

**MUSCULAR STRENGTH AND HYDROXYPROLINE CONCENTRATION
IN URINE AFTER DIFFERENT FLEXIBILITY TRAINING PROTOCOLS****MIŠIĆNA SNAGA I KONCENTRACIJA HIDROKSIPROLINA U URINU
POSLE RAZLIČITIH PROTOKOLA ZA VEŽBANJE FLEKSIBILNOSTI**

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Summary: The objective of this study was to evaluate variation in the lumbar spine extension (LSE) muscular strength index and the hydroxyproline (HP) urinary concentrations as a function of flexibility training with maximum intensity (flexibilizing) statically, by proprioceptive neuromuscular facilitation (PNF) and submaximally. The sample population – with an age of 17.13 ± 1.23 ; body mass of 63.23 ± 6.36 kg; height of 173.62 ± 5.465 cm and body fat percentage of $10 \pm 3.62\%$ – comprised 60 male individuals divided randomly into four equal groups: CG (control), StrG (stretching), SFG (static flexibilizing) and PNFG (flexibilizing by PNF). The statistical program SPSS 14.0 for Windows was used to perform a Shapiro Wilk test to verify the normality of the data and Levene's test to analyze the homogeneity of the sample, repeated measures ANOVA for multiple comparisons among groups and the Tukey's HSD Post Hoc test to determine the statistical difference within groups of the variables. A significance level of 95% ($p < 0.05$) was adopted. The results showed a significant difference in LSE between PNFG and CG ($D\% = 11\%$; $p = 0.029$). It can be concluded that the practice of PNF increased strength in the study group.

Keywords: muscle strength, hydroxyproline, muscle stretching exercises

Kratka sadržaj: Cilj studije bio je da se odrede varijacije indeksa mišićne snage ekstenzije lumbalnog dela kičme i koncentracije hidroksiprolina u urinu kao funkcija vežbanja fleksibilnosti sa maksimalnim intenzitetom (fleksibilizacija) statično, proprioceptivnom neuromišićnom facilitacijom (PNF) i submaksimalno. Ispitivanu populaciju – prosečne starosti $17,13 \pm 1,23$; telesne mase $63,23 \pm 6,36$ kg; visine $173,62 \pm 5,465$ cm i sa prosečnim procentom telesne masti $10 \pm 3,62\%$ – činilo je 60 muškaraca nasumično podeljenih u 4 jednake grupe: CG (kontrolnu grupu), StrG (istezanje), SFG (statična fleksibilizacija) i PNFG (fleksibilizacija metodom PNF). Statistički program SPSS 14.0 za »Windows« korišćen je za izvođenje testa Shapiro-Wilk kako bi se utvrdila normalnost podataka i homogenost uzorka. Za ponovljena merenja korišćena je ANOVA sa višestrukim poređenjima između grupa a Tukeyjev HSD *post hoc* test za utvrđivanje statističke razlike u okviru grupa varijabli. Usvojen je nivo značajnosti od 95 % ($p < 0,05$). Rezultati pokazuju značajnu razliku u ekstenziji lumbalnog dela kičme između PNFG i CG ($D\% = 11\%$, $p = 0,029$). Može se zaključiti da je upražnjavanje PNF pojačalo snagu u proučavanoj grupi.

Ključne reči: mišićna snaga, hidroksiprolin, vežbe za istezanje mišića

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List of non-standard abbreviations: LSE – lumbar spine extension; HP – hydroxyproline; SF – static flexibilizing; CG – control group; PNF – proprioceptive neuromuscular facilitation; PERFLEX – Perceived Exertion Scale on Flexibility; SPSS – Statistical Package for Social Science.

Introduction

The regular practice of physical exercise programs aimed at the development, maintenance or training of muscular strength and flexibility (1) is essential to reach healthy levels of physical fitness. These neuromuscular qualities are important components of the physical fitness and are related to health, and can have an extremely important role throughout life (2), in addition to being fundamental for the proper functioning of the musculoskeletal system, effectively contributing to the preservation of healthy muscles and joints (3).

Flexibility refers to the extensibility of the peri-articular tissues, to permit the normal or physiological movement of a joint or limb (3), producing the lengthening of the muscle tendon unit (4).

For flexibility training, light submaximal work (stretching) or maximum intensities that reach the threshold of discomfort (flexibilizing) can be used (5). Traditionally, flexibilizing uses methods such as the ballistic, the proprioceptive neuromuscular facilitation (PNF) and the static, with the latter being widely used due to the ease of learning and application (6).

Muscular actions, when imposed on the musculoskeletal system with overload, promote adaptations in the muscle connective tissue, inducing a rise in the hydroxyproline (HP) urine concentration, which thus serves as a biochemical marker of tissue damage in muscular collagen (7).

Hydroxyproline is an amino acid present in large quantities in collagen and does not come from dietary sources but from the hydroxylation of proline during the initial stages of its biosynthesis, entering the metabolism during its decomposition (8, 9). The existence of a relationship between a lesion of the musculoskeletal system and the increase in urine excretion of HP has been demonstrated (10).

The acute flexibilizing intervention can result in a reduction of the strength levels, according to a study by Zakas (11). However, in another study (12) it was found that the static flexibilizing did not cause any strength differences in acute training, unlike a study by Torres et al. (13), with 30 individuals, which indicated a reduction in strength after static flexion. In another study (14), the static flexibilizing and PNF exercises were found to change the strength levels, which suggests that the training program to increase flexibility can result in an immediate decrease in strength, a fact that is not observed for HP (15). Therefore, the flexibility training methods informed by the literature can lead to different changes in the levels of muscle strength and HP.

In order to clarify the cited problem situation, this study aimed to evaluate the muscular strength index variation for lumbar spine extension (LSE) and the hydroxyproline (HP) urine concentration, as a

function of the execution of flexibility training with maximum intensity (flexibilizing) – statically (S) and by proprioceptive neuromuscular facilitation (PNF), and with submaximal intensity (stretching – Str) by students from the Brazilian Air Force Air Cadets Preparatory School (EPCAR).

Materials and Methods

Sample

The sample of this study, characterized as experimental, was obtained randomly, among the 500 students from EPCAR, and consisted of 60 male volunteers, aged between 15 and 20 years, divided into four groups: CG (control) n=15, StrG (stretching) n=15, SFG (static flexibilizing) n=15, and PNFG (flexibilizing by PNF) n=15.

Ethics

All of the participants were informed about the research protocol and, in the case of minors (under 18 years of age), the parents or guardians signed a Consent Form. The study was submitted to and approved by the Research Ethics Committee from the Castelo Branco University (UCB – RJ), under protocol no. 0094/2008, and met the standards to conduct research on humans of the National Health Council, resolution 196/96, of 10/10/96 (16) and of the Declaration of Helsinki (17).

Procedure for evaluation

To determine the anthropometric characteristics of the sample, measurements of body mass, height and body fatness percentage were done based on the 3-site skinfold test by Jackson and Pollock (18). Body mass was assessed through a digital balance with 100 g resolution (Filizola, PL150 Personal Line model, Brazil), the height was measured with a wall stadiometer (Sanny, Brazil) and the skin folds were measured with the use of a plicometer (CESCOFF, Brazil), with a 0.1-mm precision. All anthropometric measurements followed the International Standards for Anthropometric Assessment (19). Data were collected on the day after the anthropometric evaluation.

To evaluate the damage produced in the muscular collagen, the biochemical marker hydroxyproline (HP) was used, by measuring its urine concentration through the colorimetric method and, for that, the HPROLI 2 h protocol (20) and the ClinRep® kit (complete kit for hydroxyproline in urine) were used. In the colorimetric method, HP is oxidized to pyrrole, followed by a coupling with paradimethylaminobenzaldehyde. The reagents are prepared in-house, being: buffer solution (pH 6.0), chloramine T solution, Ehrlich's reagent, hydroxyproline standard

solution, phenolphthalein, sodium hydroxide, isopropanol and perchloric acid.

As a precaution to preserve the validity of the gathered data, two days before the urine collection, the research participants were instructed not to ingest collagen present in certain foods. Therefore, the subjects, during the study period and within the prior 48 hours, did not use any type of ergogenic, nutritional, pharmacological substances, physiological resources or alcohol. Furthermore, in the study period, their diets lacked red or white meat, seafood, sweets, ice cream or jelly, to try to control and standardize the dietary intake of HP.

The urine samples of the present study were collected by the Nordin method (21), during a two-hour period, from 7 to 9 am, after the subjects underwent 12 hours of fasting, with the recommendation to ingest as much water as possible before the collection, in order to collect larger volumes of urine.

Properly identified one-liter bottles were provided by the São Lucas Laboratories, in Barbacena, whose quality certification is appointed by the ISO registration 9001/2000. The analyses were conducted in Belo Horizonte, under an agreement with Lab Rede.

The samples were analyzed in the HPLC system containing a gradient pump, an injector valve, a heat column (60 °C), a UV/VIS detector for 472 nm, a computer with the HPLC software and a pulse regulator.

After urine analysis, the HP values found were converted from mg/L to mg/24 h, in order to enable a direct comparison with the reference values (22).

The evaluation of muscular strength was conducted in the afternoon, using the digital dynamometry protocol, with the execution of a one repetition maximum (1RM) (23), in the extension movements of the lumbar spine (LSE). The measurement was done using a multipurpose digital dynamometer (CEFISE, Brazil, 2007) (24), with a load cell for PC, 0.1 kgf or 1 N resolution, 250 kgf or 2500 N maximum capacity and coupled with a N2000 data acquisition system.

Immediately after the muscular strength evaluation the flexibility program was implemented, organized as follows: CG (control), StrG (stretching), SFG (static flexibilizing) and PNFG (flexibilizing by PNF).

The StrG performed static stretching exercises in three series, with a holding time of 5 seconds, in submaximal intensity. The trunk flexion movement was performed in a sitting position, in pairs, slowly and gradually, in order to reach the maximum limit.

The SFG performed the same movement in three series, with a holding time of 15 seconds, gently pushing to reach the widest range of possible

movement, with maximum intensity. The PNFG executed the movement in three series, with a holding time of 8 seconds, using the passive and active method, and the support, contraction and relaxation processes to reach the widest range of movement possible, with maximum intensity.

To control the intensity of the flexibility programs, the perceived exertion scale was used as a basis (PERFLEX) (5). It was observed that the perceived effort of the StrG participants indicated forcing between the levels 31 and 60, and the SFG and PNFG were placed in the discomfort interval, between 61 and 80 of the same scale.

The result of the final average and of the standard deviation of the perceived exertion of each group was obtained by the intervention average and had the following values: CG = 15.7 ± 3.2 , StrG = 45.3 ± 4.2 , SFG = 69.9 ± 6.9 and PNFG = 79.4 ± 8.2 .

The evaluation of muscular strength was performed immediately after the intervention of the flexibility programs, with the purpose of comparing data, following the same protocols described previously. Urine collection was performed again, 24 hours after the first, following the same protocols and procedures already described.

Statistics

For the treatment of the research data, the statistical package SPSS 14.0 for Windows was used, calculating descriptive statistics (mean, standard deviation, coefficient of variation, skewness and percentage). The Shapiro Wilk test was used to verify the normality of the data and Levene's test to analyze the homogeneity of the sample. A one-way ANOVA was used for multiple comparisons of the studied group, and the Tukey's HSD test for statistical differences within groups in the independent variables. A statistical significance level of 95% ($p < 0.05$) was adopted.

Results

The *Table I* shows the anthropometric characteristics of individuals in the selected groups.

It was observed in *Table I* that the average of body fatness (10.00 ± 3.62) corresponds to a low fat rate, according to the WHO (25). Analyzing the p values of SW stated normality in the anthropometric variables of the sample.

The *Table II* shows the ratio of the variation and homogeneity test between the Lumbar Spine Extension (LSE) and hydroxyproline (HP).

The *Table II* shows, as a result of Levene's statistical calculation, a non significant difference between the research groups, confirmed by the $p = 0.055$ value in the LSE variable. The same condition was extended for the variable HP.

Table I Anthropometric characteristics and body composition of the sample.

Groups	Parameter	Mean	SD	Skewness	CV	p-value (SW)
CG	MASS	63.04	5.92	0.53	9%	0.554
	HEIGHT	171.60	5.54	-0.43	3%	0.354
	% FAT	10.55	3.29	0.25	31%	0.477
	AGE	16.87	1.19	0.00	7%	0.279
StrG	MASS	65.88	6.40	0.99	9%	0.069
	HEIGHT	175.27	6.23	0.31	3%	0.848
	% FAT	10.57	4.28	-0.88	40%	0.211
	AGE	17.27	1.22	-0.32	7%	0.080
SFG	MASS	63.72	5.29	0.37	8%	0.997
	HEIGHT	175.20	4.14	-0.34	2%	0.162
	% FAT	9.47	3.63	1.08	38%	0.095
	AGE	17.27	1.28	-0.34	7%	0.094
PNFG	MASS	60.29	7.02	-0.11	11%	0.226
	HEIGHT	172.40	5.25	-0.66	3%	0.375
	% FAT	9.42	3.41	0.83	36%	0.140
	AGE	17.13	1.30	-0.28	7%	0.161

SD: Standard Deviation; CV: Coefficient of Variation; SW: Shapiro Wilk; p-value=0.05.

Table II Variation and homogeneity between LSE and HP.

Variation and Homogeneity Test				
	Levene Statistics	df1	df2	p-value
LSE	3.802	3	56	0.055
HP	1.049	3	56	0.378

Difference values between Lumbar Spine Extension (LSE) and Hydroxyproline (HP); *p < 0.05 LSE.

Table III Inter-group comparison of the LSE variable.

(I) GROUP	(J) GROUP	Δ (I-J)	SD	p-value
CG	STRG	0.01	0.03	0.939
CG	GFE	0.07	0.03	0.062
CG	PNFG	0.07(*)	0.03	0.029*
STRG	GFE	0.05	0.03	0.206
STRG	PNFG	0.06	0.03	0.111
GFE	PNFG	0.01	0.03	0.989

CG = Control group; STRG = Stretching group; FG = Flexing group; $\Delta\%$ = Percentage change; PNFG = Proprioceptive Neuromuscular Facilitation group; SD: standard deviation; (*) p > 0.05 LSE inter-group; * p < 0.05 PNFG x GC.

The *Table III* shows inter-group comparison values for the LSE variable.

According to the data indicated in the *Table III*, only PNFG showed significant differences in the LSE

Table IV Inter-group comparison of the HP variable.

(I) GROUP	(J) GROUP	Δ (I-J)	SD	p-value
CG	StrG	-0.24	0.26	0.790
CG	FG	-0.50	0.26	0.244
CG	PNFG	-0.59	0.26	0.124
StrG	FG	-0.25	0.26	0.772
StrG	PNFG	-0.35	0.26	0.557
FG	PNFG	-0.09	0.26	0.984

CG = Control group; STRG = Stretching group; FG = Flexing group; PNFG = Proprioceptive Neuromuscular Facilitation group; SD: standard deviation; * p < 0.05 HP inter-group.

maximum strength. Nevertheless, the FG leaned towards this result.

The *Table IV* shows inter-group comparison values for the variable HP.

It is evident, according to the data in *Table IV*, that the HP did not show significant differences between the groups.

The *Table V* shows the percentage difference values of the variables LSE and HP in order to identify which variable showed significant differences.

We can observe a significant (p=0.019) percent of change between the variables LSE and HP.

Table V Percentage of change values between the variables LSE and HP.

HP				
% CHANGE				
Variables	Statistics	df1	df2	p-value
LSE	3.607	3	56	0.019
HP	1.032	3	56	0.385

Percentage change variation between Lumbar Spine Extension (LSE); Hydroxyproline (HP); *p-value=0.05.

Discussion

When analyzing the inter-group values of the LSE variable, a significant reduction was observed in the strength index only for the PNFG, which corroborates a study by Nogueira et al. (14) that also found a reduction in explosive strength from the lower limbs after implementation of flexibilizing by PNF.

In another study (26) conducted with 12 adults, a significant reduction in explosive strength was observed using the PNF method and a significant reduction of 16.98% ($p < 0.05$) in strength resistance, after the PNF routine, which can be verified with the result of this study.

In a study conducted by Murguia et al. (27), with eccentric exercises, increased levels of HP were observed, showing the occurrence of a lesion.

Studies that addressed the relationship between the stretching and flexibilizing programs and strength, and that used HP as a biochemical marker (13, 28, 29, 30), did not show immediate significant differences in the rates of this substance, which can be seen

in this study. However, other studies (9, 15, 30, 31) do not corroborate the above findings.

A study by Caetano (9), with eight military police officers suffering from lumbago, verified an immediate effect of HP after stretching in the water. It was found that the levels increased from 20.69 ± 12.76 mg/d to 27.53 ± 18.70 mg/d, indicating that there are no significant differences between the basal and post 24-h HP concentrations, a fact that is relevant to this study as an increase was found, although this was not significant.

Nevertheless, a significant reduction of the HP levels was observed in another study (30) after implementation of the stretching method (53.3 ± 22.6 and 31.6 ± 11.3 mg/d), in 8 individuals aged between 25 and 45 years. The present study has a greater number of individuals and more variables, which enables the occurrence of the observed results, and has an experimental power of 71% for a beta corresponding to 0.29.

Conclusion

The present research found a significant decrease of the static maximum strength rate of LSE, after a flexibilizing intervention by PNF, a fact not observed in stretching, static flexibilizing and in the levels of HP.

Therefore, the development of other studies related to the acute effects of stretching and flexibilizing programs, capable of verifying the variation of HP and muscle strength is recommended both for sedentary people and practitioners of physical activities and athletes.

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