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# METABOLIC ADAPTATION TO INACTIVE LIFESTYLE: FROM MUSCLE ATROPHY TO CARDIOVASCULAR RISK

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*ABSTRACT*

*Inactivity is known to principally induce muscle atrophy, determined by unbalanced protein synthesis and degradation and to enhance general cardiovascular risk. In parallel, unloading can upregulate whole body oxidative stress and the action of antioxidant factors, as physiological response. This alteration is accompanied by enhanced systemic inflammation. Growing evidence in animal models suggest that inflammation and oxidative stress can play a causative role in inactivity mediated muscle atrophy. The present review is focused on human studies linking inactivity mediated oxidative stress and inflammation to unloading related atrophy. Recent works evidencing enhanced cardiovascular risk after unloading are reported. In addition, evidence regarding the role of inflammation in cardiovascular drawbacks linked to inactivity are discussed.*

*Keywords: physical inactivity, muscle atrophy, cardiovascular risk, inflammation, oxidative stress*

# PRESNOVNE PRILAGODITVE NA NEAKTIVNI ŽIVLJENJSKI SLOG: OD MIŠIČNE ATROFIJE DO SRČNO-ŽILNEGA TVEGANJA

*IZVLEČEK*

*Znano je, da neaktivnost povzroči predvsem mišično atrofijo, ki jo zaznamuje neravnotežje med izgradnjo in razgradnjo beljakovin, in poveča splošno tveganje za srčno-žilne bolezni. Istočasno neaktivnost poviša oksidativni stres v celem telesu in kot fiziološki odgovor nanje tudi delovanje antioksidativnih faktorjev. To spremembo*

*spremlja povečano sistemsko vnetje. Vse več dokazov iz živalskih modelov kaže na to, da sta morda vnetje in oksidativni stres ključna za povzročitev mišične atrofije kot posledice neaktivnosti. Pričujoči pregledni članek se osredotoča na raziskave na ljudeh, ki povezujejo z neaktivnostjo povzročena oksidativni stres ter vnetje in z neaktivnostjo povezano mišično atrofijo. Povzame tudi nedavne študije, ki ugotavljajo povečano srčno-žilno tveganje, povezano z neaktivnostjo. Poleg tega razpravlja tudi o vlogi vnetja pri srčno-žilnih težavah, povezanih z neaktivnostjo.*

*Ključne besede: gibalna/športna neaktivnost, mišična atrofija, srčno-žilno tveganje, vnetje, oksidativni stres*

## INTRODUCTION

The impact of physical activity level on human physiology has been intensively investigated during the last fifty years. Physical exercise and in particular moderate training was demonstrated to play a positive role on health, such that the World Health Organization (WHO) is developing recommendations regarding required amount of physical activity in relation to age and gender ("Global recommendations on Physical Activity for Health" – WHO). On the contrary inactivity was shown to play a strongly negative role on human health, contributing to billions of deaths from chronic diseases, and increasing prevalence of physical disabilities, especially in elderly people (Fontana, 2009). Immobility, or low activity, are frequent conditions, differentially associated to physiologic and pathologic states. Variably prolonged periods of inactivity can be due to several illness conditions: serious trauma and neurological diseases can preclude the possibility to walk or move, possibly leading to long term immobility. Equally, diseases belonging to internal medicine and cardiology, as well as all surgical interventions, often require prolonged recovery periods in the horizontal position. Finally, muscle inactivity also characterizes the spaceflight microgravity environment where physical effort for initiating movements in the environment is drastically reduced. Notably, social changes occurring in the last years has led to the onset of the so called "sedentary lifestyle", a severe reduction of average physical activity level, with undesired consequences in health (Chaput & Tremblay, 2009). All these conditions underline the importance of fully characterizing net effects of inactivity on physiology. To reach such aims the animal model of hindlimb unloading by tail suspension can be applied to rodents. Otherwise, in humans, experimental bed rest in healthy volunteers, beside lowerlimb casting, is the most accepted model to study physical inactivity: the net impact of physical inactivity, in fact, can not be assessed in hospitalized patients due

to the significant contribution of the pathology. During bed rest studies human healthy volunteers lay in bed for different periods, performing all activities in the horizontal position. Bed rest studies, thus, allow to investigate in humans causes of metabolic and morphologic modifications occurring as a net consequence of physical inactivity.

Experimental bed rest was shown to significantly modify the endocrine milieu (Biolo et al., 2005), and several studies showed bed rest significantly induces skeletal muscle atrophy and cardiovascular alterations.

## MUSCLE ATROPHY: MOLECULAR REGULATION OF PROTEIN SYNTHESIS AND DEGRADATION

Muscle atrophy is defined as a decrease of muscle mass. The maintenance of skeletal muscle mass is determined by the balance between protein synthesis and protein degradation. Clinical importance of muscle atrophy is underlined by poor prognosis characterizing cachectic patients in several setting of diseases (Deans & Wigmore et al., 2005). Different molecular mechanisms can control the rate of protein synthesis. Activation of factors of the Akt family (also called protein kinase B) is known to up-regulate general protein synthesis in skeletal muscle: Akt factors are, in fact, serine/threonine-specific protein kinases, playing a pivotal role in muscle hypertrophy (Bodine, Latres et al., 2001). Activation of signaling cascades involving insulin-like growth factor 1 (IGF-1) and phosphatidylinositol 3-kinase (PI3K) induces Akt phosphorylation and activation (Bodine, Stitt et al., 2001). Akt, in turn, activates eukaryotic translation initiation factor 2B (eIF2B) by stimulation of glycogen synthase kinase-3 β (GSK-3 β) (Rhoads, 1999).

Several proteolytic systems, can contribute to the degradation of muscle proteins. In vitro and animal studies, showed $Ca^{2+}$-activated proteases (Calpain) and the proteasome system play important roles in muscle protein breakdown during muscle atrophy (Purintrapiban, Wang & Forsberg et al., 2003). Caspases may contribute to selected forms of muscle atrophy, releasing actin and myosin from actomyosin complexes, for subsequent degradation by the preoteasome system (Du et al., 2004). Two ubiquitin ligase enzymes as atrogin1 and muscle ring finger-1 (MuRF-1), were shown to be involved in skeletal muscle atrophy (Gomes, Lecker & Jagoe et al., 2001).

### Bed rest and skeletal muscle atrophy

Several previous works showed that physical inactivity can significantly affect molecular mediators of muscle atrophy. In the animal model of unloading, calpain activity was shown to be significantly upregulated as an early event of muscle atrophy induction (Enns, Raastad & Ugelstad et al., 2007). On the contrary, artificial overexpression of calpastatin, a calpain inhibitor, preserved sarcomere structure and the force-generating

potential of unloaded murine muscles (Salazar, Michele & Brooks, et al., 2010). Additional evidence in animal model showed that caspase-3 expression was increased in immobilized leg muscles: such modification was accompanied by increased DNA fragmentation and consequent apoptosis induction (Nagano, Suzaki & Nagano et al., 2008). In humans, after 20 days of bed rest, an upregulation of atrogin-1-mediated muscle protein ubiquitination was demonstrated, confirming the importance of the proteasome system for protein degradation secondary to immobility (Ogawa et al., 2006). Observations showing increased expression of molecular factors controlling muscle atrophy are confirmed by publications directly showing in humans changes in muscle size and architecture secondary to bed rest. A bed rest period of 20 days significantly induced a 10 % thickness reduction in postural muscles (Akima et al., 2005). Eight weeks of bed rest similarly reduced by 14-17 % the cross sectional area of thigh and knee extensors (Ferretti et al., 2001). Thickness of postural calf muscles was shown to be decreased by 9-12 % after 30 days of bed rest (Ellis, Kirby & Greenleaf , 1993) and a similar cross sectional area decrease was specifically shown in soleus and in gastrocnemius muscles after 1 month bed rest. (Berry, Berry, & Manelfe, 1993). Higher reduction (12-17 %) of cross sectional area of vastus intermedius (Ellis et al., 1993) was demonstrated after seven weeks of experimental bed rest. After extremely long term experimental periods (120 days) a 30 % decrease in calf muscle mass was measured (Leblanc et al., 1992). These and other published data demonstrate that the loss of lean body mass during prolonged periods of experimental inactivity in healthy volunteers, occurs at an average rate of 3-4% per week. This value is linearly maintained for the initial stages of the bed rest period, reaching a plateau after five or six weeks.

**Oxidative stress as potential trigger of muscle atrophy**

Muscle wasting is an important clinical feature of several chronic diseases associated with oxidative stress as pathogenetic factor (Moylan & Reid, 2007). Oxidative stress stems from an unbalanced production of free radicals which is not sufficiently scavenged by the activity of antioxidant defenses of the organism (Pastore, Federici & Bertini et al., 2003). Increased activity of antioxidant and cell damage repair systems can be evidenced after free radical production and oxidative stress onset (Hardmeier, Hoeger & Fang-Kircher et al., 1997). The most important non-enzymic antioxdant in the organism is glutathione: it is a thiolic tripeptide available in almost all human cells and synthesized by glutammic acid, cysteine and glycine (Pastore et al., 2003).

Oxidative stress is known to be involved in the regulation of complex pathways leading to protein and muscle wasting. Oxidative stress can, in fact, induce intracellular ionic calcium increase (Kondo, Nishino & Itokawa, 1994): interactions between oxidative stress and calcium availability changes can effectively trigger calpain action (Primeau, Adhihetty & Hood, 2002). Moreover, oxidative stress can induce skeletal muscle atro-

phy indirectly triggering caspase-3 activity (Primeau et al., 2002). Otherwise, oxidative stress can directly affect muscle protein degradation at proteasome level: it, in fact, has been shown to upregulate the expression of muscle atrophy F-box/atrogin1 and MuRF-1 in myotubes (Li, Chen & Li, et al., 2003). Increased expression of such E3 ubiquitin ligases in skeletal muscle can enhance proteolysis and muscle atrophy (Bodine, Latres et al., 2001). Action of free radicals on proteins is known to induce the modification of selected amino acid (proline, arginine, lysine, and threonine) by stable addition of carbonyl groups (Roth, Manhart, & Wessner, 2004). Protein carbonylation, can lead to altered enzyme structure and activity (Stadtman, 2001). Increased levels of protein carbonylation were shown in patients affected by neurological diseases such as Alzheimer and Parkinson's diseases, as well as on myopathies as Duchenne muscular dystrophy or amyotrophic lateral sclerosis (Stadtman, 2001). Carbonylation level was, previously demonstrated to be a reliable marker of oxidative stress occurrence (Greilberger et al., 2008). There is evidence that oxidatively modified proteins by carbonylation, can be selectively degraded by the 20S core proteasome without ubiquitination (Grune, Merker & Sandig et al., 2003). In addition, to avoid accumulation of damaged peptides, carbonylated proteins were shown to be more efficiently scavenged by proteolytic degradation than their nonoxidized counterparts (Grune et al., 2003). Thus, enhanced muscle protein carbonylation can directly increase rate of protein degradation and muscle atrophy.

**Inactivity and oxidative stress**

Low levels of physical activity can promote oxidative stress onset: studies performed in animals showed that regular housing, when compared to constant training, increased lipid peroxidation and ROS release (Laufs et al., 2005). Previously published evidence deriving from murine hindlimb unloading showed that inactivity can increase muscle oxidative stress with concomitantly impaired antioxidant defenses: in particular, experimental unloading increased, in soleus, lipid hydroperoxide levels and oxidation of selected target substrates (Lawler, Song & Demaree, 2003). In addition, in unloaded soleus Cu,Zn-superoxide dismutase increased while, catalase and glutathione peroxidase, significantly decreased, together with non-enzymatic antioxidant capacity (Lawler et al., 2003). Moreover, muscle unloading was shown to induce a decrease in antioxidant heat shock proteins as well as in glutathione peroxydase activity (Lawler et al., 2003). Otherwise, other published data showed that muscle unloading can upregulate heme-oxygenase response in virtue of a previously occurred oxidative damage (Hunter et al., 2001). Knowledge of direct links between oxidative stress and muscle inactivity is currently incomplete, but it seems plausible that inactivity can trigger free radicals production in muscle by interaction of at least five different oxidant production pathways (Kondo, Nakagaki & Sasaki et al., 1993): 1) generation of ROS by the xanthine oxidase pathway (Whidden et al., 2009); 2) production of NO via increased NOS

activity (Kondo et al., 1993); 3) formation of ROS by increased cellular levels of reactive iron (Kondo, Miura & Kodama et al., 1992) ; 4) potential activation of NADPH oxidase by increased availabitity of calcium through protein kinase C-ERK1/2 pathway (Javesghani, Magder & Barreiro et al., 2002) and 5) contribution of mitochondrial production of superoxide radicals (Muller et al., 2007).

**Role of oxidative stress on muscle atrophy following inactivity**

Evidence suggests that inactivity mediated oxidative stress can promote muscle atrophy, as a typical consequence of immobility. An important work investigating, by microarray approach, alterations in gene expression of unloaded muscle, demonstrated that factors promoting oxidative stress as well as ubiquitination and protein degradation are significantly upregulated by immobility (Stevenson, Giresi & Koncarevic et al., 2003). As reviewed by Powers, Kavazis and McClung (2007), oxidative stress secondary to inactivity can effectively induce muscle atrophy activating specific proteolytic pathways and apoptosis processes. Interestingly, lowered oxidative stress induction in unloaded muscles, achieved by supplementation of antioxidant vitamin E, can reduce protein wasting and muscle atrophy (Appell, Duarte & Soares, 1997). Similarly, in an animal unloading study, vitamin A supplementation significantly reduced muscle atrophy and lowered molecular mediators of protein degradation as calpain, caspases and the ubiquitin ligase MuRF-1 (Servais, Letexier & Favier et al., 2007).

The role of oxidative stress on physical inactivity mediated muscle atrophy was investigated in humans, within two bed rest studies organized in 2006 and 2007 at the Valdoltra Orthopaedic Hospital (University of Koper, Slovenia). Significant muscle thickness and pennation angle reductions, as markers of enhanced muscle atrophy, were observed in Vastus lateralis muscle at the end of the experimental period (de Boer et al., 2008). Increased bed rest mediated muscle protein carbonylation was shown, in parallel with an early stage upregulation of the antioxidant protein Heme Oxygenase-1 (Dalla Libera et al., 2009). As abovementioned, in fact, protein carbonylation was shown to be a key factor enhancing direct protein degradation by the proteasome system (Grune et al., 2003): thus, increased carbonylation levels can be considered one of the a mechanistic causes contributing to protein wasting and muscle atrophy. In a recent publication (Agostini et al., 2010) we showed, by a novel validated method involving a single biopsy and double infusion of isotopic tracers, that i) glutathione synthesis rate was upregulated in muscle after inactivity as response to tissutal oxidative stress and ii) that such change was significantly correlated to the atrophy level. Moreover, enhanced whole body inflammation and oxidative stress mediated by fat mass gain during bed rest was associated to a marked increase of muscle atrophy mediated by inactivity (Biolo et al., 2008). Such observations further underline that oxidative stress could be involved in muscle atrophy induction following inactivity in humans.

## Inflammation as additional mechanism of muscle atrophy

Previous evidence showed a direct link between inflammation and muscle atrophy. Muscle mass wasting normally occurring in healthy aging subjects was demonstrated to occur as a consequence of increased inflammation (Jensen, 2008).

Deregulation of pro- and anti-inflammatory cytokines or mediators was significantly associated to the activation of muscle wasting process. In particular upregulation of IL-1, IL-6 and tumor necrosis factor-alpha was previously shown to potentially trigger muscle sarcopenia in elderly subjects (Yende et al., 2006). In addition, an interesting study based on DNA microarray analysis showed that a subgroup of genes involved in promotion of inflammation was upregulated in sarcopenic elderly subjects (Giresi et al., 2005). Prolonged or chronic inflammation is also associated to specific pathologies linked to muscle atrophy. Previously published studies underlined that inflammation can be a possible cause of cancer cachexia: proinflammatory tumor necrosis factor alpha, known also as cachectin, was shown to be one of the factors triggering hypercatabolic processes in tumor bearing subjects (Tracey & Cerami, 1994). Tumor necrosis factor alpha, was shown in cellular model to increase total protein loss, total ubiquitination and activation of NF-kB: interestingly the process was demonstrated to be triggered by endogenous production of ROS (Kramer & Goodyear, 2007). Thus, inflammation can be considered as a potential mechanism triggering muscle atrophy in healthy subjects.

## Effects of inactivity on inflammation

Immobility was demonstrated to worsen the inflammatory condition. An observational study performed in a large sample of male and female subjects showed that persons adopting an unhealthy sedentary lifestyle were characterized by mildly increased levels of C-reactive protein (CRP), a marker of low grade inflammation: interestingly, appropriate moderate training programs and the so called "mediterranean diet" reduced the observed CRP increase (Pitsavos et al., 2007). Another epidemiologic study confirmed that, regardless of the degree of obesity, sedentary lifestyle is associated with increased levels of interleukin-6 and of CRP (Fischer, Berntsen & Perstrup, 2007). Additionally, published evidence (Bosutti et al., 2008) showed in humans that experimental bed rest can increase inflammation upregulating CRP levels and ratio between IL-6 and IL-10 cytokines. Eicosanoids, including prostaglandines tromboxanes and leukotrienes are regulators of inflammatory factors (Lewis, Austen & Soberman, 1990). Polyunsaturated fatty acids (PUFA) of the n-6 series and in particular arachidonic acid are known to be eicosanoid precursors: elevated availability of n-6 PUFA in cell membranes was previously linked to inflammatory diseases (Ueda et al., 2008). By contrast, the n-3 PUFA series is characterized by a significant anti-inflammatory action. Phospholipid content in red blood cell membranes can be considered a reliable marker of whole

body inflammation status, of fatty acid availability in plasma, and of cell membrane composition of the whole body (Harris & Von Schacky, 2004). The effect of physical inactivity on inflammation and membrane fatty acid composition, was investigated in three bed rest studies performed at the Valdoltra Orthopaedic Hospital, (University of Koper, Slovenia) in 2006, 2007 and 2008. Results (Mazzucco, Agostini & Biolo, 2010) displayed monounsaturated fatty acids total content was significantly reduced together with-linolenic and eicosapentaenoic acid levels. Otherwise, bed rest enhanced specific n-6 PUFA content and in particular, the arachidonic-to-eicosapentaenoic acid ratio was significantly increased after bed rest. In parallel, bed rest enhanced whole body inflammation reducing n-3 PUFA fraction in cell membranes. Thus, a significant bed rest mediated shift toward a proinflammatory pattern could be demonstrated (Mazzucco, et al., 2010). These observations are in accordance with previously published data showing the pro-inflammatory effect of bed rest at whole body level (Bosutti et al., 2008).

In conclusion, considering the potential of inflammation to stimulate muscle wasting, increased inflammation mediated by bed rest can reliably provide an additional mechanism explaining the inactivity mediated induction of sarcopenia.

## Cardiovascular consequences of inactivity: role of inflammation and oxidative stress

Different studies demonstrated a strong relationship between inactive lifestyle and cardiovascular risk in terms of increased diabetes (Fretts et al., 2009) and coronary artery disease (Boekholdt et al., 2006) incidence. Noteworthy, inactive lifestyle is known to be per se the primary underlying cause of metabolic syndrome (Zhu, St Onge & Heshka et al., 2004). Apart from epidemiologic studies, direct evidence directly linking inactivity and cardiovascular alterations derives from previous bed rest studies. Reported data showed that several cardiac functional parameters, as isovolumic relaxation time and myocardial performance were negatively affected by bed rest (Platts et al., 2009) suggesting a direct role of unloading on heart muscle efficiency. The effects of bed rest on cardiac muscle morphology and function were previously observed in 24 healthy young women by magnetic resonance imaging: significant reductions in left and right ventricular volumes and masses were observed as markers of cardiac atrophy leading to reduced standing stroke volume and orthostatic tolerance (Dorfman et al., 2007). This further confirms that experimental bed rest can directly impair cardiac muscle morphology and function. Internal fluids redistribution determined by changed position from the orthostatic to the supine position was shown to account for reduced blood pressure, heart rate variability and baroreceptor reflex sensitivity after bed rest, showing that physical inactivity impairs autonomic regulation of cardiovascular system (Ferretti et al., 2009). In a previously published work, 56 days of bed rest induced a significant detrimental role on the cardiovascular system increasing markers of endothelial damage

(Demiot et al., 2007). Inflammation was shown to play a key role in the development of cardiovascular pathologies (Boekholdt et al., 2006) and, in parallel, inflammation is considered to be one of the pathogenetic factors of a cardiometabolic illness as diabetes (Duncan & Schmidt, 2006). Additionally, low grade chronic inflammation is a known cardiovascular risk marker: a relation, in fact, between increased CRP levels and risk of metabolic syndrome onset was shown (Devaraj, Singh & Jialal, 2009) and, interestingly, previously published data demonstrated that increased n-6 to n-3 PUFA ratio can be related to worsened mortality for cardiovascular causes (Harris & Von Schacky, 2004). Previously mentioned publications showed that bed rest can lead to worsened inflammatory condition in terms of upregulated ratio between n-6 and n-3 PUFA (Mazzucco et al., 2010) as well as of augmented CRP synthesis (Bosutti et al., 2008): thus, experimental physical inactivity can effectively induce an enhancement of cardiovascular risk. Interestingly, proinflammatory changes in fatty acid membrane composition were paralleled by increased HOMA index for insulin resistance (Mazzucco et al., 2010). These results suggest that during physical inactivity increased inflammation can contribute to an important metabolic alteration related to cardiovascular system as insulin resistance.

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