Cellular and molecular exercise physiology: talking about past, present and future

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Abstract

Exercise physiology has evolved as a main area of investigation, in which the central goal is to better understand how the physiological systems respond to an acute bout of exercise and how these systems adapt to different types of exercise training. For many years and until now, exercise physiology field have been grounded in the fundamentals of biology and human physiology. However, during the last century, scientific knowledge has changed our understanding of biological sciences, allowing the integration of different areas, and increasing the focus on many sub-areas like cellular and molecular investigation. The development of new experimental techniques in the last years provided detailed information about cell structure and function and, as a result, we could better understand not only the human body physiology, but also many diseases and their pathophysiology. Therefore, this present review intends to discuss more about cellular and molecular exercise physiology area, focusing on historical and methodological approaches, and highlighting the future perspectives for scientific knowledge and their practical application in health and exercise.

Keywords: Exercise; Metabolism; Skeletal muscle; DNA; Protein; Molecular techniques.

Introduction

Since ancient times, physical activity and body movement have assisted humans in their development and survival. The growing interest in understanding multiple mechanical, physical and biochemical functions of the human body culminated in an emergence of a new subarea of Biology, the Physiology. The term physiology comes from the Greek "physis" = nature, function, and "logos" = word or study. Physiology intends to explain how vital functions happen, understanding their adaptations to the different stimuli coming from environment.

Exercise physiology, in turn, is a knowledge field derived from the major subject, Physiology, and aims to study how our body systems react and adapt to the stress imposed by physical exercise[1,2]. In general, exercise physiology field aims to study acute and chronic adaptations to exercise that coordinate the integrated functioning of several organic systems[3]. *Acute adaptations* resulting from physical exercise are known as adaptations occurring during and/or immediately after a single bout of exercise. *Subacute adaptations* refers to adaptations occurring within the first or second hours after a single bout of exercise. In contrast, chronic adaptations to exercise are observed 24 hours post exercise session. Thus, long-term adaptations induced by exercise training are due to the accumulation of acute and subacute effects of each physical exercise session. In addition, the intensity, duration and frequency of exercise are determining factors for functional responses in the whole body[4].

The interest in studying exercise physiology has grown significantly in the last four decades. New generations of scholars have come up with a single goal; to study the exercise based on a powerful scientific and physiological perspective. It's worth emphasizing that

physical exercise can be approached in different ways in scientific research. Some researchers use exercise as a stressor to the body and, in this case, it has a supporting role. For researchers of exercise physiology, exercise is the object of study where the knowledge of basic physiology is used to explain the body's responses to physical exercise.

The exercise physiology covers four main distinct fields: a) human performance; b) quality of life or well-being c) growth and development, and d) prevention and rehabilitation[3]. Considering this, exercise physiology can be applied both in the elaboration and organization of resources, in methods and programs aimed for athletes' physical performance, as well as in the maintenance and promotion of health.

Furthermore, in the last decades, the exercise physiology area extended even more its application field, including cellular and molecular studies to the knowledge of physical exercise. In this sense, it is relevant to mention that exercise physiology area involves two main types of research: the applied and basic researches. Applied research tests different physical exercise characteristics in different populations, and evaluates the effects of these differences on acute and chronic responses of the organic functions; while basic research aims to investigate the basic processes by which exercise adaptations occur with a deep investigation of biochemical, cellular and molecular mechanisms underlying these adaptations[5]. Therefore, to fully explain the acute and chronic effects of exercise, exercise physiology researchers need to deep investigate the adaptations occurred in response to exercise, revealing cellular and molecular mechanisms and explaining the applicability of these findings.

Exercise physiology: a historical perspective

The importance of physical exercise for health and well-being was always evident in many cultures. In Ancient Greece, Hippocrates has already advocated that any deficiency in food or physical activity would result in body sickness[6]. Therefore, recommendations were made in medical treatises believing that physical exercise would be able to expel impurities and body waste, promote tonus and muscle strength, and improve metabolism. In fact, around eight-seven treatises compilation, known as "Corpus Hippocraticum", was attributed to Hippocrates on Greek medicine[7].

The prescription of physical exercise for therapeutic purposes overlapped with the origins of exercise physiology. In 1960, a Spanish physician, Cristobal Mendez published one of the first books describing the benefits and importance of physical exercise[8]. However, the first specific book of the area was published only in the end of 19th century, "Physiology of Bodily Exercise" by Frenchman Fernand LaGrange[2]. According to Tipton, 2014, it was the first time that a publication addressed the exercise physiology subject[9].

The first physical education program at Amherst College was founded in 1860, and one of the department's first professors was Dr. Edward Hitchcock Jr. from Harvard Medical School, who has collected anthropometric and physiological data from students[10]. The programs of physical education were established to educate and train graduates as athletic clubs and gymnasia directors, besides being part of medical student's formation[3, 10]. In addition to the basic sciences, such as human anatomy, physiology and biochemistry, students were also engaged in a formal course in exercise physiology that addressed the effects of different exercises on lung capacity, muscle contraction and fatigue, changes in blood supply with exercise, energy metabolism and reaction time. Despite the emergence of other laboratories focused on exercise physiology, the Harvard Fatigue Laboratory was, for 20 years (1927-1947), the largest contributor for exercise physiology development as a major subject in the United States[3]. The Harvard Fatigue Laboratory was coordinated by Dr. Bruce Dill, conducting studies in energy metabolism, cardiovascular and hemodynamic responses to exercise, oxygen consumption and utilization of energy substrates, exercise recovery, cold and altitude, aging and health[3, 11]. During this period, more than 300 peer-reviewed articles were published on topics related to exercise, contributing to the establishment of exercise physiology laboratories in Departments of Physical Education and Medical Schools in United States.

Importantly, there was also a significant European contribution to the evolution of exercise physiology, highlighting the Nobel prize winners: the danish researcher, August Krogh (1920); the british researcher, Archibald V. Hill (1922); and the german researcher, Otto Meyerhof (1922) for their researches in skeletal muscle physiology and energy metabolism fields[12].

In Brazil, the exercise physiology began in the 1970s at University of Brazil, currently the Federal University of Rio de Janeiro - UFRJ. The exercise physiology laboratory was coordinated by Professor

Dr. Maurício Leal Rocha, where admitted university students had undergone to anthropometric measurements before starting classes season[12]. In São Paulo, specifically at the School of Physical Education and Sport of the University of São Paulo, Superior School of Physical Education at that time, professional preparation was the main focus of the school, and it could not be different since there was a need for physical education teachers. In parallel with professional preparation, the school started its research activities with the "Integrated Center for Research in Physical Education (CIPEF), with special focus on exercise physiology field. The CIPEF was created by Professor Dr. Mário de Carvalho Pini and coordinated by Professor Dr. Maria Augusta Peduti Dal'Molin Kiss, both physicians and part of the faculty members. CIPEF has developed the first researches in exercise physiology on Brazil, and graduated some of the main researchers in this field from our country[12, 13]. Posteriorly, the CIPEF lab became the "Laboratory of motor activity physiology", coordinated by Professor Dr. Carlos Eduardo Negrão, who had as main topic of research the acute and chronic effects of physical exercise on the cardiovascular system. It is important to highlight the worldwide importance that this research group reached when they published a study demonstrating the beneficial effects of aerobic exercise in heart failure patients[14].

In the past, the parameters analyzed in exercise physiology laboratories included: ergometry, cardiovascular adaptations (blood pressure and heart rate), calorimetry (oxygen consumption and substrate utilization), aerobic capacity and power, which were evaluated by field research and laboratory submaximal tests, the anaerobic capacity (Wingate's test and vertical jumps, lactate production and ventilatory responses to exercise), and muscle function (strength measures and muscular resistance). Moreover, body composition (by bioimpedance) and regulation of body temperature were also studied. All these methodologies mentioned above have still been explored until today.

Clearly, many techniques have been improved and technology has contributed to more accurate results. Furthermore, in the 90's decade, the interest in cellular and molecular adaptations in response to physical exercise significantly increased. Currently, cellular and molecular exercise physiology is a well-established research area that contributes to scientific knowledge in exercise field, and have been increasingly developed with the support of technology and the advancement of biology techniques[15].

Taking this into consideration, a more detailed discussion about the main biochemical, cellular and molecular biology techniques and their impact on exercise physiology field will be discussed.

Exercise physiology: a biochemical, cellular and molecular approach

The progress in the human biology knowledge promoted remarkable changes in exercise physiology area. As mentioned before, exercise physiology studies were mostly focused on macro physiological responses of organs and tissues during exercise and after exercise training. However, this major perspective has been expanded since enzymatic biochemistry became available and, consequently, the metabolic processes and their adaptation in response to exercise were better understood[15].

In 1966, Jonas Jergström and Eric Hultman published a seminal work, in which they developed the needle biopsy technique, demonstrating that exercise enhances skeletal muscle glycogen resynthesis, and allowing the study of other skeletal muscle adaptations to exercise[16]. Also, in 1967, John O. Holloszy was the first scientist to show that an exercise training program can increase the mitochondrial content and mitochondrial oxygen uptake in rodent's skeletal muscle, being one of the pioneers in the study of biochemical and metabolic responses during exercise[17].

Currently, Bruce Spiegelman, a renowned scientist worldwide, the first to find the key regulator of adipogenesis, PPAR-γ, said: "Exercise is not magic. It's a series of chemical reactions involving muscle. The benefit of exercise, like most things in nature, will eventually come to down to chemistry and biochemical reactions"[18]. In fact, today it is well known that a single bout of exercise can modulate many organelles, metabolic and molecular regulators, like sarcoplasmic reticulum, calcium ion (Ca^{2+}), mitochondria, reactive oxygen species (ROS), ATP turnover, partial pressure of oxygen (PiO_2), enzymes, proteins and transcription factors[4]. In long term, all these intracellular responses induced by regular exercise will adjust the body physiology, increasing performance and health[4, 15].

In order to expand this knowledge in the exercise physiology field, many scientific methods have

been improved or developed. Among the methods currently used to meet this purpose, some have been gaining attention in our research group. One of them is the *ex vivo* evaluation of skeletal muscle function in an organ bath system. This method consists in evaluating the contraction force after electrical stimuli of a specific harvested muscle that is attached to electrodes by the proximal and distal tendons and bathed by a Krebs solution. After performing a protocol with several consecutive electrical stimuli, it is also possible to evaluate how fatigable and resistant the muscle is. Besides that, the *ex vivo* organ bath allows us to test in real time the effects of drugs, substrates or any other component that could be added to the system and that could promote acutely modifications on the muscle contractility[19, 20].

Beyond the muscular function, the well-known enzymatic analyzes like hexoquinase and citrate synthase activity, and the maximal oxygen consumption (VO_2 max) as markers of muscle metabolism[21, 22], the direct measurement of mitochondrial respiration has been widely used to evaluate bioenergetics and mitochondrial function and efficiency in different organs and tissues. The mitochondrial oxygen consumption rate can be analyzed by high-resolution respirometers/oxygraph in different cell types, in isolated mitochondria from several human and animal tissues and also in permeabilized fiber bundles isolated from muscles[23-25]. Application of this assay provides important information about oxidative phosphorylation (OXPHOS analysis), a component of metabolic phenotyping, extending conventional bioenergetics to the level of mitochondrial physiology for functional diagnosis in health and disease[26].

The methods described above certainly can help scientists make new discoveries about how our body work under exercise and how exercise connects organs and systems. Indeed, over the last years there have been significant advances in the scientific knowledge and technologies, which uncovered networks of signaling pathways and regulatory molecules that coordinate adaptive responses to exercise[4].

As an extension of exercise physiology field, cellular and molecular exercise physiology studies can connect physical exercise with its responses, adaptations and benefits, clarifying the big black box that lies between these conditions (FIGURE 1). In this context, Frank Booth published, in 1988, the first article about advances and perspectives on cellular and molecular exercise physiology, bringing this topic for discussion in the literature and highlighting the importance of molecular biology for exercise field[27]. Currently, the molecular biology techniques keep helping us to uncover the cellular and molecular mechanisms underlying exercise adaptations.

As defined by Nature at Nature.com, "Molecular Biology is the field of biology that studies the composition, structure and interactions of cellular molecules such as nucleic acids and proteins that carry out the biological processes essential for the cells functions and maintenance". Most of the molecular biology techniques were developed after the "Central dogma of molecular biology", first stated by Francis Crick in 1958 and re-stated in a Nature paper published in 1970[28], five years after the discovery of DNA structure by him and James Watson[29]. The classic view of the central dogma of biology states that "the coded genetic information hard-wired into DNA is transcribed into individual transportable cassettes, composed of messenger RNA (mRNA); each mRNA cassette contains the program for synthesis of a particular protein (or small number of proteins)"[30]. To make it simple to understand, the central dogma of molecular biology explains the flow of genetic information, from DNA to RNA, to make a functional product, the protein.

After this important discovery, two of the main molecular biology techniques were developed, Western Blot for detecting protein expression[31] and Northern blot, later replaced by PCR (Polymerase chain reaction) for detecting mRNA levels[32], respectively. In the last 30 years, these two methods have been widely used and explored by scientists from around the world, and were the basis for many scientific articles in biology field[33-36]. More recently, Western blot and PCR have also been supporting the exercise physiology area, helping physical exercise researchers to better understand signaling pathways modulated by acute and chronic exercise in health and disease[4, 15].

However, as a result of genomic studies in recent years, many exceptions to the classic view of central dogma of molecular biology are now known. For example, much of the DNA that does not encode proteins can encode many types of functional RNAs[37, 38], like microRNAs. MicroRNAs are small non-coding RNA molecule (containing about 22 nucleotides) that promotes RNA silencing and acts as a post-transcriptional regulator of gene

expression, targeting mRNAs for cleavage or translational repression[39, 40]. Recently, microRNAs are becoming focus of study of some exercise scientists, who have demonstrated that cellular adaptations and responses to physical exercise can be modulated by specific microRNAs[41-43].

The transcription process, conversion of DNA to RNA, can also be regulated by some proteins, called transcription factors, which bind to a gene or to specific DNA sequences, orchestrating gene activity and transcription apparatus function[44]. The key concepts of transcriptional control were first established in prokaryotic organisms in 1961 by Jacob and Monod[45], and currently it is known that a single gene can be regulated in a range of ways, from altering the number of copies of RNA that are transcribed, to the temporal control of transcription process[44, 46]. Considering this, the peroxisome proliferator activated receptor gamma coactivator-1alpha (PGC-1α) is a well-known transcription factor, discovered in 1998 by Bruce Spiegelman[47], and known to be a key regulator of many proteins and other transcription factors, as the nuclear transcription factors NRF-1 and NRF-2, and the mitochondrial transcription factor A (Tfam), responsible for mitochondrial DNA replication[47, 48]. Because of the considerable role of PGC-1α on tissues' metabolism and mitochondria function, after its discovery, scientists started to investigate the relationship between PGC-1α and exercise, and they were able to show that only a single bout of aerobic exercise can quickly increase the activity and the expression of PGC-1α on skeletal muscle [49-51]. Today it is well known that PGC-1α plays a central role in the activation of nuclear and mitochondrial genes that are required for mitochondrial biogenesis induced by a physiological stimuli as exercise, and it has been one of the main focus of several studies in cellular and molecular exercise physiology area[4].

However, not only mRNA transcription can be modified inside the cell, but many proteins, after translation, can undergo into a wide variety of chemical modifications, that are called post-translational modifications[52]. The post-translational modifications can be, among others, phosphorylation, ubiquitination, methylation, acetylation, glycosylation, oxidation and nitrosylation, and many of these are critical to protein function[53]. There are several methods available today that can be used to predict or evaluate the post-translational modifications in proteins of many tissues, and they go from a simple protein expression by Western Blot, and immunohistochemistry, to elegant and sophisticated methods like protein crystallography, fluorescence resonance energy transfer (FRET) and mass spectrometry[54-57]. In this context, exercise researchers have been demonstrating that exercise significantly promotes post-translational modifications in proteins, changing their function, which plays a role in the responses and adaptations to exercise[58-60].

In contrast to the short past, where we could only evaluate the expression of a limited number of proteins or genes, nowadays we can simultaneously compare the total set of genes, mRNA and proteins of several biological molecules, and this is possible with the "omics" technology (the English-language neologism "omics" informally refers to a field of study in biology ending in -omics, such as genomics or proteomics. The suffix -ome, as used in molecular biology, refers to a totality of some sort; similarly, "omics" has come to refer generally to the study of large, comprehensive biological data sets)[61]. Genomics, transcriptomes, metabolomes and proteomics are large-scale techniques that allow scientists to have an overview of the effects of their experimental conditions on expression profiling of genes, mRNAs, metabolites and proteins, respectively. In addition to the expression of well-known molecules, the large-scale methods enable the discovery of new targets (genes and proteins) that had not yet been studied under certain experimental conditions, opening several new windows of possibilities for researchers. For example, today there are many new targets that have been studied for drug development and that were discovered by a large-scale approach[62-64]. Thus, the discovery and the better understanding of new genes and proteins can assist the treatment of many diseases through the development of novel therapies that can have these molecules as potential targets. For exercise research, these new targets can help scientists to uncover the big puzzle that is composed of acute responses and adaptations to exercise, supporting the application of exercise programs for health promotion and for prevention and treatment of several diseases.

Another recent and promising technology for molecular biology field and also for exercise studies is the CRISPR–Cas9 technique that can promote gene/genome editing. Developed in 2012, this technology allows cell's genome to be cut at a desired location, allowing existing genes to be removed and/or new ones to be added[65, 66]. Despite the bioethical concerns behind it, CRISPR–Cas9 genome editing

technique leads to a new era in molecular biology, arising many potential applications, including in medicine[67, 68]. For exercise field, we can certainly expect future applications of CRISPR technology that could go from edition of major genes activated by exercise or physical inactivity for promotion of health and adjuvancy in diseases' treatment, to genome edition for an enhanced physical performance.

It is important to highlight that the methods and technologies discussed so far are only a part of many other molecular biology techniques, such as gene knockin, gene knockout, transgenic mice, different cell cultures, use of cloning vectors, animals' cloning, gene therapy, ChIP-sequencing (method used to analyze protein interactions with DNA), and pharmacogenetics (drugs designed to address specific genes or DNA mutations) that have also been highly helpful for the scientific advances and the development of cellular and molecular exercise physiology researches[21, 69-71]. Therefore, the application of novel approaches in biochemistry, cellular and molecular biology in exercise physiology, as aforementioned, allows us to characterize and reveal the complexity of intracellular signaling networks triggered by exercise.

Despite all this, some questions may be remained: Why studying cellular and molecular exercise physiology matters? Why is so important to better understand the mechanisms by which exercise promotes adaptations in our body? And what is the relevance of this type of knowledge for Physical Education?

To answer these questions, first we should clarify that understanding exercise mechanisms as a way of building knowledge is not the only goal of cellular and molecular exercise physiology researchers. For sure, knowledge improvement is extremely important for Science in a general way, without any distinction of area, and this is the reason why basic research needs to exist. Considering this, below are other reasons why we believe that cellular and molecular exercise physiology research is important:

1) As stated by Frank Booth, 2007, "molecules drive policies for better health"[72]. This means that the discovery of new molecules associated with physical inactivity (being the cause of diseases), or molecules associated with the benefits of exercise (promoting health), can encourage governments to increase public policies implementation for physical activity promotion. Also, providing more information for society about the reasons why physical exercise is beneficial or why physical inactivity is detrimental, could convince more people to engage in an exercise program, since we all know that exercise is good, but the majority of the population still does not practice it.

2) The knowledge obtained from cellular and molecular exercise physiology research can be applied in prevention and pharmacological treatment of diseases, especially for those who are unable to exercise, such as paraplegics, the frail, people with muscular dystrophies, or healthy with medical conditions preventing physical activity. Drugs will never completely replace exercise practice for those individuals who can undertake a physically active lifestyle, but they can work well as an adjuvant therapy for several syndromes and diseases[72-74].

3) New molecules targets that are triggers for diseases associated with physical inactivity could be used as early prognostic markers in the future, allowing the early disease detection and, consequently, making possible earlier clinical intervention, and increasing the chance of successful treatment[72].

4) Understanding how our body responds to exercise is necessary for the improvement of physical performance and health. Therefore, expanding cellular and molecular exercise physiology knowledge should broaden our minds, helping the development of novel exercise programs and improving traditional training protocols for performance, prevention and rehabilitation[15].

5) Last but not least, the use of molecular biology, as the combination of transcriptomic and genomic technologies, is a powerful predictor of athletic performance and genetic variations, which can be associated with better athlete suitability for different types of sports/exercise (e.g. endurance, strength or speed). Also, this approach could predict the athletes vulnerability to sports-related injuries and their specific nutritional requirements, overcoming problems arising from intense exercise trainings, from the pressure under competitions, and avoiding

potential health risks (hypertension, cardiovascular diseases, and musculoskeletal injuries) related to exercise, training and competition[75-77].

Taking these arguments into consideration, we can say that the study of cellular and molecular mechanisms in exercise area is extremely relevant and should be expanded by exercise physiologists and researchers from Physical Education Universities. Below, FIGURE 1 illustrates and summarizes the information and the discussion presented in this article.

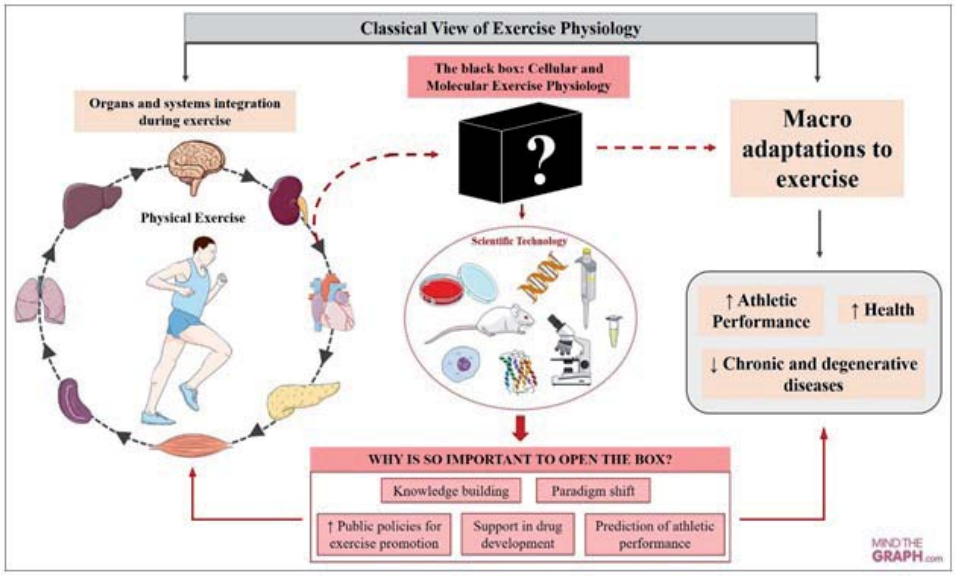

FIGURE 1 - The classical view of Exercise Physiology and the Cellular and Molecular Exercise Physiology: a perfect marriage. Cellular and molecular exercise physiology studies, assisted by molecular biology techniques, can support the improvement of health and exercise performance, corroborating or changing the theoretical basis of the classical view of Exercise Physiology.

Conclusion and perspectives

Although there have been progress in molecular biology techniques and in scientific knowledge, much work needs to be done in order to elucidate the meaning of the findings. The study of mechanisms, specially the large-scale analysis like genomics and proteomics, usually provides us a high number of different information that needs to be interpreted within specific contexts. Using an analogy, mechanisms discovery is comparable to a big mixed puzzle that needs to be solved. This includes the investigation of function and regulation of single genes and proteins, and the elucidation of how they interact with each other and with the environment under certain conditions[15].

Therefore, the next challenge for molecular biology and cellular and molecular exercise physiology researchers consists in better understanding and interpreting the "big data" provided by scientific technology and, consequently, applying this knowledge in the promotion of health and exercise performance.

Considering this, we can conclude that cellular and molecular exercise physiology field is an important branch of its major subject, the exercise physiology, collaborating for the scientific enrichment in this area, working on the frontier of knowledge, and opening the innovation doors for exercise sciences and for Physical Education as a discipline and carrier.

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