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From physical inactivity to immobilization: Dissecting the role of oxidative stress in skeletal muscle insulin resistance and atrophy

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ABSTRACT

In the literature, the terms physical inactivity and immobilization are largely used as synonyms. The present review emphasizes the need to establish a clear distinction between these two situations. Physical inactivity is a behavior characterized by a lack of physical activity, whereas immobilization is a deprivation of movement for medical purpose. In agreement with these definitions, appropriate models exist to study either physical inactivity or immobilization, leading thereby to distinct conclusions. In this review, we examine the involvement of oxidative stress in skeletal muscle insulin resistance and atrophy induced by, respectively, physical inactivity and immobilization. A large body of evidence demonstrates that immobilization-induced atrophy depends on the chronic overproduction of reactive oxygen and nitrogen species (RONS). On the other hand, the involvement of RONS in physical inactivity-induced insulin resistance has not been investigated. This observation outlines the need to elucidate the mechanism by which physical inactivity promotes insulin resistance.

1. Introduction

The terms physical inactivity and immobilization are a source of confusion in the literature. Most of the conclusions drawn on physical inactivity are based on results from immobilization experiments [1,2]. Physical inactivity is a behavior characterized by a lack of physical exercise, whereas immobilization is a clinical state in which one limb or whole body is mechanically unloaded. Although immobilization belongs to the continuum of physical inactivity, it is an extreme situation, requiring a distinct experimental design. On the one hand, immobilization is investigated in human through several models such as bed rest, casting and unilateral lower limb suspension. In rodents, hindlimb unloading remains the reference model of immobilization [3]. On the other hand, physical inactivity is experimentally reproduced with the reduction of the daily number of steps from 10,000 to 1500–3000 in human or with the locked-wheel model in rodents [4,5]. From physical inactivity to immobilization, decline of muscle load promotes insulin resistance and atrophy [6,7], pathological states in which the overproduction of reactive oxygen and nitrogen species (RONS) seems a common denominator [8,9]. Herein, we will focus this review on the role of RONS on skeletal muscle insulin resistance and atrophy in the context of physical inactivity and immobilization. To avoid confusion, we chose to make a clear distinction between physical inactivity and immobilization (see Fig. 1).

1.1. Physical inactivity: definition, causes and consequences

Physical inactivity is basically defined as a lack of physical activity [10]. The World Health Organization (WHO) established a threshold, separating inactive *vs.* active humans, based on the metabolic equivalent of task (MET), one MET being the minimum power required to maintain the basal metabolism. According to WHO, active adult performs at least 150 minutes of moderate-intensity (3.0–5.9 MET) physical activity per week or at least 75 min of vigorous-intensity ($\geq$ 6.0 MET) physical activity *per* week or an equivalent combination of moderate- and vigorous-intensity activity achieving 600 MET-minutes score per week [11]. In children and adolescents (5–17 years old), physical inactivity is defined as not meeting 60 min of moderate to vigorous-intensity physical activity daily [11]. Based on these definitions, the worldwide prevalence of physical inactivity reaches 31% in adults and 80% in adolescents [12]. This high proportion of inactive people contrasts with the singular capacity of human for long endurance exercises [13].

In the genus Homo, a high level of physical activity was an adaptive behavior required for food procurement, escape from predators, social interactions and search for shelter. During the last

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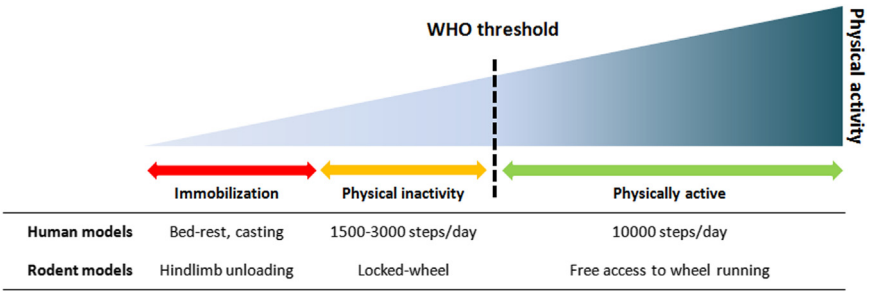

Fig. 1. Human and rodent models used to study immobilization and physical inactivity. WHO: World Health Organization.

two centuries, the scientific progress radically changed conditions which drove hominid evolution for 7 million years. By replacing human work with machines, the industrial revolution initiated a drastic reduction of physical activity. Since then, the development and democratization of new technologies have strengthened this phenomenon. In modern society, physical activity, instead of vital, became a leisure which is not practiced by a large part of the population. In the beginning of the 20th century, the sedentary behavior was firstly encouraged by the scientific community which pointed out the hazards of exercise [14]. A turning point operated when, in 1953, Morris and Heady published a large scale epidemiological study highlighting the deleterious effect of physical inactivity on health. In this study, the authors concluded: "*physical work may be a way of life conducive to good health*" [15].

First seen as a progress, the reduction of physical activity is now recognized as a major factor contributing to the burden of non-communicable diseases [12]. After smoking, physical inactivity is the second risk factor for non-communicable diseases, responsible for 5.3 million deaths per year worldwide [16]. In addition, Pedersen proposed a "diseasome of physical inactivity", gathering cardiovascular disorders, different types of cancer, type 2 diabetes, depression and dementia [4]. Worldwide, Lee et al. [16] estimate that physical inactivity causes 6% of the coronary heart disease, 7% of type 2 diabetes and 10% of breast and colon cancers. Among these diseases, the most alarming is likely type 2 diabetes, a pathological state characterized by insulin resistance. In the United States, diabetes affects 9.3% of the population and the total cost reaches 245 billion dollars per year [17].

1.2. Immobilization: definition, causes and consequences

Immobilization is a deprivation of movement for medical purpose of either a limb or whole body. It is noteworthy that the cause is independent of the will and the consequences on biology are almost immediate, thus contrasting with physical inactivity. Due to medicine progress and aging of the population, more and more people are immobilized in hospital or at home. In the United States, hospitalization related to aging increased by 11.8% between 2005 and 2015 [18]. For instance, osteoporotic hip fracture is estimated to reach 300,000 cases annually in the United States [19]. Given that the proportion of elderly will increase, the number of hospitalizations is expected to rise in the future [20].

Whatever the cause, the major complication for bedridden patients is the rapid development of skeletal muscle atrophy [21–23], a collateral damage which poses challenging health issues. Indeed, skeletal muscle atrophy is associated with a loss of strength, a situation which promotes functional deficits, exacerbates illness and complicates patient recovery, especially in the elderly [24]. In this population, immobilization constitutes a major risk factor for functional decline and loss of autonomy [25]. Consequently, the prevention of skeletal muscle atrophy is crucial for patients, medical team and healthcare system [24,26].

2. Skeletal muscle oxidative stress in immobilization and physical inactivity

2.1. Source of RONS in skeletal muscle

From immobilization to strenuous physical exercise, RONS production in skeletal muscle follows a U-shaped curve [27]. This representation brings out the RONS paradox, good friends when associated with physical activity but bad guys when induced by an absence of physical activity. Herein, we will present the main mechanisms leading to RONS production in skeletal muscle.

Sequential univalent reduction of dioxygen produces oxidant molecules collectively named reactive oxygen species (ROS). The primary ROS generated, superoxide ($O_2^{\bullet -}$), gives rise to others ROS, *e.g.*, hydrogen peroxide (H_2O_2) and the highly toxic hydroxyl radical ($HO^{\bullet}$). In skeletal muscle, ROS are produced by (1) mitochondria, (2) nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOX), (3) phospholipase A2 (PLA2), (4) xanthine oxidase (XO) and (5) endoplasmic reticulum (ER).

In the mitochondria, electrons from NADH and $FADH_2$ are transferred from electron donor to electron acceptor molecules in a process coupled with energy production. Electrons are transported through four enzymatic complexes (I, II, III, and IV) known as electrons transport chain. During this process, a small part of the electrons leaks, mainly through complex I, reduced dioxygen thus leading to $O_2^{\bullet -}$ formation [28]. According to *in vitro* experiments, it has been proposed that 0.12–2 % of dioxygen consumed by mitochondria is converted into $O_2^{\bullet -}$ [28]. However, these values cannot be generalized to the *in vivo* situation, and depend on several factors such as oxidized substrate, mitochondria respiratory states, fiber types and electron donor concentration [27,28]. Whatever the exact proportion of dioxygen converted into $O_2^{\bullet -}$, mitochondria is a major source of ROS in skeletal muscle [29].

The enzymatic complex NOX catalyzes the NADPH-dependent reduction of dioxygen to produce $O_2^{\bullet -}$. In immune cells such as neutrophils and macrophages, NOX2 (also called gp91phox) is used as a "superoxide gun" to kill pathogens during phagocytosis [30]. In addition to the phagocyte NOX2, six non-phagocytic NOXs have been identified: NOX1, NOX3, NOX4, NOX5, DUOX1 and DUOX2 [31]. Skeletal muscle expressed NOX2 and NOX4, located in the sarcoplasmic reticulum, the sarcolemma and transverse tubules [29,32]. It has been reported that NOX4 is constitutively active and directly produces hydrogen peroxide [33]. Although NOXs contributes to skeletal muscle ROS production both at rest and during exercise, their physiological functions remain unidentified in myocytes.

PLA2 hydrolyzes membrane phospholipid and releases arachidonic acid. This lipid serves as a substrate for the lipoxygenases, a reaction coupled with the reduction of dioxygen into $O_2^{\bullet -}$ [34]. Furthermore, PLA2 could stimulate NOXs and mitochondria $O_2^{\bullet -}$ production [27]. Gong et al. proposed that PLA2-dependent process generates $O_2^{\bullet -}$ in skeletal muscle under resting and exercise conditions [35].