GENDER DIFFERENCES IN THE SENSITIVE THRESHOLD TO ELECTRICAL NERVE STIMULATION IN YOUNG ADULTS

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ABSTRACT

Objective: To investigate gender differences in neuronal sensory threshold (NS) for transcutaneous electrical nerve stimulation (TENS) among young adults, and the probable effects of thermotherapy. Methods: 30 young healthy students (15 men and 15 women) with 22±2 years old were divided by gender. TENS was applied in both right and left knees of subjects with a frequency of 20 Hz and pulse duration of 230μs. The amplitude of the electric current (m□) was gradually increased to record the perception threshold (ST) and tolerance threshold (TT), before/after thermotherapy. The warm R-knee was performed by infrared light (250W) ≈70cm perpendicular, and cooling L-knee with icepack, both carried out for 15 minutes. The tissue temperature was recorded by digital thermometry. The data were analyzed and differences established at $p<0.05$. Results: The tissue temperature after heat treatments was different ($p\leq0.05$) between genders. The basal ST for TENS was not different between genders, however, thermotherapy raised the NS in both men and women. At the baseline TT was lower ($p \leq0.05$) in women, however, after thermotherapy it increased ($p\leq0.05$) in both sexes. Conclusion: Neuronal sensory threshold for TENS is gender- thermo-dependent in healthy young adults.

Keywords: Transcutaneous eletric nerve. Hyperthermia induced, Cryotherapy, Sensory threshold.

Citation: *Rocha WA, Facini MP, Santuzzi CH, Freitas GKF, Pereira RRR, Araujo MTM, et al. Gender differences in the sensitive threshold to electrical nerve stimulation in young adults. Acta Ortop Bras. [online]. 2011;19(2):74-8. Available from URL: http://www.scielo.br/aob.*

INTRODUCTION

In recent years, the neuronal sensory system (NSS) has been the target of countless investigations in different biomedical areas.[1-5] Particularly in rehabilitation medicine, as the main analgesic and non-pharmacological therapeutic methods, such as electrotherapy, thermotherapy (including cryotherapy) and phototherapy, depend directly on the sensorimotor pathways and on their adaptability.[1-7] To this effect, the evaluation of the sensory perception threshold (ST), that is, the lower quantity of energy capable of evoking a perception, and of the tolerance threshold (TT), the higher quantity of energy tolerated before pain, are essential measurements for studies of the superficial, thermal tactile and painful sensory pathways.[1,2] Recent investigations have evidenced that the NSS can undergo direct and indirect influences of various endogenous and exogenous physical and chemical factors for the regulation of sensory perception and of pain thresholds.[1-4] The discovery that the circulating levels of sex hormones (SH), especially estrogen E2 and progesterone P2, besides the reproductive[8] and cardioprotective[9] roles, can exercise modulatory effects on the neuronal functions and alter the superficial cutaneous sensitivity[1,2], gave rise to intriguing questions about the parameters of conformation/therapeutic modulation in analgesia in both genders, particularly in associations with thermal resources.[5-11] Considering that transcutaneous electrical nerve stimulation (TENS) appears as one of the most reliable and effective non-pharmacological resources for analgesia[3-7,10-15], it became worth investigating the variations of the NSS between genders, and whether previous changes in skin temperature induced by heating/cooling would interfere in the adjustment/peripheral control of the NSS.

Recently, experimental and clinical investigations showed that there are differences in the perception of the nociceptive stimulus, in the pain tolerance threshold and in cutaneous sensitivity to electrical stimuli between genders.[10-14] Studies by Santuzzi *et al*.[12] in anesthetized rats, showed that the combined use of TENS and cryotherapy reduced the previously high femoral nerve activity through the isolated use of TENS. Hopkins et al.[13] showed that in the knee joint of men, after

All the authors declare that there is no potential conflict of interest referring to this article.

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Article received on 8/9/09 and approved on 1/31/10.

effusion, both cryotherapy and TENS significantly reduced arthrogenic muscle inhibition, suggesting that the use of thermal resources in association with or prior to electrical resources, mainly cryotherapy, appears to interfere selectively in the neural activity of the sensory fibers (A and C delta) in animals and humans.[12-14] Some authors also show that in 87% of cases of acute and chronic pain, thermal resources (including cryotherapy) are the types of non-pharmacological analgesic therapeutic approaches frequently used prior to or in association with electrical resources[3,5,14]. Although from the electrophysiological perspective this common practice does not seem adequate, due to factors such as: changes in skin impedance, variations of tissue water content, alterations of the enzymatic and energetic metabolism and actions in the impulse transmission speed in the nerve fibers, among others caused by thermotherapy.[4,5,12-14] Nevertheless, there are few experimental and/or clinical studies demonstrating the differences of gender in the perception and tolerance of transcutaneous electrical stimuli, as well as the implications of SH in the regulation of final skin temperature after the use of thermotherapy. Therefore, the aim of this study was to investigate gender differences in the perception and tolerance thresholds (ST/TT) for TENS in healthy young adults, and whether previous thermotherapy (induced tissue heating or cooling) modifies cutaneous sensitivity thresholds.

METHODS

Subjects and experimental groups

A convenience sample of 30 healthy youths of both genders (15 men and 15 women), undergraduate students of a certain institution[4], was invited to take part in the study. The inclusion criteria were: young age, (between 19 and 25 years), normal body mass index (between 20 and 24.9 Kg.cm^2), sedentary (> 2 months), absence of neurological, cardiovascular, respiratory, metabolic, dermatological and musculoskeletal dysfunctions in the last two months. The women (volunteers) were also supposed to be in the follicular phase (proliferative), that is, from the 6th to the 11th day of the estrous cycle after menstruation and not making use of contraceptives. The exclusion criteria were: athletes, pregnancy, cancer, recent surgery (>3 months), epilepsy, allergies, besides other diseases that could impair the results. The study was conducted according to national and international standards for research on humans (Resolution 196/96-CNS and CIOMS/OMS), and approved by the Institutional Review Board of the Integrated Healthcare Center - CEP/CIAS-UNIMED (under opinion 84/2006). The individuals were provided with explanations about the experimental protocol and validated their voluntary participation, signing the informed consent term for experiments with humans (CMS-196/96).

All the participants were instructed to do not modify their eating behaviors, not to consume products with caffeine, alcoholic beverages or systemic medications (sympathomimetic stimulants) in the 24 hours preceding the data gathering.[1,2]

Anthropometric measurements, body composition and vital signs

After recruitment, the individuals were divided by gender: 1-Men (n= 15) and 2-Women (n= 15). The data gathering was performed in the physiotherapeutic resources sector of the institution of origin[(d)], in a well-lit environment, ventilated with controlled temperature (22˚C) for 20 non-consecutive days in the period of one month. The first step was to check the body weight (kg) and stature (cm) of the volunteers on scales with a stadiometer (Filizola®, Campo Grande-MS, Brazil), followed by the measurement of body temperature using digital thermometry. Corporal composition (BMI, body mass index and % of body fat) was also evaluated with an adipometer (Sanny®, São Bernardo do Campo-SP, Brazil), where the investigators used the protocols proposed by Jackson and Pollock[16] of three skin folds (pectoral, thigh and abdomen) and the Siri equation.[17]

The vital signs (SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate and RR, respiratory rate) were monitored at three particular times, that is, 5 10 and 15 minutes during the application of segmental thermotherapy/cryotherapy. Mean blood pressure (MBP) was calculated by the equation: MBP=DP+(SP-DP)/3 and the levels were classified according to the guidelines of Sociedade Brasileira de Hipertensão (Brazilian Hypertension Society).[18]

Evaluation of the perception and tolerance thresholds for TENS

The volunteers were positioned in supine with lower limbs in abduction and knees extended. Isolating material (electrical, heat and light) was used between the right (R) and left (L) knees, in order to prevent any interference among the body segments. The participants used two TENS Neurodim® II (IBRAMED Ind. Ltda., Amparo-SP, Brazil) equipment (one for each knee) with two channels and four silicon carbide electrodes (new). After local asepsis, the electrodes were covered with a conductive gel substance (H_2O) and fixed in a cross, five centimeters above and below the joint line of both knees, covering them laterally and medially (L3-L5 dermatomes). The stimulation was configured with pulse frequency set at 20 Hz and pulse duration at 230μs. Initially, the electrical current amplitude was gradually increased for determination of the perception threshold (ST) in each joint, then raised to the maximum limit tolerated (TT) by the individuals before pain.[1,2,4] The values of LS and TT for electrical current (mÁ)of each knee were acquired and recorded, and soon afterwards superficial thermotherapy was applied with rerecording of the sensitive thresholds.

Superficial cutaneous thermotherapy/cryotherapy

The heating was performed on the right knee (R), without humidity, using an infrared light (250 W bulb) from Kroman® Ind. Ltda., São Paulo, Brazil, positioned perpendicularly at a distance of≈70 cm from the left knee (L). The distance of the light bulb was set at ±8 cm, according to the report on the thermal sensation of perceptible or pleasant heat. Simultaneously, tissue cooling was performed in the knee (L) by cryotherapy using a crushed ice pack wrapped in transparent plastic film (Ice-bag), placed directly on the skin of the knee(excluding the posterior region, S1-S5 dermatomes), fixed with an elastic bandage to avoid displacements. The thermotherapy/cryotherapy in the knees was performed for 15 minutes, according to therapeutic practice.[5,19,20] For determination and monitoring of skin temperature (thermometry) during the cutaneous thermotherapy/cryotherapy, the participants used an infrared laser

digital thermometer (INCOTERM® Ind., Porto Alegre, Brazil), with thermal precision of ± 0.1˚ C, temperature range between -20˚and 200˚ C and laterally and perpendicularly positioned at a distance of 75mm from the knee joint. The basal skin temperature (initial) was recorded first in degrees Celsius, followed by (final) thermotherapy on both knees. If the tissue temperature exceeded the maximum values established for heating (45˚C) and cooling (10˚C), they were interrupted immediately.[5,13]

Data analysis

Initially the Statemat® 2.0 software (GraphPad, San Diego, CA, USA) performed the ideal estimate for sample with test power of 90% and alpha error of 0.05, which indicated n= 16 volunteers. The data were considered normal by the Shapiro-Wilk test and are expressed as mean ± standard deviation (SD). Prism® 5.0 software was used to verify differences among the variables, and the Student's t-test was applied for independent samples, with two-way analysis of variance (ANOVA), followed by the Tukey/Krames Test as post hoc analysis. A multivariate analysis was carried out by means of linear regression and Pearson's coefficient to determine the correlation between the tolerance threshold (TT) and skin temperature levels after thermotherapy/cryotherapy. The minimum level of significance adopted was $p \leq 0.05$.

RESULTS

The anthropometric characteristics of corporal composition and vital signs in young adults were determined in Table 1, where no differences were observed between men and women (genders), demonstrating homogeneity in the sample.

As presented in the Table 2, in the initial skin temperature there were no significant differences between the genders. However, after heating, the degrees of final skin temperature attained were higher ($p \leq 0.05$) in the men than in the women. As was the case in cooling, the loss of tissue heat was also higher ($p \leq 0.01$) in the men than in the women.

The perception (ST) and tolerance (TT) thresholds for TENS in knees of men and women, before and after thermotherapy/cryotherapy were represented in Figure 1, which showed no differences in the basal ST between men (7.9 ± 0.7 mÅ) and women (9.1 ± 0.8 mÅ). However after skin heating there was an increase ($p \leq 0.01$) in the men (13.2 ± 0.7 mÅ), but not in the women (10.4 ± 1.1 mÅ). After skin cooling there was a reduction in the ST (Figures 1A and 1B) of both, men (4.7 ± 0.5 mÅ) and women (5.3 ± 0.6 mÅ). It is also possible to observe that the basal TT found in the men (31.4 ± 1.5 mÅ) was higher ($p \leq 0.05$) than in the women (22.0 ± 2.2 mÅ) and that after cryotherapy/thermotherapy the TT (Figures 1C and 1D) for TENS increased ($p \leq 0.01$) in both genders, male (45.6 ± 3.3 mÅ) and female (32.5 ± 3.5 mÅ).

In the correlations between skin temperature levels and the LTs of men and women after heating (Figure 2A) and cooling (Figure 2B), it was ascertained that in heating the correlation was negative for both, yet it proved significantly strong in men ($r^2 = 0.62^*$; $p = 0.012$) and weak in women ($r^2 = 0.08$; $P = 0.45$). However, in cooling the correlation was strong negative for the men ($r^2 = 0.21$; $P = 0.21$) and strong positive for the women ($r^2 = 0.10$; $P = 0.39$), although without any differences between the genders.

Table 1. *General characteristics and vital signs in healthy young adults.*

Variables	Men (n = 15)	Women (n = 15)	*p*
Age (years)	23 3	22 2	0.125
BMI (Kg.cm^{-2})	23.5 0.5	22.7 0.5	0.132
Body fat (%)	21 2	27 3	0.087
Body temperature (°C)	36.6 0.4	37.1 0.6	0.384
MAP (mmHg)	106 3	100 4	0.095
HR (bpm)	86 5	90 4	0.124
RR (rpm)	17 2	18 1	0.241

Values presented as mean ± SD (standard deviation). BMI, body mass index; MAP, mean arterial pressure. HR, heart rate. RR, respiratory rate. Student's t-test for independent samples.

DISCUSSION

In this study, the differences between ST and TT were determined for the application of TENS between genders, demonstrating that SHs play an important role in the control/adjustment of the neuronal sensory thresholds in young adults, and that prior thermotherapy modifies this transcutaneous electrical sensory pattern. These results showed that the basal ST was the same between men and women and that the basal TT was higher in the men, yet after superficial cutaneous thermotherapy, both thresholds (ST/TT) changed between genders.

The changes found in the electric sensory thresholds between young men and women corroborate findings in literature, which describe that the speed of sensorimotor functions can change with oscillations in circulating hormone levels, especially levels of E_2 and P_2 through the estrous cycle.[2,11] Lund et al.[10] investigated differences in the electric sensory threshold and pain threshold before, during and after the use of TENS, and observed an increase of ST in men and women after the use of TENS (high frequency), although there was a greater variation in the women's group. In the studies of Avila et al.[15], using another kind of neuromuscular electrical stimulation, the tolerated electrical current amplitude, that is, TT, was also higher for men than for women. The authors suggest gender differences in the composition of the type of nerve fibers; or direct neuroactive actions of the SHs on the nerve impulse trigger and transmission speed. However, in the studies described previously there was no association and/or previous use of thermal resources,

Table 2. *Effects of thermotherapy/cryotherapy on skin temperature in healthy young adults.*

	Men (n = 15)	Women (n = 15)
R-Knee heating-°C		
Initial	36.5 0.2	37.1 0.3
Final	44.5 0.4^{++}	40.9 0.4^{++*}
L-Knee cooling-°C		
Initial	36.3 0.4	36.9 0.2
Final	14.1 1.6^{++}	19.1 1.2^{++**}

*Values expressed as mean ± SD, in degrees Celsius (ºC). Two-way ANOVA and Tukey/Krammer test as post hoc analysus. *p≤0.05 and **p≤0.01 vs. genders; $^{++}p \leq 0.01$ when initial vs. final*

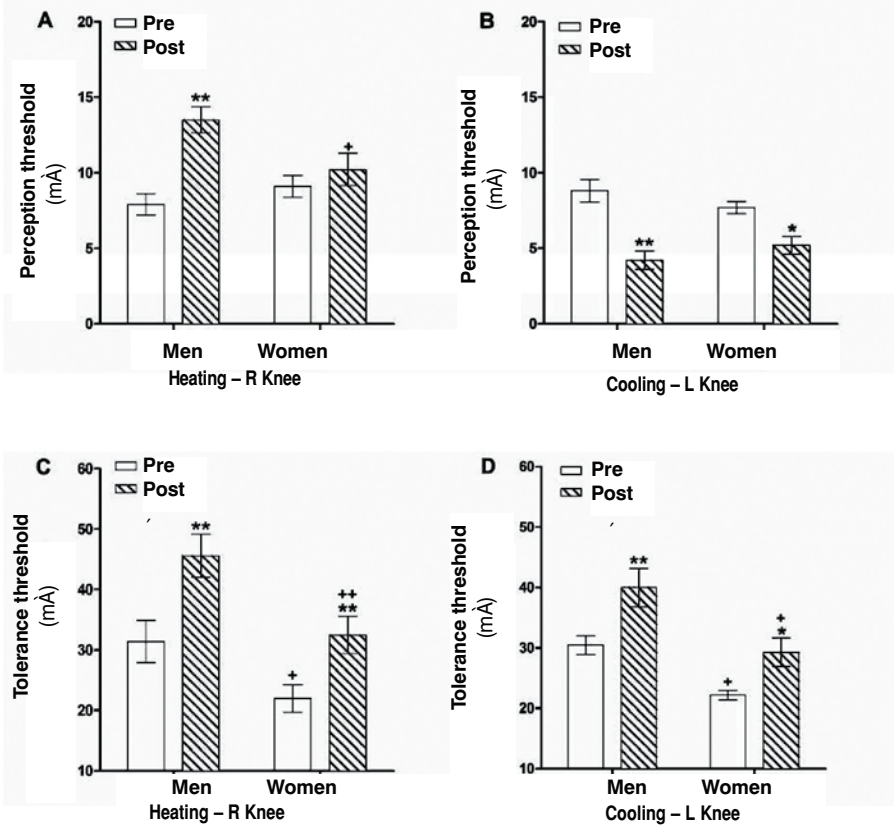

Figure 1. *Effects of infrared thermotherapy (panel A and C) and cryotherapy (panel B and D) on the sensory thresholds (ST/TT) for transcutaneous electrical nerve stimulation (TENS) in knees of healthy young men (n = 15) and women (n = 15). Values expressed in mean ± SD. Two-way ANOVA and Tukey/ Krammer as post hoc. Significant differences established at *p<0.05 and **p<0.01 pre/post; +P<0.05 and ++p<0.01 man/woman.*

which could theoretically alter the cutaneous ST and TT.[5,12]

According to studies by Santuzzi *et al*.[12] and other authors[5,13,14,19,20], cold, that is, the removal of heat by cryotherapy (multiple techniques), initially increases the triggering of fast sensory nerve fibers (A and C delta type), then gradually reduces nerve activity over time until transmission is blocked at tissue thermal levels below 2º centigrade in animals and humans, indicating that the decrease of superficial skin temperature can modulate the trigger speed and the amplitude of neural impulses. The results of this study showed that during the superficial heating or cooling of the knees, the degrees of final skin temperature obtained, both for heating (infrared) and for cooling (ice pack) were higher in the males, which suggests the participation of SHs in the peripheral thermoregulation control/adjustment processes, probably through the stimulation of localized skin thermogenesis or thermolysis by routes and mechanisms of action still unknown.

Also considering that SHs interfere in the central and peripheral regulation of the sensorimotor neuron functions[2], and according to the results found, it is possible to infer that induced and controlled changes of skin temperature (thermotherapy) significantly alter the enzymatic and energetic processes of the skin, interfering differently in the electric sensory thresholds (ST and TT) of men and women. In this regard, Palmer *et al*.,[14] in examining the effects of two kinds of electrical stimulation (TENS and interferential current)on thermal perception thresholds in women, observed that there are no changes of thermal sensitivity for both kinds of stimulation, and that the action mechanism of these types of electrical stimulation analgesia mediated by sensory fibers (A and C delta) are very complex. They also showed that transcutaneous electrical stimulation does not alter superficial and deep thermal sensory perception. Although the results of this study show that the reciprocal is not true, since numerous data indicate that superficial thermotherapy/cryotherapy modify electric, tactile and painful sensitivity.[10-14]

Current investigations in thermoregulation[20-24] showed that the circulating levels of the hormone testosterone (T), besides interfering in aggressive behavior, act in the central control of body temperature in vertebrate animals. Oppas *et al*.[24] demonstrated that E_2 and its tissue receptors (REα and REβ) play an important role in the control of thermoregulation in the tails of mice by still unknown mechanisms; and suggest that oscillations in the tissue levels of SHs (T, E_2 and P_2) appear to interfere directly and differently in skin thermogenesis/thermolysis. They also indicate that functional aging, that is, greater variations of synthesis, secretion and of the circulating levels of SHs, might significantly affect peripheral thermoregulation.[21-25] In this regard the correlations between LTs for TENS, and the levels of final skin temperature were very enlightening, as they revealed that in the male gender, cutaneous heating interferes directly in the TT for TENS. In cooling, in spite of the existence of strong correlations between levels of superficial skin temperature and TT, these were not significant between the genders.

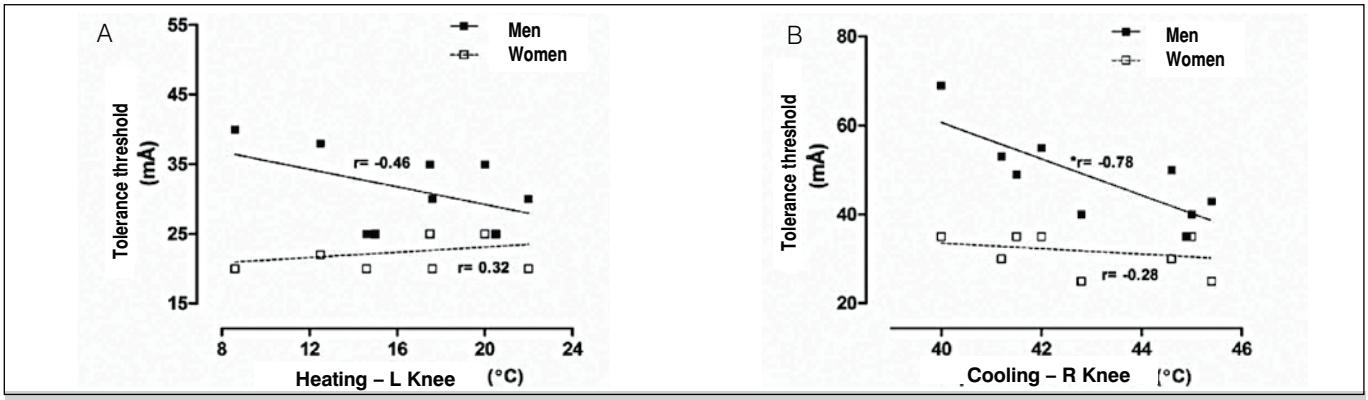

Figure 2. *Linear regression model and Pearson's correlation between tolerance threshold (TT) for transcutaneous electrical nerve stimulation (TENS) and skin temperature after cryotherapy (A) and thermotherapy (B) in knees of healthy young men (■, n = 15) and women (□, n = 15). Values expressed in mean ± SD. Significant differences established at *p<0.05.*

CONCLUSION

Based on the results obtained, together with findings in literature, we concluded that the cutaneous sensory functions (ST/TT) for TENS are gender thermo-dependent, where the perception and tolerance thresholds for TENS, either isolated or in association with heating, is higher for the male gender, and when associated with cooling, the tolerance threshold for TENS is higher in the female gender. Indicating that the probable control and/or adjustment mechanism of the peripheral NSS appears to occur through the action of SHs in the cutaneous thermogenesis/thermolysis processes. Accordingly, attention and caution are recommended in daily therapeutic practice for the application of electrical stimulation with regards to gender and to superficial skin temperature.

ACKNOWLEDGMENTS

The authors are grateful to Prof. Viviam Maria Carvalho Matos for her help in recruiting the volunteers, and to Engineer Manoel Ramos Penha for his help in calibrating the equipment of this study. Survey conducted with the help of recent doctoral degree recipient CDV-FACITEC (Of. 028/2008, Renewal /2010)

REFERENCES

1. Galvão Mde L, Manzano GM, Braga Nde O, Nóbrega JA. [Determination of electric current perception threshold in a sample of normal volunteers]. Arq Neuropsiquiatr. 2005;63:289-93.
2. Barbosa MB, Montebelo MIL, Guirro ECO. Determinação dos limiares de percepção sensorial e de resposta motora nas diferentes fases do ciclo menstrual. Rev Bras Fisioter. 2007;11:443-9.
3. Paxton SL. Clinical uses of TENS. A survey of physical therapists. Phys Ther. 1980;60:38-44.
4. Bolfe VJ, Ribas SI, Montebelo MIL, Guirro RRJ. Comportamento da impedância elétrica dos tecidos biológicos durante estimulação elétrica transcutânea. Rev Bras Fisioter. 2007;11:153-9.
5. Nadler SF, Weingand K, Kruse RJ. The physiologic basis and clinical applications of cryotherapy and thermotherapy for the pain practitioner. Pain Physician. 2004;7:395-9.
6. Ozawa M, Tsuchiyama K, Gomi R, Kurosaki F, Kawamoto Y, Aiba S. Neuroselective transcutaneous electric stimulation reveals body area-specific differences in itch perception. J Am Acad Dermatol. 2006;55(6):996-1002.
7. Ozawa M, Tsuchiyama K, Gomi R, Kurosaki F, Kawamoto Y, Aiba S. Neuroselective transcutaneous electrical stimulation reveals neuronal sensitization in atopic dermatitis. J Am Acad Dermatol. 2009 Apr;60:609-14.
8. Creinin MD, Keverline S, Meyn LA. How regular is regular? An analysis of menstrual cycle regularity. Contraception. 2004;70:289-92.
9. Santos RL, Abreu GR, Bissoli NS, Moysés MR. Endothelial mediators of 17 beta-estradiol-induced coronary vasodilation in the isolated rat heart. Braz J Med Biol Res. 2004;37:569-75.
10. Lund I, Lundeberg T, Kowalski J, Svensson E. Gender differences in electrical pain threshold responses to transcutaneous electrical nerve stimulation (TENS). Neurosci Lett. 2005;375:75-80.
11. Riley JL 3rd, Robinson ME, Wise EA, Myers CD, Fillingim RB. Sex differences in the perception of noxious experimental stimuli: a meta-analysis. Pain. 1998;74:181-7.
12. Santuzzi CH, Gonçalves WLS, Rocha SS, Castro MEC, Gouvêa SA, Abreu GR. Efeitos da crioterapia, estimulação elétrica transcutânea e da sua associação na atividade elétrica do nervo femoral em ratos. Rev Bras Fisioter. 2008;12:441-6.
13. Hopkins J, Ingersoll CD, Edwards J, Klootwyk TE. Cryotherapy and transcutaneous electric neuromuscular stimulation decrease arthrogenic muscle inhibition of the vastus medialis after knee joint effusion. J Athl Train. 2002;37:25-31.
14. Palmer ST, Martin DJ, Steedman WM, Ravey J. Effects of electric stimulation on C and A delta fiber-mediated thermal perception thresholds. Arch Phys Med Rehabil. 2004;85:119-28.
15. Avila MA, Brasileiro JS, Salvini TF. Electrical stimulation and isokinetic training: effects on strength and neuromuscular properties of healthy young adults. Rev Bras Fisioter. 2008;12:435-40.
16. Jackson AS, Pollock ML. Generalized equations for predicting body density of men. Br J Nutr. 1978;40:497-504.
17. Siri WE. Body composition from fluid spaces and density. Washington: National Academy of Science 1961.
18. a Silva MA, Rivera IR, de Souza MG, Carvalho AC. Blood pressure measurement in children and adolescents: guidelines of high blood pressure recommendations and current clinical practice. Arq Bras Cardiol. 2007;88:491-5.
19. Algafly AA, George KP. The effect of cryotherapy on nerve conduction velocity, pain threshold and pain tolerance. Br J Sports Med. 2007;41:365-9.
20. Kanlayanaphotporn R, Janwantanakul P. Comparison of skin surface temperature during the application of various cryotherapy modalities. Arch Phys Med Rehabil. 2005;86:1411-5.
21. Hampson E, Kimura D. Reciprocal effects of hormonal fluctuations on human motor and perceptual-spatial skills. Behav Neurosci. 1988;102:456-9.
22. Hau M, Dominguez OA, Evrard HC. Testosterone reduces responsiveness to nociceptive stimuli in a wild bird. Horm Behav. 2004;46:165-70.
23. Sokolnicki LA, Khosla S, Charkoudian N. Effects of testosterone and estradiol on cutaneous vasodilation during local warming in older men. Am J Physiol Endocrinol Metab. 2007;293:E1426-9.
24. Opas EE, Gentile MA, Kimmel DB, Rodan GA, Schmidt A. Estrogenic control of thermoregulation in ERalphaKO and ERbetaKO mice. Maturitas. 2006;53:210-6.
25. Struezel E, Cabello H, Queiroz L, Falcao MC. Análise dos fatores de risco para o envelhecimento da pele: gerais e nutricionais. Rev Bras Nutr Clin. 2007;22:139-45.