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HYDROXYPROLINE LEVELS IN YOUNG ADULTS UNDERGOING MUSCULAR STRETCHING AND NEURAL MOBILIZATION

NIVOI HIDROKSIPROLINA KOD MLADIH PACIJENATA NA TERAPIJI MIŠIĆNOG ISTEZANJA I NEURALNE MOBILIZACIJE

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Summary: This study aimed to assess the acute effect of stretching and neural mobilization on urinary hydroxyproline (HP) levels in young adults. The sample, composed of physical therapy students from Teresina (PI), was divided into three groups: a neural mobilization group (NMG; n=15; age= 22±3 years; BMI=24.75±3.09); a static stretching group (SSG; n=15; age=23±4 years; BMI: 25±4.33) and a control group (CG; n=15; age: 24±4 years; BMI: 23.91±3.09). The NMG underwent neural mobilization of the sciatic nerve while engaged in hip flexion with knee extension in a direct, oscillatory and strenuous manner for 60 seconds. The SSG performed passive static stretching, which consisted of the maintenance of a high amplitude posture, without exceeding the limits of the movement, for a period of tension ranging from four to six seconds. Urinary HP was evaluated at the baseline and 24 hours after the intervention using the colorimetric method. Repeated measures ANOVA showed significant intragroup increases in the NMG (Δ=7.38 mg/24h; p=0.0001) and the SSG (Δ=3.47 mg/24h; p=0.002) and inter-group increases in the NMG (Δ%=118.89%) when compared to the SSG (Δ%=60.32%; p=0.006) and the CG (Δ%= –0.91%; p=0.0001). These results indicate that the NMG worked with tension beyond the ordinary amplitude arches of articular movement, thus causing a restructuring of collagen.

Keywords: articular movement amplitude, connective tissue, hydroxyproline

Kratak sadržaj: Cilj studije bio je da se proceni akutni efekat istezanja i neuralne mobilizacije na nivoe hidroksiprolina (HP) u urinu mlađih odraslih osoba. Uzorak, koji su činili studenti fizikalne terapije iz Terezine, podeljen je u tri grupe: grupa za neuralnu mobilizaciju (NMG; n=15; starost: 22±3 godine, indeks telesne mase (BMI): 24,75±3,09); grupa za statičko istezanje (SSG; n=15; starost: 23±4 godine; BMI: 23,91±3,09). NMG podvrgnuta je neuralnoj mobilizaciji išijatikusa tokom fleksije kuka uz ekstenziju kolena uz direktan, oscilatoran i naporan pokret tokom 60 sekundi. SSG izvodila je pasivno statičko istezanje, sastavljeno od održavanja položaja s velikom amplitudom, ne prelazeći granice pokreta, za period naprezanja od četiri do šest sekundi. HP u urinu određen je na početku i 24 časa posle intervencije pomoću kolorimetrijske metode. Ponovljena merenja pomoću ANOVA pokazala su značajne poraste u NMG (d=7,38 mg/24h; p=0,0001) i SSG (Δ=3,47 mg/24h; p=0,002) i poraste u NMG (Δ%=118,89%) u poređenju sa SSG (Δ%=118,89%) u poređenju sa SSG (Δ%=60,32%; p=0,006) i CG (Δ%=–0,91%; p=0,0001). Rezultati ukazuju na to da je NMG radila s tenzijom izvan uobičajenih lukova amplitude artikularnog pokreta, što je izazvalo restrukturaciju kolagena.

Ključne reči: amplituda artikulatornog pokreta, vezivno tkivo, hidroksiprolin

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List of non-standard abbreviations:

PNF – proprioceptive neuromuscular facilitation
HP – hydroxyproline
NMG – neural mobilization group
SSG – static stretching group
BMI – body mass index
CG – control group
PERFLEX – Perceived Exertion Scale on Flexibility
SPSS – Statistical Package for Social Science.

Introduction

The considerable number of injuries in the locomotor system due to imbalances of the muscular chains has led the American College of Sports Medicine to include flexibility in its recommended fitness program (1). Flexibility training may be developed though submaximal or maximal exercise. Stretching is characterized as a submaximal exercise due to its use of muscular extension and suspension within the limits of the articular movement amplitude. Maximal exercises are characterized by stress beyond limits and may be performed statically, dynamically or through proprioceptive neuromuscular facilitation (PNF) (2). On the other hand, the neural mobilization technique restores mobility and flexibility to the nervous system, promoting and improving its normal functions, with a resulting increase in amplitude (3).

These methods of training, if used properly, may provide muscular relaxation, relief from stress, improvement of body aptitude, relief from muscular pain and reduced risk of injuries or back pain (4).

Flexibility exercises of low magnitude for long periods increase the plastic deformation of non-contractile tissue, allowing gradual remodeling of collagen bonds and redistribution of water to the surrounding tissues (5). Thus, systematic stretching up to the limit of the articular amplitude may cause pain (6) when it requires myofibrils, favoring the development of micro-traumas associated with muscular pain (7).

The deformation of the connective tissue occurs in different degrees and intensities of strength. It requires the rupture of collagen bonds and the realignment of fibers such that the stretching is permanent and there is an increase in flexibility. The collapse of the tissue begins as a micro-collapse of fibrils and fibers, before the full collapse takes place. This may occur as a consequence of a single maximal effort event or of repetitive submaximal overloads (8).

One of the indicators for this type of tissue damage may be the level of hydroxyproline (HP), a biochemical marker for bone formation and reabsorption (9). HP is formed through the oxidation of proline in collagen binding and is inserted in metabolism during its decomposition. This decomposition gives rise to peptides containing HP, which in most cases do not undergo hydrolysis and are eliminated in the urine, and their levels of excretion are increased whenever there is an injury in the connective tissue, especially on the same day, after 24 hours and particularly 48 hours after exercise (10).

These changes in the tissue can contribute to inducing adaptations in mechanical properties, consisting of alterations in load resistance and an increase in tolerance to maximal exercise (11).

Urinary HP levels, as an important indicator of the level of tissue disorder, may be used to coordinate appropriate exercise prescription and prevent injuries during physical activities. Therefore, the objective of this study was to assess the acute effect of stretching and neural mobilization on urinary levels of hydroxyproline in young adults.

Materials and Methods

Sample

Forty-five physical therapy students from the city of Teresina (Piauí), who were sedentary, male and between 17 and 30 years old, were invited to take part in this research study.

The study excluded subjects with the following characteristics: neural, muscular and associated skeletal alterations; use of ergogenic-nutritional, pharmacological or physiological aids, alcohol or any other substance that could influence HP levels in urinary excretion; body mass index (BMI) above 40; metabolic diseases; pain; or those who adopted high-protein diets.

After applying these criteria, the sample was divided into three groups: a neural mobilization group (NMG); a static stretching group (SSG) and a control group (CG) (*Table I*).

Table I Characteristics of the sample.

Group	n	Age (years)	BMI
NMG	15	22±3	24.75±3.09
SSG	15	23±4	25.12±4.33
CG	15	24±4	23.91±3.09

NMG = Neural Mobilization Group; SSG = Static Stretching Group; BMI = Body Mass Index.

The subjects signed the Informed Consent document in accordance with resolution 196/96 of the National Health Council (12) and the Declaration of Helsinki (13). The study was approved by the Committee of Ethics in the Research of Human Beings from the *Faculdade Integral Diferencial* (protocol n° 129/09).

Procedure for evaluation

Initially, a recall questionnaire was used to obtain information about eating habits, medications taken and medical history. Baecke's questionnaire on habitual physical activity was used for grown up men, to learn about their physical activity statuses (14). Body structure and mass evaluation in order to identify BMI (15) was carried out by means of Filizola™ mechanical scales with stadiometer, in accordance with recommendations from the International Society for the Advancement of Kinanthropometry (16).

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 (17), 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 *Laboratório Álvaro*, Curitiba.

To determine the urinary concentration of HP, the ClinRep kit (complete kit for hydroxyproline in urine) was used, applying the colorimetric method (17). During this process, HP is oxidized to pyrrole, followed by an engagement with paradimethylaminobenzaldehyde. The reagents are prepared in house, namely: buffer solution (pH 6.0), Erlich's Chloramine-T, Standard Solution for hydroxyproline, phenolphthaleine, 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 472 nm, a computer with HPLC software and a pulse regulator.

After the biochemical analysis, the obtained HP values were converted from mg/L to mg/24 h in order to make a direct comparison with the proposed reference values (17). 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.

Procedure for intervention

For the neural mobilization and the static stretching, the subject was positioned in dorsal decubitus on a 4040 couch at the *Instituto São Paulo* (Brazil), and the therapist engaged the subject in hip flexion, with knee extension up to the maximum resistance of the tissue. This procedure was repeated three times in each method.

The static stretching, done by passive extension, consisted of maintenance of a posture of great amplitude without exceeding the limit of the movement during a period between four to six seconds (2).

The neural mobilization of the sciatic nerve was performed in a direct, oscillatory and stressful way for 60 seconds. The therapist performed the hip flexion with knee extension and performed the oscillations in dorsal flexion for 60 seconds, which corresponds to 20 oscillations (3).

The Perceived Exertion Scale on Flexibility (PERFLEX) was used to evaluate the exertion perceived during the interventions (2). This scale has various levels of intensity (from 0 to 110), categorized using five verbal descriptors to specify the perception of effort toward movement amplitude: normality (from 0 to 30); forcing (from 31 to 60); discomfort (from 61 to 80); bearable pain (from 81 to 90); and severe pain (from 91 to 110).

The sample showed a perception of exertion that corresponded to a sensation of forcing (46.49 ± 6.51) and discomfort (72.68 ± 3.02) for stretching and neural mobilization, respectively.

Statistic analysis

The data were analyzed using Microsoft Excel and SPSS (version 14.0) and are represented using the mean, standard deviation and absolute (Δ) and relative (Δ%) differences. The normality and homogeneity of variance of the sample were analyzed using the Shapiro-Wilk and Levene tests, respectively. For the intra-group comparisons, the paired t-Student test was used. The study used the analysis of variance of repeated measures followed by Tukey's post-hoc test to identify the possible differences in the inter-group comparisons. The study used $p<0.05$ as the cutoff for statistic significance.

Results

Table II shows the results of the intra-group comparisons for the sample, referring to the HP levels. By analyzing *Table II*, it is possible to observe that the HP levels increased significantly in the NMG and the SSG between the period immediately before and 48 h after the intervention. The CG showed no alteration.

Table II Analysis of the intra-group HP levels before and 24h after the intervention.

	Mean (pre)	SD	Mean (post)	SD	Δ	Value-p
NMG	6.21	3.05	13.59	5.50	7.38	0.0001
SSG	5.75	2.17	9.21	2.83	3.47	0.002
CG	6.45	1.20	6.39	1.28	0.06	0.906

NMG: neural mobilization group; SSG: static stretching group; CG: control group; mean values in mg/24h; Δ: absolute difference; SD: standard deviation.

Figure 1 shows the inter-group comparisons in terms of percentage differences. The NMG showed a significant increase when compared to the SSG ($p=0.006$) and CG ($p=0.0001$). No significant differences ($p=0.097$) were found between the SSG and CG.

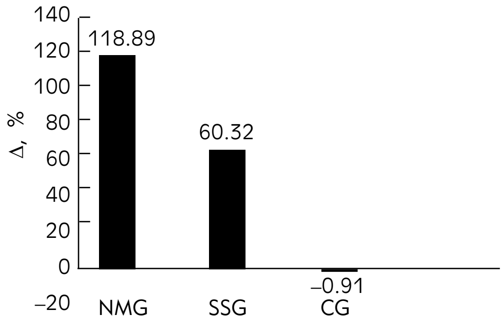

Figure 1 Analysis of urinary levels of hydroxyproline.
NMG: neural mobilization group; SSG: static stretching group; CG: control group; Δ%: percentage differences.
* $p<0.05$; NMG vs. SSG; NMG vs. CG.

Discussion

The findings of this study reveal significant increases in the intra-group HP levels in both interventions. In inter-groups comparisons, the NMG obtained a significant increase in HP levels when compared to the SSG and the CG. The 24-hour waiting period after the intervention to collect urine may have been one of the factors that led to such a result. Immediately after the degradation of collagen caused by the intervention, there is a need for the instant regeneration or rearrangement of the collagen cells and only after several hours is proline hydrolyzed and released in the urine as HP. Thus, the waiting time during which higher HP concentrations in the urine can likely be found is between 24 and 48 hours after the stimulus (18).

Fitness seems to influence the responses of HP. Silva et al. (19) reported in their study that there was no significant alteration in HP levels after a stretching program involving sniper war-veterans (baseline: 20.69±12.76 and 24h: 27.53±18.70). This suggests that there was no damage to the connective tissue due to the low intensity of the exercise. However, these individuals were active, which differs from the present study with sedentary subjects, and the minimum intensity used on the SSG can show an increase in HP levels. In the NMG, this increase was higher, probably due to the higher intensity.

Nascimento et al. (20) investigated the effects of maximal flexibility training through the method of dynamic flexing in aquatic and terrestrial environments. They found out that in both environments this kind of exercise resulted in damage to the connective tissue because of the significant increase in HP levels from the baseline to 24 hours later; nevertheless, no differences between environments were found (baseline: 39.07±15.27; 24h-terrestrial environment: 71.28±12.15; 24h-aquatic environment: 66.74 ±10.31). This is in agreement with the results for the NMG, which also used the maximum articular amplitude to apply the neural mobilization technique.

HP levels can decrease when the interventions involve a longer period of treatment. Caetano et al. (21), after 10 sessions of hydrokinetic, therapeutic treatment in patients with acute lower back pain, reported a decrease in HP levels ($\Delta = 53.3 \pm 22.6$; $p=0.008$) and in pain, as measured by the CR10 Borg scale ($\Delta = 3$; $p=0.03$). Faria-Souza et al. (22) 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 ($\Delta = -15.3$ mg/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.

The neural mobilization technique has reduced pain in the posterior muscular chains (23), but the evaluation is performed by observing the restoration of the normal functions of the nervous system (24), the responses from the nervous tissues (23) and the increase in the articular movement amplitude (26). However, there are no studies with this type of intervention, which evaluates damage to the connective tissue as measured with the biochemical marker HP. Therefore, the present study showed a higher HP increase for the NMG in relation to the other groups when using a forcing technique that goes beyond the limits of the articular movement, causing possible damage to the connective tissue; nevertheless, this analysis was performed 24 hours after the intervention.

In conclusion, the NMG, which used the neural mobilization technique, showed higher levels of HP than the SSG and the CG. This suggests that muscle actions performed using this technique can affect the metabolism of collagen and even the muscular and tendon structures of the connective tissue. Thus, the need for a greater synthesis of collagen suggests an adaptive response in the muscular tissue and, consequently, an increased excretion of hydroxyproline in the urine. Ultimately, this study may spur further research that analyzes chronic interventions using this technique with other articular movements in order to confirm the findings presented in this paper.

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