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# Biochemical biomarkers are not dependent on physical exercise in patients with spinal cord injury

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ABSTRACT

*Aims:* This work presents an evaluation of the impact of physical exercise of the upper limbs in patients with muscular atrophy in the lower limbs by analysis of specific biomarkers.
*Methodology:* It is a cross-sectional study. Patients were recruited using convenience sampling: control group (C: n = 12) and two groups of wheelchair users: non-athletes (NAth: n = 12) and athletes (Ath: n = 13, professional basketball players). Plasmatic biomarkers analyzed: fibrinogen, TBARS and NO. Comparisons were assessed by one-way ANOVA and Newman–Keuls Multiple Comparison post-hoc.
*Results:* Plasma fibrinogen values were not different between Ath (3.67 ± 0.44 g/L) and NAth (3.44 ± 0.38 g/L) groups. It was observed difference between fibrinogen levels from both wheelchair user groups (Ath and NAth) when comparing to control group (C: 2.27 ± 0.08 g/L) and standard values of fibrinogen (1.8 g/dL–3.1 g/dL). The TBARS values were not different between the wheelchair users Ath (3.21 ± 0.24 nmol/mL) and NAth (3.66 ± 0.27 nmol/mL). Independently of practicing physical activity, the TBARS values from both wheelchair users, Ath and Nath, were different when compared to the TBARS values from control group (C: 24.11 ± 1.75 nmol/mL). The plasma levels of NO were not different among the groups.
*Conclusion:* Under SCI conditions, the upper body exercise practicing did not alter plasma levels of NO and ROS production neither rheological changes in viscosity indicated by blood clotting studies (fibrinogen levels).


## 1. Introduction

Important clinical conditions in spinal cord injury (SCI) are related to damages resulted from loss of functions such as mobility and sensitivity. Frequent causes of SCI are trauma (car accident, gunshot, falls, etc.) or disease (polio, spina bifida, Friedreich's Ataxia, etc.) [1]. While the atrophy is established after a SCI, there are intrinsic skeletal muscle changes, such as in mitochondrial oxidative capacity, which is the main contributor to the metabolic abnormalities [2]. Independently of the type of SCI injury, complete or incomplete [3], it is observed removal of the supply trophic substances from the nerve to muscle and also decreased muscle electrical/contractile activity, leading to a sharp drop in the rate of synthesis of muscle proteins and increased rate of degradation [4].

Classical studies about SCI demonstrated muscle and vascular changes below the level of injury [5–13]. These peripheral circulatory and skeletal muscle adaptations contribute to the increased risk of cardiovascular disease in SCI patients [14]. In fact, cardiovascular disorders are the substantial causes of morbidity and mortality in both acute and chronic stages of SCI [15–17]. The muscle atrophy and extensive physical deconditioning combined with reduced cardiac output impair the demand of oxygen to the muscle, leading to vascular atrophy [18].

Following SCI, blood flow to inferior limbs diminishes about 50–67% mainly due to the loss of the autonomic nervous system control and to the reduction of the local blood flow [19]. Changes in the sympathetic activity especially into large vascular beds, as in skeletal muscle vessels, the abolished compensatory vasoconstriction associated with reduced venous blood return, contribute to low blood pressure. In fact, venous thromboembolism has been detected in acute spinal cord injury patients [20].

Regarding the vascular events in the chronic phase of SCI, among other issues, there is reduced blood volume, decreased muscle or tissue pressures in the extremities, or functional alterations in the sympathetic nervous system [21]. There are well-reported imminent risks for developing deep vein thrombosis, which is lower in 8–12 weeks, but highest in 7–10 days after the injury and during the early phases of recovery and rehabilitation [19]. Causal factors related to deep venous thrombosis are venous stasis in inferior limbs after muscle paralysis and lack of muscle pump activity. In addition, there is hypercoagulability as consequence of

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reduction of fibrinolytic activity and raised activity of factor VIII from blood coagulation cascade [22].

An important mechanism that may contribute to the vascular injuries associated to hemodynamic alterations in inactive individuals with SCI is the reduced NO availability, due to endothelial dysfunction and increasing humoral or local vasoconstrictors, such as reactive oxygen species (ROS) [23]. On the other hand, exercise is an important stimulus for regulating blood flow, which is partially due to enhanced metabolic rate and NO production, affecting vascular relaxation and inhibition in platelet aggregation [24]. While regular physical activity increases the bioavailability of NO [25,26], the cardiovascular decondition is associated with the altered nitric oxide (NO) metabolism under SCI [27,28]. According to Buck and Chojkier [29], physical activity practice increases endothelial oxidative stress and stimulates the release of NO, which leads to vasodilatation.

Mitochondrial signaling contributes to disuse muscle atrophy due to oxidative stress [30]. In fact, in muscle tissue the elevated metabolic rate associated with physical exercise increases mitochondrial $O_2$ consumption and energy production during cellular metabolism. Oxygen- and nitrogen-derived free radicals are then generated and are involved in oxidative damage to cell components.

Oxidative stress, produced by mitochondrial activity, can be evaluated in plasma through reaction of lipid peroxidation products with 'Thiobarbituric Acid Reactive Substances' (TBARS) [31]. Djordjevic et al., [32] demonstrated athletes with higher VO2max, compared to athletes with poorer aerobic power, had higher levels of TBARS as an accepted index of lipid peroxidation. The authors conclude that TBARS supported the positive correlation found between muscle percentage and TBARS as a consequence of the higher working capacity and consequently increased oxidative stress in working musculature of these athletes.

It is already known reactive hyperemia [33] and arterial blood flow are extensively used to determine hemodynamic parameters in SCI patients, mainly male aged 20 and 40 years [5]. In such condition, resting metabolic demands are so low that resting blood flow might be of little diagnostic value. Blood flow during exercise is influenced by cardiac output, making changes in peripheral vascular function [5,34].

Thus, analysis of factors that inform about blood clotting, intrinsic vascular function and the cellular metabolic damages might be useful as additional tools to understand cardiovascular aspects in SCI, mainly when the interest is to know the impact of physical conditioning in such condition.

In face of the data stated above there is a potential positive association among mitochondrial metabolism, NO production and blood clotting after SCI. Considering the disuse of the lower limbs muscles leads to decrease of the local muscle mass and consequent metabolic alterations in installed blood flow reduction, we hypothesized in such condition there is lack of hemodynamic homeostasis which should be detected by biochemical biomarkers. To explore the mentioned potential, the aim of this study was to evaluate, as biomarkers, the blood levels of TBARS, NO and fibrinogen of SCI patients, who are professional basketball players, in comparison with SCI patients that did not practice physical exercise after the injury.

## 2. Materials and methods

### 2.1. Subjects/groups, inclusion and exclusion criteria

At the first contact with the potential subjects, they received an explanation concerning the objective of the study and, then, they were invited to be part of the research. Thirty-two volunteers attended to the study.

In any type of procedure, volunteers were held solely and exclusively after approval and agreement, by means of signing the Informed Consent Form (ICF) on a voluntary basis. The study was previously approved by the Ethics Committee of The National Ministry of Health/University of Ribeirao Preto (CAAE: 18388513.7.0000.5498/protocol: 462.531/2013).

It was included subjects aged between 20 and 60 years old. The sample was assembled in the following groups: wheelchair users who regularly practiced physical exercises (athletes: professional basketball players); wheelchair users who did not practice physical exercises (non-athletes); able bodied (control group).

Description of the groups:

Group 1: Ten wheelchair users who are professional basketball players since the 2rd year after the SCI. The members of this group were called athletes (Ath). Along the last 7 years before the present research, all of them have regularly played basketball 3 times a week, on the Municipal Sports Center located at 627, Camilo Matos Street, Ribeirão Preto, São Paulo, Brazil (zip code: 14090-210).

Group 2: Ten wheelchair users who did not practice physical activities regularly. The members of this group were called non-athletes (NAth). Along the last 7 years before the present research all of the patients were being clinically followed up in the Universitary Physiotherapy Clinic — University of Ribeirão Preto, (UNAERP), which is located at 2201, Costabile Romano Avenue, Ribeirão Preto, São Paulo, Brazil (zip code: 14096-030).

Evidence of the muscle atrophy for groups 1 and 2: All included patients suffered damage to the spinal cord due to trauma that resulted in a loss of functions of mobility and sensitivity of lower limbs. In all wheelchair users, the type of injury was complete In the studied cases, the injuries were to the five Lumbar vertebra (the vertebra in the lower back between the thoracic vertebra, where the ribs attach, and the pelvis; L-1 thru L-5) or also to the fifth Sacral Vertebra (from the Pelvis to the end of the spinal column S-1 thru S-5). The patients' injury caused loss of functioning in the hips and legs.

The wheelchair users included as volunteers had had the complete SCI at least seven years before the development of the present work. The medical records of patients since the trauma had occurred included detailed physical and medical history, sensor-motor neurological examination to confirm the level, completeness of the lesion and was useful to get the time-course from the trauma at the moment of the present research. The evidence for the skeletal muscle atrophy was certified by previous diagnosis of the time of SCI, respective levels of injury and also on literature data [1].

Group 3: Twelve participants able-bodied. This was the control group (C). None of them were athletes.

Exclusion criteria: Acute coronary syndromes, coronary artery bypass grafting or percutaneous intervention during the first three months of these events were excluded as well as those with renal insufficiency (serum creatinine > 2.0 mg/dL), hepatic insufficiency and uncontrolled hypothyroidism. Those who were chronic users of vitamin C, vitamin E and beta-carotene supplementation were also excluded.

### 2.2. Study procedures

#### 2.2.1. Protocol

Subjects were submitted to blood collection for the analysis of biochemical markers: fibrinogen, TBARS and nitric oxide. All the blood collection took place between September 2014 and October 2014.

#### 2.2.2. Fibrinogen

Blood was collected by venipuncture, using vacuum collection disposable tubes containing 2.7 mL of sodium citrate, immediately centrifuged at 2000 rpm for 15 min. After the centrifugation process, the supernatant plasma was collected for storage at −20 °C. The Multifibren® commercial kit was used to measure the serum fibrinogen