exercise tolerance are poorly understood and will require transdisciplinary biological and neurobehavioral research to identify and understand both genetic and non-genetic determinants of exercise and PA adherence and lasting lifestyle modification.

Discovery of the Molecular Transducers of Adaptations to PA: Role of OMICS Technologies

A clear mechanistic understanding of the interplay between PA and health requires comprehensive knowledge of network dynamics in the context of sedentary, active, and post-active states. To this end, the advent of "omics" technologies, such as genomics, epigenomics, proteomics, and metabolomics, affords tremendous capacity to investigate tissue/cell-specific molecular responses to PA. Application of these technologies not only permits rigorous characterization and mapping of the physiological network, but also creates new opportunities for unbiased discovery of novel molecules and regulatory control mechanisms that are uniquely engaged during and after PA.

Despite enormous potential, these technologies are still continuing to evolve, and the logistical and methodological caveats of this approach must be recognized and carefully considered. For example, application of proteomics and metabolomics technology to exercise physiology research has been confounded by a number of challenges, including a lack of standard protocols and molecular standards, the number of tissues involved, and the transient nature of the response to exercise. All such targets require validation testing. However, current tissue-specific, cell-based assays, while catalytic in some disciplines, may not be suitable for screening molecules relevant to exercise outcomes because cell culture systems (a) cannot be artificially exercised and/or (b) are missing the integrative element of "…..kines," hormones, neural input, gravity, temperature changes, etc. present in the intact organism.

The rate-limiting impediment to discovery of molecular transducers and their function is not the "omic" core technology, but the bioinformatics to extract the most useful signals and generate the most appropriate biological interpretation, including those associated with exercise adaptation. Robust computational and bioinformatics analytical tools allowing integration of large datasets from a multiplicity of "omics" platforms with in vivo exercise physiology assays and measurements would contribute greatly to our understanding of the response to acute bouts of exercise and long-term adaptation to regular exercise exposure. In this regard, the development of detailed molecular profiles in cells and tissues in response to acute and chronic exposures to exercise ("the exercise responsomes") would provide the benchmark against which all other exercise-related conditions, including aging, sex differences, disease states, etc., could be compared for commonality and specificity. The development of procedures for data and resource sharing should also be encouraged and may include, for example, exercise/PA research repositories or coordinating centers; addition of expertise and resources to permit further analyses of data and/or biospecimens collected in existing trials (e.g., a collaborating institution with specific "omics" expertise); development of searchable databases to facilitate inter-institutional partnerships; integration of measures of PA and fitness into both pre-clinical investigations and clinical trials focused on drugs/devices; and collection of both biological samples and behavioral outcomes into all exercise/PA intervention studies. Such resources will enable the field to more rapidly identify biomarkers of exercise adaptation or PA behavior.

Mechanistic Research in Animal and Cell Models

Animal models have proven abundantly useful and relevant in understanding the health effects of exercise in humans. For example, animal research has been critical for discovery of basic molecular, cellular, and integrative mechanisms of action. However, with the exception of a few attempts in recent years, there have been limited efforts to integrate human clinical trials with basic biological investigations in lower animals. Validated exercise animal models that are relevant to human growth, development, aging, and disease are needed. Establishing mechanisms for such seamless integration would significantly propel the field forward. Also lacking are appropriate and established animal models of aerobic and resistance training, and cellular systems that mimic physiological conditions during or after exercise. These models could be used to test the role of novel molecules identified in discovery-based cell culture studies.

Exercise Physiologists Trained in Integrative Biology and Interdisciplinary Teams

Exercise physiologists bring a unique skill set and perspective to the study of complex multi-system organisms. The future of PA research depends on a sufficient pool of appropriately trained scientists with strong expertise in exercise physiology and state-of-the-art technologies. The field therefore requires a plan to

rebuild the discipline while also developing the next generation of integrative physiologists. Resources are needed not only to fund new trainees, but also to restructure current programs in a manner that combines studies in integrative physiology and bioenergetics with training in basic biochemistry, cellular and molecular biology, and bioinformatics. Additional resources are needed to establish mechanisms for assembling and supporting interdisciplinary teams that are able to catalyze and sustain exercise research. The field would likewise benefit from a program to support a multi-site consortium of exercise scientists with complimentary expertise and resources that together are well positioned to tackle the large, challenging problems relevant to the overarching mission.

Synopsis and Conclusions

Deciphering the mechanisms that underlie the acute and adaptive responses to PA holds enormous discovery potential for human health and medicine. A major step toward this goal will be to identify all molecules that are altered in key organs/tissues in response to PA (i.e., the "exercise responsome"). Understanding how these acute responses integrate over time and link to health outcomes will provide novel insight on the mechanisms critical to maintaining health, uncover potential novel mechanisms contributing to disease processes, and identify potential new therapeutic targets to aid in the prevention and treatment of disease. Ultimately, this research will catalyze the advent of personalized exercise medicine by promoting health through improvements in PA prescriptions, adherence, and adjunct therapies.

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