# Effect of the vanadyl sulphate on the muscular metabolic compromising induced by immobilization of posterior limb of rats

Gabriel Borges Delfino[1], João Luiz Quagliotti Durigan[2], Karina Maria Cancelliero[3] and Carlos Alberto da Silva[4]


## ABSTRACT

The purpose of this study was to evaluate the metabolic performance of immobilized skeletal muscle in rats treated with vanadyl sulphate. Male *Wistar* rats were divided in groups (n = 6): control (C), immobilized (I), treated with vanadyl sulphate (VS, 0,25 mM) and immobilized treated with vanadyl sulphate (I + VS) during seven days. The concentration of vanadyl sulphate diluted in water was 0,25 mM. After experimental stage, the glycogen content (GC) was evaluated in soleus (S), white gastrocnemius (WG), red gastrocnemius (RG), tibialis anterior (TA) and extensor digitorum longus (EDL) muscles, besides S and EDL weight. The statistical analysis was realized by the ANOVA followed by Tukey test ($p < 0,05$). In VS group, the results showed a significant increase in GC (S 110%, WG 71%, RG 85%, TA 125%, EDL 108%) and in the weight (S 9%, EDL 11%). The immobilization reduced significantly the GC (S 31.6%, WG 56.6%, RG 39.1%, EDL 41.7%, TA 45.2%) and weight (S 34.2% and ELD 27%), and in I + VS group, there was a increase of the GC in all muscles (S 211%, WG 115%, RG 148%, EDL 161.9%, TA 147%), besides hindering the weight loss in S (75%) and EDL (46%). The vanadyl sulphate treatment promoted an increase in the glycogen content of control and immobilized groups, besides hindering the weight loss, showing that the insulino-mimetic effect is represented by glycogenic action associate to a possible anti-catabolic action.


**Keywords:** Muscular disuse. Vanadium compounds. Rehabilitation.

## INTRODUCTION

Immobilization is a classical condition of muscular disuse found in physical therapy clinical practice, especially in situations of ligament ruptures, bone fractures, muscular and medullar lesions, muscular and articular degenerative pathologies, inflammations, surgeries, among others, in which there should be the restriction of a body segment. This condition does not favor the maintenance of the dynamic balance of the anabolic and catabolic reactions which contribute to the muscular homeostasis, leading to alterations of atrophic, morphological and chemo-metabolic character, which hence converge to muscular hypotrophy.


1. Discente do Curso de Fisioterapia e bolsista de iniciação científica CNPq – Universidade Metodista de Piracicaba, Piracicaba, SP.
2. Mestre em Fisioterapia – Universidade Metodista de Piracicaba, Piracicaba, SP.
3. Mestra em Fisioterapia – Universidade Metodista de Piracicaba, Piracicaba, SP e Doutoranda em Fisioterapia – Universidade Federal de São Carlos, São Carlos, SP.
4. Professor Doutor do Programa de Pós-Graduação em Fisioterapia da Universidade Metodista de Piracicaba, Piracicaba, SP.


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**Correspondence to:** Gabriel Borges Delfino, Rua João Batista Calmazine, 10, Bairro Parque Cidade Nova – 13840-000 – Mogi Guaçu, SP. E-mail: delfinogb@gmail.com

The hypotrophy of the skeletal muscle may be defined as the loss or decrease of muscular mass, besides the decrease of the energetic substrates availability, being these elements important for the maintenance of the muscular metabolic balance. There are several factors that contribute to the degree of muscular hypotrophy, such as age; sex; time of immobilization; type of fiber; length in which the muscular group is immobilized and muscular group. (extensor/flexor)[1].

In the literature, the resistance to insulin scenario has been studied in the muscular disuse condition. Therefore, Hirose *et al.*[2] studied the insulin signaling via in rats which had the left paw immobilized for seven days and verified reduction in the intracellular signal transduction estimated by the hormone, suggesting thus deficit in the activation of the IR (insulin receptor) and in the molecules activated from it, including the IRS-1 phosphorylation (substrate of the insulin receptor one) and the PI3-K activation (phosphatidylinositol three kinase). The non-activation of the PI3-K via results in the compromising of several mechanisms, among them, the protein synthesis, the glycogen synthesis and translocation of the GLUT4 transporters (type four glucosetransporters) for the cell membrane.

It has been proposed that several substances, such as the clenbuterol, the metformin, the creatine and the glutamine may help in the maintenance or improvement of the metabolic conditions of the skeletal muscles during the muscular disuse period, mainly with the purpose to maintain or increase the glycogen sources, besides the inhibition of muscular weight reduction[3-5].

Studies have suggested that inorganic combinations such as molybdate, pervanadate, tungstate and vanadyl help in the glucose tissue metabolism, besides being used as potential elements with anti-catabolic and metabotropic applicability in the endocrinology and orthomolecular medicine fields[6].

Vanadyl is a trace-element found in physiological conditions in $10^{-10}$ and $10^{-9}$ M concentrations, and is believed to be important for the regulation of the activity of the enzymes regulators of the cellular metabolic vias[7-8].

Clark *et al.*[9] demonstrated that the vanadyl in the skeletal muscle alters the glucose metabolism similarly to the insulin's. This element increases the glucose entrance, glycogen synthesis and glycolysis in a breadth lower than insulin. It is worth mentioning that in the XVIII century, Lyonnet *et al.*[10] showed evidence of the insulino-memitic effect of the vanadyl combinations, even before insulin discovery.

More expressive results involving vanadyl combinations were reported with the use of the vanadyl sulphate utilization ($VOSO_4$), possibly because vanadyl is the active intracellular form of vanadium[7-8,11]. Vanadyl sulphate is the oxidative form of vanadium, which *in vitro* and in diabetes animal models promoted a decrease in the hyperglycemia and in the insulin resistance[12].

Once the muscular disuse is a frequent condition in the skeletal-muscular rehabilitation clinical practice, and the search for therapies during the functional-kinetic limitation period is constant, the utilization of vanadyl sulphate became suggestive. This substance presents insulino-mimetic actions, since the muscular disuse is an insulin-resistance model.

Thus, the aim of this study was to evaluate the effect of the vanadyl sulphate over the muscular metabolic profile of posterior limb of rats submitted to articular immobilization during seven days.

## METHODS

Albino *Wistar* rats, with age range between 3 and 4 months, weighting 286,6 ± 17 g, were fed with food and water as libitum and were submitted to a photoperiod cycle of 12 h light/dark, under controlled temperature (23°C ± 2). The animals were treated according to the recommendations by the Guide for Care Use of Laboratory Animals[13].

The animals were divided in four experimental groups (n = 6): control; immobilized; treated with vanadyl sulphate and immobilized treated with vanadyl sulphate. Both the immobilization period and the treatment were of seven days.

The rats were anesthetized with sodium pentobarbital (50 mg/Kg) for the immobilization, followed by the left paw's immobilization with an acrylic resin orthosis, which kept the ankle articulation in neutral position (90°), leaving the knee and hip articulations free (figure 1).

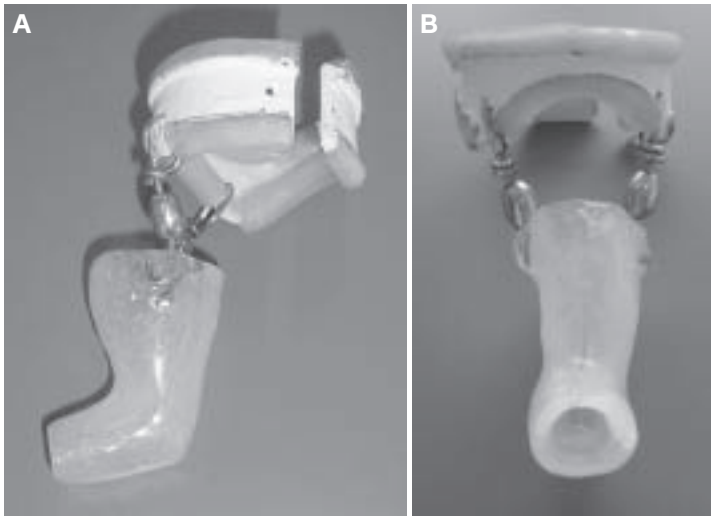


***Figure 1*** *– Acrylic resin orthoses: lateral view* ***(A)*** *and anterior view* ***(B)***

The treatment with vanadyl sulphate was conducted through the administration of the substance diluted in water in the 0,25 mM concentration, made available in the water to be drunk during 24 hours per day in amber containers in order to avoid photolysis.

After the experimental period, the animals were sacrificed through cervical dislocation and samples of the soleum, red gastrocnemius, white gastrocnemius, tibialis anterior and extensor digitorum longus muscles of the toes were isolated, removed and sent for the determination of the muscular glycogen content through the sulfur phenol method[14]. Such method consists of the digestion of the muscle samples in KOH 30% to hot and the precipitation of the glycogen from the passage through ethanol to hot. The samples were centrifuged to 3000 rpm during 15 minutes and the precipitated glycogen was submitted to acid hydrolysis in phenol presence between the two phases of the precipitation. The values were expressed in mg/100 mg of humid weight. The weight evaluation of the soleum and extensor digitorum longus muscles of the toes was performed through an analytical scale.

The statistical analysis of all the variables was initially performed through the Kolmogorov-Smirnov normality test and the homocysteate test (Barlett criterion). ANOVA was used followed by the Tukey test after the observation that the variables contemplated the parametric methodology. A significance index of 5% was established for all the calculations.

## RESULTS

Initially, it was observed that the immobilization promoted metabolic alteration in the skeletal muscles during 7 days, represented by significant reduction ($p < 0,05$) in the glycogen content (mg/100 mg) of all analyzed muscles, being 31,6% in the soleum (average ± epm, C: 0,38 ± 0,03 and I: 0,26 ± 0,02), 56,6% in the white gatrocnemius (C: 0,46 ± 0,02 and I: 0,20 ± 0,02), 39% in the red gastrocnemius (C: 0,41 ± 0,01 and I: 0,25 ± 0,03), 41,7% in the extensor digitorum longus of the toes (C: 0,36 ± 0,03 and I: 0,21 ± 0,02) and 45,2% in the tibialis anterior (C: 0,31 ± 0,03 and I: 0,17 ± 0,02) (figure 2).

The immobilization also promoted alteration in the muscular weight (mg) characterized by the significant reduction in the soleum (34%), and in the extensor longus of the toes as well (27%) (table 1).

**TABLE 1**
**Average ± epm index of the muscular weight (mg) of the soleum and extensor longus of the toes muscles, from the control; immobilized; control treated with vanadyl sulphate and immobilized treated with vanadyl sulphate groups**

| | S | ELD |
|---|---|---|
| Control | 123,5 ± 2,1 | 120,6 ± 8,5 |
| Immobilized | 81,3 ± 1,89* | 88,1 ± 7,8* |
| Control + vanadyl sulphate | 135 ± 4,6 | 134 ± 5,2 |
| Immobilized + vanadyl sulphate | 143 ± 9,76# | 128,6 ± 7,2# |

n = 6, $p < 0,05$, * compared with control group and # compared with the immobilized one.

Concerning the group treated with vanadyl sulphate, a significant increase in the glycogen sources of all analyzed muscles was observed, being 110% in the soleum (average ± epm, C: 0,38 ± 0,03 and SV: 0,80 ± 0,04, $p < 0,05$), 71% in the white gatrocnemius (C: 0,46 ± 0,02 and SV: 0,79 ± 0,03, $p < 0,05$), 85% in the red gatrocnemius (C: 0,41 ± 0,01 and SV: 0,76 ± 0,04, $p < 0,05$), 108% in the extensor longus of the toes (C: 0,36 ± 0,03 and SV: 0,75 ± 0,06, $p < 0,05$) and 125% in the tibialis anterior (C: 0,31 ± 0,03 and SV: 0,70 ± 0,05, $p < 0,05$) (figure 2). Concerning the weight of the soleum and extensor longus of the toes muscles, the treatment with vanadyl sulphate did not promote any significant alteration (table 1).

Nonetheless, when the treatment with vanadyl sulphate was administered to the immobilized group, its efficiency in avoiding the muscular decrease of the analyzed muscles was observed (table 1), as well as the reduction in the glycogen sources, increasing them in 211% in the soleum (I: 0,26 ± 0,02 and I + SV: 0,81 ± 0,07, $p < 0,05$), 115% in the white gastrocnemius (I: 0,20 ± 0,02 and I + SV: 0,43 ± 0,04), 148% in the red gastrocnemius (I: 0,25 ± 0,03 and I + SV: 0,62 ± 0,04), 161,9% in the extensor longus of the toes (I: 0,21 ± 0,02 and I + SV: 0,55 ± 0,05), and 147% in the tibialis anterior (I: 0,17 ± 0,02 and I + SV: 0,42 ± 0,06), (figure 2).

## DISCUSSION

The insulin actions over the proteins and amino acids metabolism converge concerning anabolic reactions. The insulin, after interaction with the membrane receptor, stimulates the glucose transporters (GLUT-4), facilitating the entrance of the hexoses in the cell, besides anabolically acting over the protein metabolism.

Immobilization is a condition characterized by the decrease of strength and size of the muscle, being a procedure widely used in lesions such as: bone fractures; ligaments ruptures or articulation degenerative diseases[15]. An inversion in the metabolic balance in the muscular tissue, where the catabolic reactions surpass the anabolic ones, is observed, due to immobilization. Such procedure increases the hypotrophy and mainly weight loss[16].

The morphological, physiological and biochemical events triggered by immobilization have been the focus of several studies[17-19]. Within this context, the decrease of the muscular tissue re-

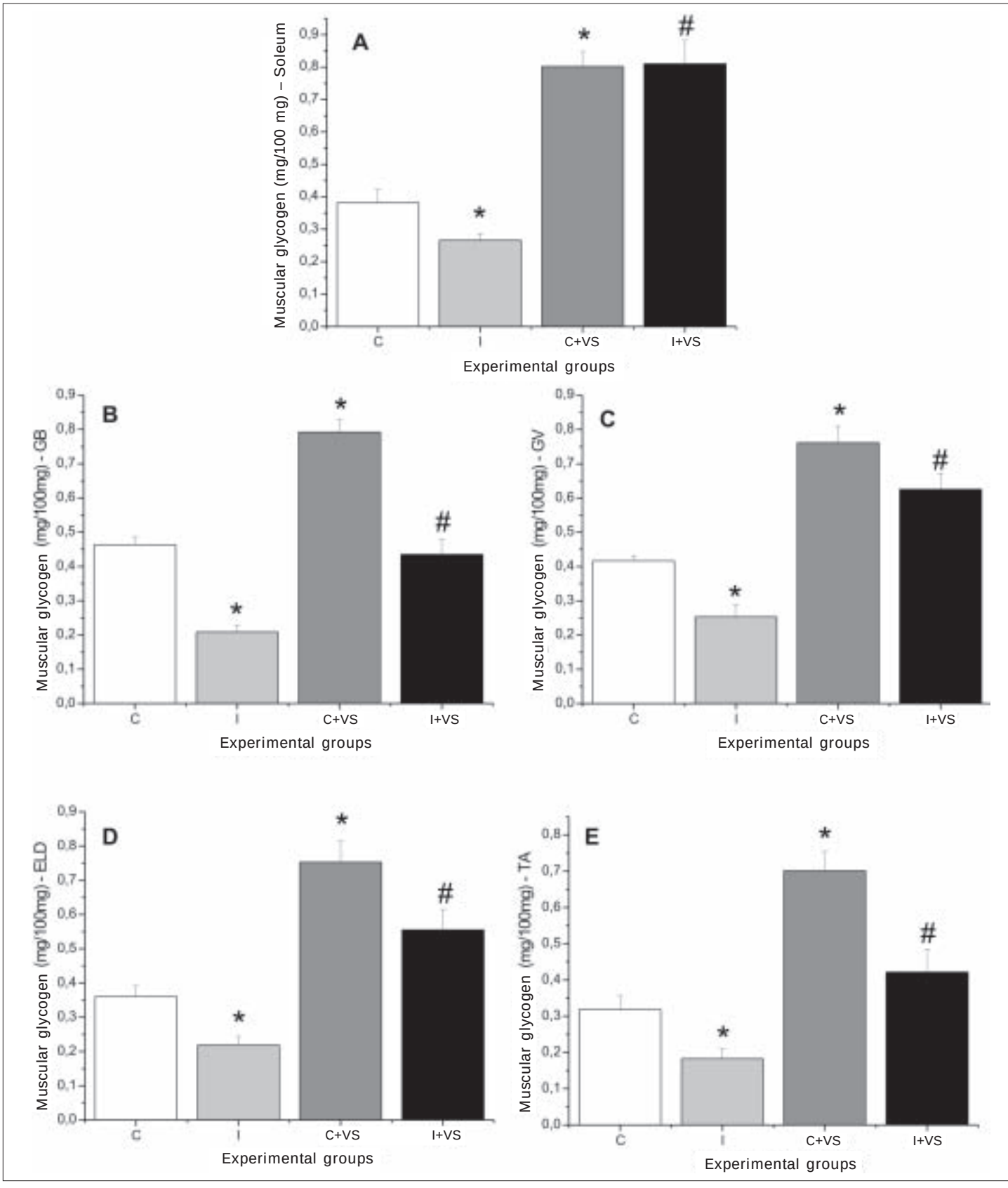


***Figure 2*** *– Average ± epm index of the glycogen concentration (mg/100 mg) of the soleum* ***(A)****; white gastrocnemius* ***(B)****; red gastrocnemius* ***(C)****; extensor longus of the toes* ***(D)****; tibialis anterior* ***(E)*** *muscles of the control (C); immobilized (I); control treated with vanadyl sulphate (C + SV) and immobilized treated with vanadyl sulphate groups (I + SV).*

n = 6, p < 0,05, * compared with control group and # compared with immobilized group.

sponse to insulin in the immobilization period has been demonstrated, presenting alterations in the glucose metabolism[2,20-21].

Our results follow the studies that suggest reduction in the glycogen sources of the immobilized muscles, demonstrating the functional relations between the muscular contraction and the glucose rapport and metabolism, highlighting that the white portion of the gastrocnemius muscle was the most compromised concerning the lowest energetic sources. These observations show that in the immobilization model used in this study, the white fibers (type II) were the most affected, corroborating Herbisson *et al.*[22], Jaffe *et al.*[23] and Mcdougall *et al.*[21]. Moreover, besides the metabolic alterations, weight loss was also observed, which may suggest reduction in the fibers number and/or size, which is an expression related with a negative protein balance[16,24].

Experimental physical therapy has been searching alternatives which focus the improvement in the energetic profile of the immobilized muscle group, with the purpose to improve the physiological condition of such muscles, while improving the methodology of the physiotherapeutic intervention and thus, minimize the rehabilitation time. Therefore, it has been demonstrated that supplementation is a viable option which helps in the maintenance of a differentiated energetic standard[3-4].

Further studies including substances containing the trace-element vanadium are needed due to this viability of supplementation in the muscular immobilization period. Vanadium has demonstrated in studies to have insulin-mimetic effects, among them the increase of glucose transport; glucose oxidation and glycogen synthesis, besides hampering lipolysis and gluconeogenesis[25-28]. It is also worth mentioning that one of the most important effects of the vanadium salts is the GLUT4 transporter translocation of its intracellular compartment, to the cell's surface, increasing hence, the glucose capture[29].

Among the different vanadium combinations, the vanadyl sulphate utilization was chosen in this study due to its more significant results[7-8,11].

When the glycogen sources of the group treated with vanadyl sulphate are evaluated, there is an increase of them as well as of weight, which corroborate the studies that showed evidence of increase in the glycogenesis during treatment with vanadyl sulphate[30-31]. It is important to mention that such increase of the glycogen sources was significant when compared with the control group. However, when compared with the muscles of the treated group among themselves, no significant difference was observed, showing hence, that there is no specificity concerning the type of fiber of the analyzed muscles.

Conversely, in the results concerning the treated immobilized muscles, the metabolic behavior was differentiated by the type of fiber, once the muscles with fibers type-1 (oxidative) presented higher sensibility represented by the increase of the glycogen sources. A possible explanation for this difference in the response may lie in the fact that these muscles present higher number of insulin receptors and that there is a need for further studies for the distinction of the muscular fiber type more sensible to the treatment with vanadyl sulphate.

It is worth mentioning that studies involving the association of models of muscular disuse with the treatment with inorganic combinations, especially related with the vanadyl sulphate, are scarce yet.

## CONCLUSION

The treatment with vanadyl sulphate promoted increase in the muscular glycogen sources both in the control and immobilized groups, besides avoiding the reduction of the muscular weight. Therefore, this study suggests the maintenance of a differentiated nutritional standard in the muscular tissue in disuse improving thus the conditions for the rehabilitation period.

*All the authors declared there is not any potential conflict of interests regarding this article.*

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