# URINARY CONCENTRATION OF HIDROXIPROLINE, ON MEN WITH LOW BACK PAIN SUBMITED TO HYDROKINESIOTHERAPY

# CONCENTRACION URINARIA DE HIDROXIPROLINA EN HOMBRES CON LUMBALGIA SOMETIDOS A HIDROKINESIOTERAPIA

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**PERNANBUCO, S.C., LARANJEIRA C., MESQUITA G.M., da CONCEIÇÃO M.C., GOMES de SOUZA V.R. & DANTAS M.E.H.** Urinary concentration of hidroxiproline, on men with low back pain submited to hydrokinesiotherapy. ***Mot. Hum*., 11(2): 80-86, 2010.**

**RESUMEN**

*El objetivo del estudio fue analizar los efectos de la hidrokinesioterapia en las concentraciones urinarias de hidroxiprolina en hombres con lumbalgia. Los participantes fueran 23 hombres con 23 a 50 años, con diagnóstico de lumbalgia y fueran orientados a participar en el programa de Hidrokinesioterapia. La recolección de orina para evaluar la concentración de HP ocurrió en la primera, quinta y decima sesión utilizando HPROLI 24H. Los resultados fueran 14,45 mg/d, en la quinta sesión fue 14,09 mg/d y finalmente la decima sesión el grupo obtuvo 16,22 mg/d. La conclusión fue que la hydroxyprolina es un gran marcador del sangre para tejidos musculares lesionados.*

**Palabras Clave:** Hidroterapia; ejercicios de estiramiento muscular, hidroxiprolina; dolor en la región lumbar.

## INTRODUCTION

The pain has a role in alerting the body to communicate that something is wrong and when it comes to chronic pain, it creates a stress in the body and even a physical disability. Therefore, pain is a problem that demands immediate attention and the best strategy to eliminate any type of pain is to remove the causal factor (1).
Among the possible pain that affects the human, is a pain in the lumbar region. Low back pain is described as a muscular alteration that most contributes to the increase in costs and downtime in the United States. It is estimated that 70% -85% of adults will experience a significant episode of back pain at some point in their lives (2,3).

Chronic back pain should be treated as a public health problem, especially since it affects the economically active age population, can be highly disabling and is one of the leading causes of absenteeism. It is estimated that 75-90% of costs relate to patients with chronic low back pain. (4).

Pain in the spine are most often the result of postural problems that can cause discomfort from relatively mild to severe lesions, such as osteoarticular diseases (5).
Increased physical activity and exercises are being planned by the population accompanied with the increase of muscle-skeletal system. The risk of these injuries are proportional to the intensity and duration of training at any age (6).
Interventions aimed at stretching increase flexibility and range of motion (AAM) have been advocated as an important component in different areas related to motor function. Its beneficial effects are demonstrated in functional activities, injury prevention, training in posture and muscle relaxation, and athletic performance. (7,8).

Flexibility refers to the extensibility of the ends of joint tissue that allow normal movement or physiological joint, resulting in the extension of the muscle or tendon unit (9,10).

Flexibility exercises of low intensity for long periods promote the deformation of the plastic and non-contractile tissues allowing a gradual remodeling of collagen and distribution of the fluid that circulates in the tissues (11).

The connective tissues can be injured during physical activity above the structures can withstand higher than usual even in adults. This injury can be measured by the metabolism of collagen through one of its components, hydroxyproline.

The identification of the levels of collagen the biochemical components of a structure is crucial in the regeneration of injuries by increasing their molecular bioactivity.
Muscle actions when imposed on skeletal muscle overload cause damage to tissues and increased urinary excretion of hydroxyproline (HP) indicates that damage tissue. Collagen has a key part of HP that is formed mainly through the oxidation of proline and enters the metabolism during the degradation of collagen itself. This degradation is formed containing peptides that HP does not undergo hydrolysis in part later and are thus eliminated in the urine and can be measured in laboratory collection (12).

The aim of this study is to determine the levels of urinary excretion of hydroxyproline in male subjects with low back pain, treated with emphasis on static stretching hydrokinesiotherapy

## METHODS

*Selection of Subjects:*

The subjects of the study consisted of a group of 23 men aged 23-50 years, living in the western state of Rio de Janeiro, volunteers, indicating diagnosis of low back pain and medical direction to the practice of hydrotherapy. The study took place in a pool with the following measures: 8x5 m, 1.20 deep and with 1° C ± water at 29° C.

The subjects signed the Informed Consent document in accordance with resolution 196/96 of the National Health Council and the Declaration of Helsinki (2008). The study was approved by the Committee of Ethics in the Research of Human Beings from the Universidade Castelo Branco (UCB-RJ) under protocol n° 129/07.

*Hydrokinesiotherapy:*

The aquatic therapy program consisted of ten sessions twice a week. Warm-up exercises were performed by running back and bicycle suspension with the aid of floats in the axillary. For the exercises were chosen the following protocols:
• Warm-up running back, bike to float in the axillary region;
• Flex static: hip flexion with knee bent and supported by floats in the popliteal fossa in the standing position, trunk flexion with knee extended and your feet flat on the edge of the pool, with the support of both hands on the bar, with trunk flexion knee flexion, with the support of both hands on the bar (fetal position), lateral bending of the trunk with the patient sitting, standing upright bascule hip, and finally
• Relaxation: buoyancy vest with the support of the neck and spaghetti in the popliteal fossa.

The static Flexibilizing was used as a basis for treatment and formal object of this study is to arrive slowly limit of normal articular arc of the individual, gently pushing beyond this limit, wait about six seconds and perform new insistence smooth reaching a higher arc motion possible at this point, the arch articular obtained should be held for 10 seconds. The routine should be repeated three to six times with an interval of relaxation between them.

*Hidroxiproline:*

For the analysis of the urinary concentration of HP, the subjects underwent collection of urine before and 24 hours after the intervention. The subjects were oriented to avoid the use of any type of ergogenic, nutritional and pharmacological substance or alcohol during the period of the study and in the week prior to the tests. In order to control and standardize the dietary intake of HP, red and white meat, seafood, sweets, ice cream and gelatin were eliminated from subjects' diets.

Before each collection of urine, performed via Nordin's method (12), participants fasted for 12 hours. All of the samples were collected and stored in Empasul™ plastic sterilized containers and immediately transported and analyzed at the Laboratory.

To determine the urinary concentration of HP, the ClinRep kit (complete kit for hydroxyproline in urine) was used, applying the colorimetric method. During this process, HP is oxidized to pyrrole, follo wed by an engagement with paradimethyla minobenzaldehyde. The reagents are prepared in house, namely: buffer solution (pH 6.0), Erlich's Chloramine-T, Standard Solution for hydroxyproline, phenol phthaleine, sodium hydroxide, isopropanol and perchloric acid. The samples were analyzed in the HPLC system, containing a gradient pump, an injection valve, a heat column (60°), a UV/VIS detector for 472nm, a computer with HPLC software and a pulse regulator.

The collections of HP were measured before the first session of hydrokinesiotherapy, an analysis at the 5th session and after treatment (10th session).
After the biochemical analysis, the obtained HP values were converted from mg/L to mg/24h in order to make a direct comparison with the proposed reference values. The HP level from 5 to 40 mg/24 h was used as a benchmark, once it was considered to be the appropriate cutoff value, according to the method applied, for individuals who are over 20 years old.

*Statistical Procedure:*

We used descriptive and inferential statistics, with mean, median, standard deviation, standard error, coefficient of variation and percentage change. For homogeneity of the sample was used Shapiro-Wilk, due to the n sample group. To compare the results of continuous quantitative variable HP test was used for ANOVA one way, whereas for a significance level $p < 0.05$ for the variable measured in this study and the Beta error between 10% and 20%.

## RESULTS

The variable HP1 variation coefficient of 41%, and use the median (14.95 ± 5.88 mg / 24h) as the best measure of central tendency. The HP2 variable with average equal to 140.9 ± 5.29 with the coefficient of variation of 38%. HP3 and the variable had a mean 16.22 ± 5.86 and coefficient of variation of 36% (Table 1).

One can thus observe a slight decrease in the mean between the levels of HP1 and HP2, as reflected in urinary excretion of HP. But this did not occur between HP1 and HP3, where the increase in the average reflects an increased excretion of urinary HP between the collections of the fifth. the 10th. session and between collections pretest and posttest, demonstrating increased catabolism of collagen.

| ***HP*** (mg/24 h) | ***M*** | ***ε*** | ***Md*** | ***CV*** | ***sd*** |
|---|---|---|---|---|---|
| **HP1** | 14,45 | 1,20 | 14,95 | 41% | ±5,88 |
| **HP2** | 14,09 | 1,08 | 13,3 | 38% | ±5,29 |
| **HP3** | 16,22 | 1,22 | 16;6 | 36% | ±5,86 |

TABLE 1: Characteristics of the sample group of HP in the pretest, 5th session, 10th session (posttest).
HP1 Where: Level Hydroxyproline baseline (pretest); HP2: Hydroxyproline Level in 5 session; HP3 Hydroxyproline Level in 10 session; M: avarege; ***ε:*** standard error; Md: median, CV: coefficient (posttest) of variation, sd: standard deviation. Value of reference 5-40 mg/24 h.

Note also that the dispersion parameter of the variables for HP1 HP2 was lower, showing that the group became more similar, concentrating the results. This did not occur in relation to variables for HP2 HP3, ditto with the HP1 compared with HP3, where increasing values of the deviations mean group dispersion.

According to data found in the survey was not significantly different ($p < 0.05$) in the excretion levels of HP in the urine of patients with low back pain undergoing treatment in ten sessions of HP, with an emphasis on static flexion (TABLE II).

| | Media variables of Squares | F | P |
|---|---|---|---|
| HP | 32,32 | 0,93 | 0,39 |

TABLE II: Statistical difference between Means of HP - ANOVA
HP: level of hydroxyproline; P: p <0.05, F: Anova

As shown in Table III the rate of percentage decrease in the levels of Hydroxyproline baseline in the fifth session was 2.5%, between fifth and tenth session was an increase of 13.10%, and between the index baseline and the tenth session was 10.92%.
These results showed that there was a decrease in the excretion of HP in the 5th session of hydrokinesiotherapy and a further increase was observed at the 10th session.

| | Basal – 5 | 5/10 | Basal/10 |
|---|---|---|---|
| Δ | -0.35 | 2.12 | 1.77 |
| Δ% | -2.5% | 13.10% | 10.92% |

TABLE III: Change in index Group Sample HP.
Where: Basal / 5 features of the index variation of HP's pre-test session 5, 5 / 10 features content varying from 5 HP to 10 characteristic Basal/10 session and the index of variation of pre-to post-test test.

## DISCUSSION

The lesions caused by stretching static promoted changes in the metabolism of hydroxyproline observed in this study group. Proline Plays important roles in protein synthesis and structure, metabolism (Particularly the synthesis of arginine, polyamines, and glutamate via pyrroline-5-carboxylate), and nutrition, as well as wound healing, antioxidative reactions, and immune responses (13).
The overload also induced a meaning from hydroxylproline accumulation and pro-interleukin colágeno1 mRNA in muscle, it was observed when comparing the training group underwent resistance training and the control group (15).

Overloading (OV) healthy skeletal muscle produces myofibre hypertrophy and extracellular matrix remodelling, and these processes are thought to be interdependent for producing muscle growth. Inflammatory cytokine interleukin-6 (IL-6) gene expression is induced in overloaded skeletal muscle, and the loss of this IL-6 induction can attenuate the hypertrophic response to overload (OV).

Overload also induced a significantly greater accumulation of hydroxyproline and procollagen-1 mRNA in Interleukin 6 (IL) muscle, when compared with WT muscle after 21 day Overload. Transforming growth factor-beta and insulin-like growth factor-1 mRNA expression were also induced to a greater extent in IL-6 muscle when compared with WT muscle after 21 day Overload

The role of physical activity in affecting the composition of extracellular matrix and mechanical properties of tendons has been well studied. On study made by Almeida (2) for three-month-old Wistar rats were divided into three groups: the control, animals were not submitted to stretching procedures; groups that had their calcaneal tendons manually stretched three or five times a week, for 21 days. Afterward, the calcaneal tendons were removed and assayed for hydroxyproline content and biomechanical test. The hydroxyproline content in the stretched groups was higher, suggesting that more collagen was present in the tendons of these groups. These tendons also showed higher values of maximum stress and modulus of elasticity or Young's modulus. These results indicate that stretching leads to alterations in the synthesis of the extracellular matrix components and in the mechanical properties of tendons. This results found on winstar rats followed the same tendency of the human being group studied and observed in this study, who used the same stretching method.

The content of the major components of the extracellular matrix was studied in 60 patients with spinal osteochondrosis over time during complex rehabilitative therapy including radon-containing mineral baths, The complex rehabilitative treatment using radon baths in patients with spinal osteochondrosis was shown to result in a reduction in the serum level of glycosaminoglycans (GAG) and total oxyproline by the end of a course of therapy, which suggests the lower rate of destructive processes in the extracellular matrix. The determination of GAG concentrations and oxyproline forms in patients with osteochondrosis may be used to evaluate the efficiency of the

treatment performed (16). On this study founded the reduction of collagen after muscles be stressed, suggesting that the process made by other way promoted collagen elimination change with muscle tissue regeneration.

HP levels can decrease when the interventions involve a longer period of treatment. Caetano et al. (17), after 10 sessions of hydrokinetic, therapeutic treatment in patients with acute lower back pain, reported a decrease in HP levels ($\Delta$= 53.3 ± 22.6; p=0.008) and in pain, as measured by the CR10 Borg scale ($\Delta$= 3; p=0.03).

Faria-Souza et al. (18) reported that patients with shoulder impact syndrome treated using maximal flexibility exercises in kinesiotherapy had an increase in the range of movement and a decrease in the sensation of pain after chronic treatment, as they had a significant decrease in their HP levels ($\Delta$= – 15.3mg/day; p=0.005). However, those studies analyzed the chronic effect of the aforementioned intervention, unlike the present investigation, which analyzed the acute effect. This suggests that the adaptation process to interventions can reduce the levels of pain and HP.

## CONCLUSION

On this study were possible observe, based on results of this study that HP marker could demonstrated muscles lesions on subjects Who were submited a static strecht.
For other studies suggests that use other elements and a costume marker, like ultrasonography exam.

## BIBLIOGRAPHY REFERENCES

1. Heredia, E. P.; Rodrigues, F. O Tratamento de Pacientes com Fibrose Epidural pela Reeducação Postural Global – RPG. Revista Brasileira de Neurologia 2008; 44 (3): 19-26.

2. Giesecke, T., Gracely, R.H., Grant, M.A.B., Nachemson, A., Petzke F., Williams D.A., Clauw, D.J. Evidence of augmented central pain processing in idiopathic chronic low back pain, Arthritis & Rheumatism 2004; 50: 613–623.

3. Nelson-Wong,E.; Callaghan, J. P. The impact of a sloped surface on low back pain during prolonged standing work: A biomechanical analysis Applied Ergonomics 2010; 41(6): 787-795.

4. Pacheco, J.L; Rossi, A.C.S; Gomes, E.C. Benefícios da Auriculoacupuntura em motorista da polícia militar. 22-04-08. Disponível em:<http://www.fisioterapeutasonline.com/2008030287/Artigos-de Fisioterapia/Acupuntura-na-Fisioterapia/beneficios-da-auriculoacupuntura-em-motoristas-da-policia-militar-de-pernambuco-com-dor-lombar.html>.Acesso em: 11/12/08.

5. Paccini, M. K.; Cyrino, E. S.; Glaner, M. F. Efeito de exercícios contra-resistência na postura de mulheres. Revista de educação física 2007:18: 169-175.

6. ACSM – AMERICAN COLLEGE OF SPORTS MEDICINE, 2003.

7. Gajdosik R.L., Vander Linden D.W., Mcnair P.J., Williams A.K., Riggin R.J. Effects of an eight-week stretching program on the passive-elastic properties and function of the calf muscles of older women. Clin Biomech. 2005; 20(9): 973-983.

8. Gama, Z. A. S.; Dantas A. V. R.; Souza T. O. Influência do intervalo de tempo entre as sessões de alongamento no ganho de flexibilidade dos isquiotibiais. Revista Brasileira de Medicina do Esporte Rev Bras Med Esporte 2009: 15 (2): 110-114.

9. Cramer J.T., Housh T.J., Johnson G.O., Miller J.M., Coburn J.W., Beck T.W. Acute effects of static stretching on peak torque in women. Journal of Strength and Conditioning Research 2004; 8 (2), 236–241.

10. De Almeida, F. M., T. C. Tomiosso, et al. "Effects of passive stretching on the biochemical and biomechanical properties of calcaneal tendon of rats." Connect Tissue Res 2009; 50(5): 279-284.

11. Mafra O. R., Silva E. B., Giani T. S., Neves C. E. B., Lopes R. S. D., Dantas, E. H. M. Hydroxyproline levels in young adults undergoing muscular. Stretching and Neural Mobilization. JMB 2010; 29: 1–5.

12. Nogueira, A.C., Simão, R.; Carvalho, M.C.G.A., Vale, R.G.S., Dantas, P.M.S. Concentração de hidroxiprolina como marcador bioquímico do nado músculo esquelético após treinamento de resistência de força. R. Bras. Cie. E Mov. 2007; 15(2):33-38.

13. Wu, G., F. W. Bazer, Et Al. "Proline and hydroxyproline metabolism: implications for animal and human nutrition." Amino Acids.Epub 2010: 0939-4451

14. Nordin Be, Hodgkinson A, Peacock M. The measurement and the meaning of urinary calcium. Clin Orthop Relat Res 1967; 52: 293–322.

15. White, J. P., J. M. Reecy, et al. "Overload-induced skeletal muscle extracellular matrix remodelling and myofibre growth in mice lacking IL-6." Acta Physiol (Oxf) 2009: 197(4): 321-332.

16. Kim, L. B., I. V. Zhiliakov, et al. "Serum levels of glycosaminoglycans, hydroxyproline, and fibronectin in patients with spinal osteochondrosis." Klin Lab Diagn 2009: (8): 16-18.

17. Caetano Lf, Mesquita Mg, Lopes Rb, Pernambuco Cs, Silva Eb, Dantas Ehm. Hidrocinesioterapia na redução lombar avaliada através dos níveis de hidroxiprolina e dor. Fitness & Performance Journal 2006; 5 (1): 39–43.

18. Faria-Souza Apg, Dantas Ehm, Silva Eb, Martinho Ko, Mesquita Mg. Kinesiotherapy Associated with Laser GaAs Application in Shoulder-Rotator Cuff Syndrome. Laser Physics 2007; 17 (3): 286–289.

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## ABSTRACT

*The aim of this study is to determine the levels of urinary excretion of hydroxyproline in male subjects with low back pain treated with emphasis on static stretching hydrokinesiothery. The subjects of the study consisted of a group of 23 men aged 23-50 years old, living in Rio de Janeiro, volunteers, indicating diagnosis of low back pain and medical direction of the practice of hydrotherapy.*

*The collections of HP were measured before the first session of hydrokinesiotherapy, an analysis at the 5th session and after treatment (10th session) the protocol HPROLI 2h. The results: pre test of HP was 14,45 mg/d, fifth session – 14,09 mg/d; tenth session – 16,22 mg/d. Conclusion was that Hydroxyproline was a good marker for muscle tissue lesion.*

**Key words:** hydrotherapy; muscle stretching exercise, hydroxyproline; low back pain**.**

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