

Mini-Review

The importance of animal studies in Exercise Science

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Abstract — The validity and relevance of research with animals for the development of knowledge in Exercise Science have for long been discussed. Given the complexity of the biological systems, the use of animal models offers a significant contribution to uncover new findings about acute and chronic effects of exercise, particularly when these studies in humans have limitations and ethical implications. There have been notable findings using experimental animals either in basic sciences or in clinical studies involving physiology, pharmacology, genetic, biochemistry, urology, endocrinology and cancer. This article presents a brief review of scientific research using animal models with a focus on exercise training as an effective tool for the prophylaxis and treatment of different pathological processes, which are the basis of many concepts taught and used in undergraduate courses and graduate programs, as well as in new researches showed in scientific conference meetings in numerous areas of science.

Keywords: animal studies, exercise science, physical education, sport, knowledge evolution.

Introduction

For some time now in Brazil, researchers have been discussing the validity and relevance of using animal models in studies focusing on Exercise Science. It should be noted that most

of the experimental trials undertaken to assess the effects of exercise have so far aimed at evaluating their impact on intact organisms, and the few trials using human models have been generally said to be more reliable. However, the more frequent use of humans in these studies would not be either possible

or desirable in most cases, since they would require a much extended life-long follow-up of humans, which would not be feasible (mainly because of the high percentage of participants expected to drop out), or the use of invasive procedures, which would raise ethical questions. Studies examining the impact of exercise training on function of different cells or tissues are therefore carried out in animals whenever questions about the feasibility of using humans are raised. Besides, researches using animal models are also conducted for further understanding of the biology of the animals themselves, as well as improving their health.

Studies using animal models in which one can control environmental, psychological and emotional factors have contributed to scientific fields, such as medicine, pharmacy, nutrition and psychology. These results have been fundamental in developing scientific knowledge and improving the quality of life of people. Given the complexity of biological systems, the use of animal models has provided a significant understanding of the various adaptive mechanisms undergoing acute and chronic physical exercise, particularly when ethical considerations make the use of human models unfeasible.

Thus, this review paper aimed to survey a corpus of studies using animal models, which have grounded many of the concepts taught at Physical Education and Sports undergraduate courses, and have contributed to a range of research studies in this area. The review also aimed to raise awareness among scholars and professionals about the importance of this largely consolidated approach and emphasize fundamental ethical principles concerning the use of animals in building a solid knowledge base on Exercise Science.

Research using animal models in Exercise Sciences: a brief overview

Long time ago studies have been conducted using animals to a better understanding of the structure and functioning of the body. One of the first formal studies using animal models found in the literature was conducted by Harvey (1628), who described that the heart pumped the blood in a circular, unidirectional way, from the arteries to the veins and back to the heart ¹. In 1849, Regnault and Reiset estimated the respiratory quotient (RQ) of animals using a closed-circuit spirometry ². Based on findings obtained from the study, a relationship between body weight and metabolic rate was established, which gave birth to the Surface Area Law and allometric scaling, still currently used.

A hallmark in Exercise Physiology was the first volume of the *American Journal of Physiology*, published in 1898, and edited by Porter, a cardiovascular physiology specialist. Three papers on Exercise Physiology using animal models were featured in this first issue: “*Spontaneous physical activity in rodents and the influence of diet*”, “*Neural control of muscular movement in dogs*” and “*Perception of muscular fatigue and physical activity*” ³. In the following four issues of the Journal (1898-1901), six papers covered topics related to Exercise Physiology from labs using animal models ³. Some years later, other important

Journals emerged: the *Physiological Review*, in 1921; the *Journal of Applied Physiology* in 1948; and later, the renowned *Medicine and Science in Sports*, whose name was changed to *Medicine and Science in Sports and Exercise* in 1980.

Almost a century later in Brazil, the *Revista Paulista de Educação Física* (later called *Revista Brasileira de Educação Física*) was first published in January, 1988. In 1995, the Journal published the first study using animal models: “*Dosagem histamínica muscular em ratos exercitados*”. The *Journal of Physical Education-Motriz* was first published in 1995, and a study featured in this first issue “*Effects of protein-calorie malnutrition in response to acute exercise (single session) – metabolic parameters*” ⁴ data from animal models.

From the 20th century onwards, the knowledge base obtained by animal studies contributed significantly to consolidate Exercise Science. Fletcher and Hopkins, in 1907, were the first to report and account for the anaerobic metabolism *in vivo* of a frog muscle ⁵. Another important researcher in the advancement of Exercise Physiology was Krogh, who was awarded the Nobel Prize in 1920 for determining the blood flow in the frog skeletal muscles during contraction and at rest ^{6,7}.

Hill was awarded the Nobel Prize in 1922 for the discovery of the chemical and mechanical processes involved in muscular contraction. He also used frogs as animal models ⁸. Benedict, who had developed, along with Harris, a formula to estimate basal energy expenditure, published in 1938 a study on the relative caloric expenditure among different species, from birds to large mammals such as the elephant ⁹.

Current knowledge on energy metabolism is owed to Krebs, who described for the first time in 1937 the citric acid cycle (Krebs Cycle). The cycle emerged from studies carried out in the pectoral muscles of pigeons ¹⁰. This discovery remains a hallmark in metabolic biochemistry, and Krebs was granted a Nobel Prize in Medicine in 1953. Later, in 1950, Lehninger and Kennedy, using isolated rat liver mitochondria, demonstrated that the reactions in the citric acid cycle take place in this organelle ¹¹.

By the same time the renowned Swedish physiologist Astrand conducted several experimental studies examining effects of exercise training on hemodynamic and autonomic nervous system in rats ^{12,13}. While Astrand and other researchers prioritized clinical studies, they also undertook many studies using animal models to understand human physiology better.

Tipton, an important researcher in Exercise Physiology, has been working for decades with both humans and animals, investigating the effects of exercise training on heart rate focus on bradycardia ¹⁴. He also studied the effects of training on the blood flow of dogs, and the impact of microgravity on the physical performance of rats ^{15,16}, along with some comparative research ^{17,18}, which contributed towards a fuller understanding of the circulatory system.

Costill is another important American researcher who made use of both human and animal models. His studies shed new light on glycogen storage in skeletal muscle cells of trained rats and variations in water ¹⁹, as well as glucose intake ²⁰, and changes in oxidative capacity, glycogen content and skeletal muscle tone ²¹. The American researcher McArdle carried out

key studies on muscle contractility and oxidative stress using both human and animal models^{22,23}. Saltin, who has long been studying the metabolic process involved in exercise training, assessed oxygen consumption in camels at rest and during exercise²⁴, metabolic responses to maximal exercise²⁵. He also researched the physiological and biochemical responses of horses undergoing exercise training at varying speed and duration²⁶.

In 1998, Kraemer studied the effects of resistance exercise on the bone tissue of rats²⁷. Among his many studies, Faulkner also dealt with contractility and muscle regeneration in older²⁸ and younger rats²⁹. Holloszy, a well-known researcher in the field of skeletal muscle, has been working on animal models for years in an attempt to understand the response of skeletal muscle to exercise training in rats³⁰. Zatsiorsky, a leading researcher in Biomechanics, analyzed the muscle demands and reactions of cats performing consecutive steps³¹.

Several relevant findings were obtained in studies using animal models, such as blood and cardiac disorders, hemodynamic adaptations, changes in the arterial baroreceptors, O₂ consumption and heart rate of trained dogs³²⁻³⁵. Taking together, these studies offered a solid basis for a clearer understanding of the adaptations promoted by exercise training in humans.

Basic electric properties of the myocardium were first found in the ventricle of frogs³⁶, and one of the first descriptions of the processes involved in the ATP degradation in the mitochondria was carried out in rats³⁷. Likewise, the techniques to isolate the nuclei of muscle fibers, the key to understand adjustments promoted by exercise, were firstly tried out in the diaphragm of rats³⁸.

Research using animal models contribute to the understanding of the metabolic mechanisms and the use of glucose during exercise³⁹. Heat and exercise-induced changes in plasma enzymes were detected in studies using dogs. More recently, the mechanism controlling muscle development and growth via myostatin was first explained by studying lineage determination in beef cattle^{40,41}.

The use of animal models was also useful in studying diseases affecting athletes, e.g., cardiac hypertrophy. The first studies in the area estimated the diameter of rat cardiomyocytes presenting cardiac hypertrophy⁴²; the association between cardiomyopathy and the hypertrophy of type II fibers was first demonstrated in dogs, while cardiac hypertrophy following muscle stress was assessed in rats⁴³. Furthermore, genetically modified animals have been used to provide a better understanding of the intracellular pathways signaling activated by physiological cardiac hypertrophy induced by exercise training⁴⁴.

The usefulness of animal models has long been acknowledged in the studies undertaken in the area of Exercise Science, being regarded as a key element in promoting understanding and improving the health status of athletes and the population in general. Animal models are currently widely used and supported by research centers worldwide, including the renowned American College of Sports Medicine (ACSM), which every year awards the most relevant studies and notable researchers⁴⁵. Among the recipients of the ACSM awards, we may find Hagberg, Cooper, Laughlin, Pollock, Dempsey, Wilmore,

Saltin, Costill, Tipton, Faulkner, Holloszy, amongst other great researchers.

Experimental research with animals in the field of Exercise Sciences in Brazil

When compared to other academic areas, research in Exercise Sciences in Brazil is quite recent. The first studies were published almost forty years ago. Moreover, the first researchers of this area achieved their postgraduate degrees — Master (i.e., MSc) and Doctoral (PhD)—in other areas, such as Pharmacy, Engineering, Psychology, and more often, Physiology and Medicine. From the 1990s onwards, research in Exercise Science started to build its own identity, with many researchers emerging from undergraduate courses in the area.

Currently, there are not many institutes for basic research on Exercise Science in Brazil, since it is a recent field of interest in research and has been little widespread. However, many researchers have collaborated for the growth of this area in Brazil, publishing papers, as well as members of the editorial board of high-impact national and international journals, and integrating the committee of scientific societies in Physical Education and related areas worldwide. Furthermore, research collaboration has been established with prestigious academic-scientific laboratories from different countries in an attempt to examine deeply the molecular mechanisms by which physical exercise can exert on the cells using molecular biology tools.

Moreover, many researchers supervise graduate students and an increasing number of studies using animal models have been undertaken, reinforcing thus the key role of animal models in investigations associated with physical activity and sports in Brazil. Brazilian official funding agencies have acknowledged the importance of the studies being conducted, granting more scholarships and support to research groups working with animal models.

Figure 1A shows the global growth in the number of publications in *Medline* involving the crossing of the keywords “Physical activity” and “animal”. Similar behavior can be observed when it was included the keyword “Brazil” to this survey (Figure 1B). In this Figure, it can be observed a sharp increment in research published from 1997 onwards in Brazil (Figure 1B), while for Exercise Science worldwide this increase may be observed from 1967 onwards (Figure 1A). These numbers clearly show how recent the area is in Brazil. This later growth may be also due to the emergence of Graduate Programs in the 90s in Brazil, which boosted research in general and the number of published articles⁴⁶.

As can be seen in Table 1, the different crosses of the keywords: “physical activity”, “exercise” or “sport” to “animal”, “rat” or “mice” in “Brazil” resulted in a significant number of articles published in *Medline*. In the international ranking of scientific production in all academic areas, Brazil occupied the 13th position in 2009 (nearly 2.7% of the articles published worldwide)⁴⁶.

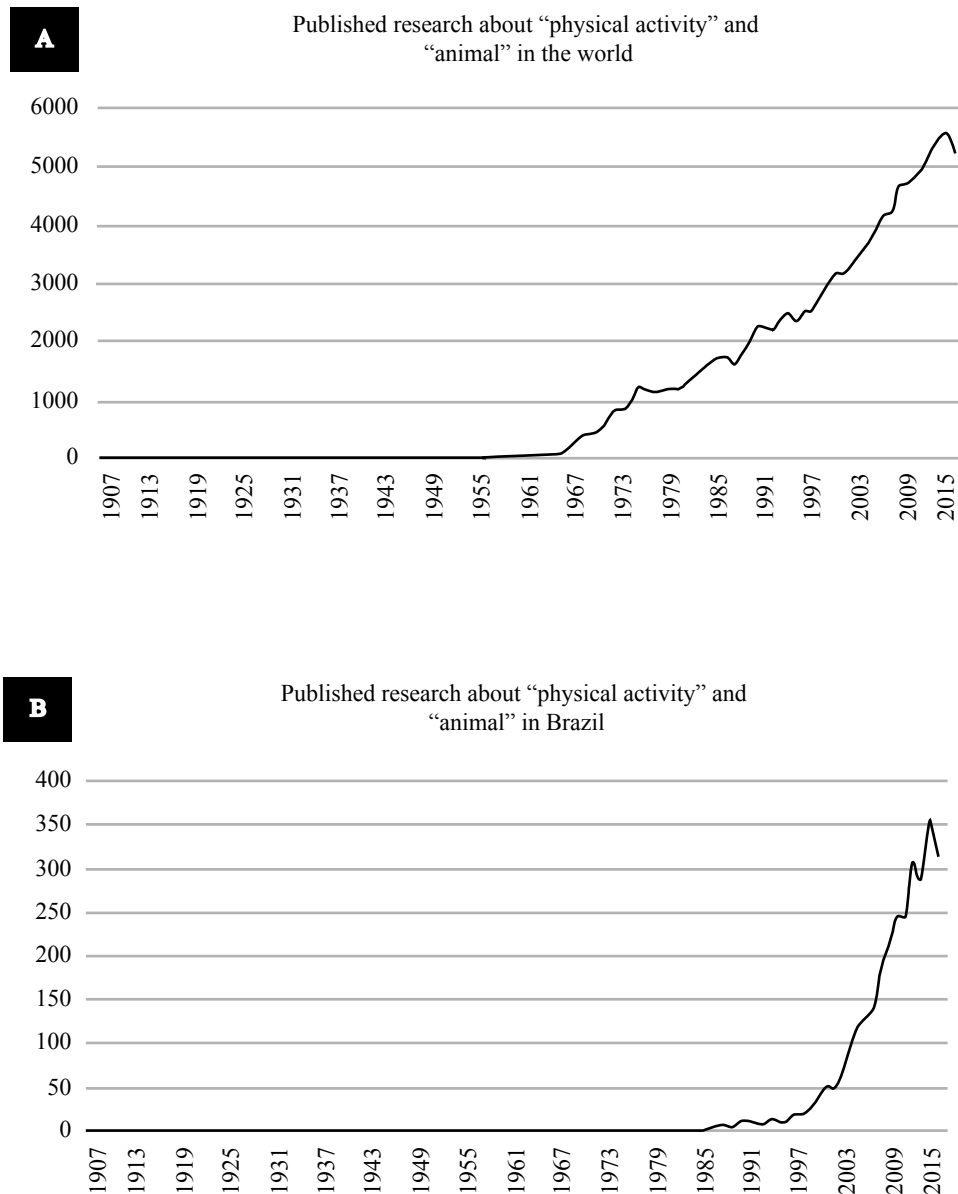


Figure 1. (A) Illustrates the increase (1907-2015) in the number of articles available at Medline using the keywords “Physical activity” and “animal”, our search yielded 120,628 articles. (B) When the word “Brazil” was added in the survey, we obtained 2,993 articles in this period, i.e., nearly 2.5% of all articles found.

Table 1. Search result aggregating the key-words: “physical activity”, “exercise” or “sport” with “animal”, “rat” or “mice” in “Brazil”.

	Animal*	Rats	Mice
Physical activity	2993	1802	868
Exercise	1487	993	327
Sport	825	478	226

* humans were not included

Research using animal models: ethical considerations

The use of animal models plays a key role in scientific knowledge, as it allows a better understanding of Physiology,

Biochemistry, and behavior, and enables researchers to understand the aetiopathogeny of diseases and improve methods of prevention, diagnosing and treatment (both pharmacological and non-pharmacological). A first ethical consideration should be the choice of suitable species for answering a research question. In this sense, rodents such as rats and mice are the most commonly selected for experimental research. They share some physiological features with humans and have a very short lifespan (two to three years)⁴⁷. The short life of rodents makes it easier to study age-related diseases or chronic degenerative disorders. Furthermore, the small physical structure of the rodents favors their production and maintaining.

Although small species have been largely used in research, large animals can also be useful mainly based on the high similarity with humans. Thus, horses are an excellent model for health and performance studies, whereas pigs are primary used for lipid metabolism, obesity and metabolic syndrome. Moreover, sheep are an excellent model for studying the interaction of pregnancy and exercise. In addition to these animals, dogs are also used, despite having higher heart and ventilatory capacity, higher proportion of slow-twitch fibers (i.e., type I) and lower lactate concentration than humans. Furthermore, rabbits, hamsters, cats and snakes, which generally require the utilization of reward to perform the exercise (like food), were also used in studies in Exercise Science⁴⁸.

One clear advantage that deserves attention about the utilization of animal models in research is the fact that they can be maintained in extremely controlled environments, enabling thus the isolation of variables responsible for the functioning of the organic system; which is not possible in human beings. This characteristic enables the utilization of a low sample size to reach reliable results. Recently, advances in genetic engineering and biomolecular techniques have been extensively collaborating in the creation of new opportunities to study the complex phenotypes within the context of the intact animal. In fact, there is a growing interest in the study of genetically modified animals, since — with this model — it is possible to investigate candidate genes responsible for high responsiveness to physical stimulus, collaborating to the understanding of the superior adaptability showed by some people, regarding physical performance and health.

However, it should be clear that the decision to use animals in research studies requires critical thinking, sound judgment, and careful analysis. In this sense, we should remember that the use of animals is a privilege granted to the research community by the society, which have expectations that such a choice will generate new knowledge and contribute to animal and human welfare^{49,50}.

The *replacement, refinement and reduction* (the 3Rs) strategy put forward by W.M.S. Russell e R.L. Burch in 1959 is an effective method for implementing the principles above described. *Replacement* refers to the possibility of substituting animal by other methods, such as in vitro studies, mathematical models, cell/tissue culture, computer simulation, among others. *Refinement* emphasizes the importance of developing new techniques to mitigate the pain and suffering of animals, and involves proper analgesia and asepsis in the pre-, trans – and post-operative measures. *Reduction* would imply fewer studies using animals, fewer animals in the studies, and improved statistical treatment⁵¹.

Currently, the 3Rs principle is a globally accepted approach to the decision-making process of using animals in a study, in designing study groups, and in using techniques and protocols, thus complying with ethical considerations in studies involving animals.

In Brazil, the law 11,794, the decree 6,899, and other complementary normative established by the *Conselho Nacional de Controle de Experimentação Animal* (CONCEA-MCTIC), regulating the production, maintaining and utilization of animals

of the phylum Chordata, subphylum Vertebrata, except human beings, in teaching activities and scientific research throughout the country⁵². One of the main regulations refers to the necessity of a previous evaluation and authorization of the Ethics Committee on Animal Use to starts researchers with animal models. In this sense, it is important to emphasize that research projects using animals will be mainly evaluated on the basis of the following aspects by the Ethics Committee on Animal Use: 1) a proper rationale for the use of animals; 2) whether the species and number of animal will be appropriate for generating new data; 3) availability of proper facilities and equipment for handling animals ethically; 4) description of procedures to minimize animal discomfort and pain, such as the use of anesthetics and analgesics.

Final remarks

The use of animal models has largely contributed to a fuller understanding of the complex physiologic processes involved in the adaptation of the organism to physical exercise. It also shed light on the interactions between physical exercise and pathologies, and on the prevention and treatment of diseases.

The choice for animal models is warranted not only because very often the use of humans is too restricted or outright improper, but also because animals may offer a great contribution to assessing the role of the genetic profile in the response to exercise training. A new generation of exercise physiologists, pharmacologists and biochemists may, therefore, profit from the chance of studying the molecular mechanisms involved in a range of diseases and the role of genetics in human health and physical performance, thus consolidating a solid knowledge base in Exercise Sciences.

Physical Education in Brazil has reached a solid status, and professionals should embrace their key responsibility of producing and spreading knowledge, values and experiences, becoming thus agents of change in society. We should not be passive recipients of knowledge produced abroad, but innovative professionals informed by our own practices and studies in both humans and animals. In this sense, it is critical to disrupt any remaining prejudices to give basic research in Exercise Sciences the status it deserves. This is indeed an educational endeavor that should involve Physical Education professionals, undergraduate students from public and private universities and the population in general to reappraise the contributions of the area and to support research using animal models.

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