**JMPT logo**

**JMPT TITLE PAGE FORM**

|  |  |
| --- | --- |
| **ARTICLE INFORMATION** | **Fill in information in each box below** |
| **Article Title**  An accurate and succinct description of the article so that the reader knows exactly what the article contains.  The title includes key words to assist readers with finding the article.  Avoid declarative statements or conclusions in titles. Avoid titles written as questions. If possible, include variables, who/what was studies, circumstances of data collection, and research design. | A NEW APPROACH TO MANUAL THERAPY FOR THE IMMUNE SYSTEM: AN EXPERIEMTNAL STUDY |
| **MeSH** terms that best match your paper. Only use terms that are in MeSH  Please visit <http://www.nlm.nih.gov/mesh/meshhome.html> | Stress; Immune System; Interleukins; rats; manual therapy |
| **Running head** - no more than 40 letters/ spaces  (this is the heading that runs across the top of each page) | MANUAL THERAPY FOR THE IMMUNE SYSTEM |
| **How many words are in the abstract?** (approx 250 words or less) | 197 |
| **How many words are in the text?** (do not count abstract, figure legends, references) | 2897 |
| **Practical Applications of your study**  List 3 to 5 short sentences that highlight the practical use of the *findings* of the study. Do not include commentary or background. Do not repeat abstract.  Statements should relate to practical application of the new knowledge.  Statements should relate directly to the study findings.  Describe how the findings add value to existing evidence.  Describe the implications of the study combined with existing evidence for practice or policy. | Micro-physiotherapy promoted a reduction of interleukin 4 and lowest level of TNF-α;  Micro-physiotherapy had positive effects in the immune system;  A possible positive influence in treating stress disorders could be seen regarding this technique. |
| **Human Subjects and Animals**  If human subject or animals were used, state the name of IRB, Research Ethics Committee or equivalent in the Methods *and* here.  Required at time of initial submission: Upload a copy of the IRB/Ethics approval or exemption letter with your submission materials. | Animal Use Ethics Committee of Midwest State University (Unicentro) under protocol number 034/2014. |
| **Clinical Trial Registry**  If the study was a clinical trial, please include clinical trial registration number in the Methods and here. | Not applicable |
| **Permission to Acknowledge**  List the names of people (non-authors) or entities who you are acknowledging and specifically how they contributed to the study. List permissions here.  Required at time of initial submission:  Upload a signed letter of permission from *each* person and/or entity stating they give permission to the JMPT to print their name. | Not applicable |
| **Permission to reprint**  For all previously published figures or tables, a signed letter of permission from the copyright holder stating they give permission to the JMPT to reprint must be uploaded to the website at the initial time of submission. List permissions here. | Not applicable |
| **Funding sources**  State funding sources (grants, funding sources, equipment, and supplies). Include name and number of grant if available. Clearly state here if study received direct NIH or national funding. All sources of funding should be included. | No fundings |
| **Conflicts of interest**  List all potential conflicts of interest for all authors. Include those listed in the ICMJE form. These include financial, institutional and/or other relationships that might lead to bias or a conflict of interest. If there is no conflict of interest state none declared. | The authors declare no conflict of interest |
| **Contributorship** | For each author, list initials for how the author contributed to this manuscript.  **List author initials for each relevant category. For example, John Frank Smith should be “JFS”** |
| *Concept development* (provided idea for the research) | DG; ASIS; |
| *Design* (planned the methods to generate the results) | DG; RBP; LAC; IIK; |
| *Supervision* (provided oversight, responsible for organization and implementation, writing of the manuscript) | DG; ASIS; RBP; LAC; IIK; EC; AH; ALM; JCG |
| *Data collection/processing* (responsible for experiments, patient management, organization, or reporting data) | IIK; EC; AH; ALM; JCG |
| *Analysis/interpretation* (responsible for statistical analysis, evaluation, and presentation of the results) | RBP; IIK; EC; AH; ALM; JCG |
| *Literature search* (performed the literature search) | ASIS; RBP; LAC; |
| *Writing* (responsible for writing a substantive part of the manuscript) | DG; ASIS; RBP; IIK; EC; |
| *Critical review* (revised manuscript for intellectual content, this does not relate to spelling and grammar checking) | DG; ASIS; RBP; LAC; IIK; EC; |
| *Other* (list other specific novel contributions) |  |

**CORRESPONDING AUTHOR CONTACT INFORMATION**

|  |  |
| --- | --- |
| For the corresponding author (responsible for correspondence, proofreading, and reprints) | Fill in information in each box below |
| First name, middle initial, last name and degrees | Rodolfo B Parreira; MSc |
| Email address – this is where your proofs will be sent | rodolfo@institutosalgado.com.br |
| Postal mailing address – this is where one complimentary copy will be shipped if your paper is accepted | Rubens Carlos de Jesus, st. #355 zip code: 86055-240; Lonrina – PR / Brazil |
| Phone number | +55 43 33754712 |
|  | +55 43 33754709 |

- - - **ORDER OF AUTHORS AND AUTHOR INFORMATION - - -**

(for additional authors, please copy, paste the author information box, then fill in)

**First author**

|  |  |
| --- | --- |
| Given / First name and middle initial | Daniel |
| Family / Last name | Grosjean |
| Degree(s). Only highest academic and professional degrees. Do not include certifications. Please refer to AMA style guide for more information | Th.M. |
| Name of department(s) and institution(s) to which work should be attributed for this author (eg, Kinesiology Department, University of Georgia) | Private clinic |
| City, State/Province, Country | Pont-à-Mousson, Nantes, France |

**Second author**

|  |  |
| --- | --- |
| Given / First name and middle initial | Afonso S. I. |
| Family / Last name | Salgado |
| Degree(s). Only highest academic and professional degrees. Do not include certifications. Please refer to AMA style guide for more information | PhD. |
| Name of department(s) and institution(s) to which work should be attributed for this author (eg, Kinesiology Department, University of Georgia) | School of Manual and Postural Therapy, Salgado Institute of Integral Health |
| City, State/Province, Country | Londrina, Paraná, Brazil |

**Third author**

|  |  |
| --- | --- |
| Given / First name and middle initial | Rodolfo B. Parreira |
| Family / Last name | Parreira |
| Degree(s). Only highest academic and professional degrees. Do not include certifications. Please refer to AMA style guide for more information | MSc. |
| Name of department(s) and institution(s) to which work should be attributed for this author (eg, Kinesiology Department, University of Georgia) | School of Manual and Postural Therapy, Salgado Institute of Integral Health |
| City, State/Province, Country | Londrina, Paraná, Brazil |

**Fourth author**

|  |  |
| --- | --- |
| Given / First name and middle initial | Lisandro A. |
| Family / Last name | Ceci |
| Degree(s). Only highest academic and professional degrees. Do not include certifications. Please refer to AMA style guide for more information | MSc. |
| Name of department(s) and institution(s) to which work should be attributed for this author (eg, Kinesiology Department, University of Georgia) | School of Manual and Postural Therapy, Salgado Institute of Integral Health |
| City, State/Province, Country | Londrina, Paraná, Brazil |

**Fifth author**

|  |  |
| --- | --- |
| Given / First name and middle initial | Ivo I. |
| Family / Last name | Kerppers |
| Degree(s). Only highest academic and professional degrees. Do not include certifications. Please refer to AMA style guide for more information | PhD. |
| Name of department(s) and institution(s) to which work should be attributed for this author (eg, Kinesiology Department, University of Georgia) | Laboratory of Neuroanatomy and Neurophysiology, Universidade Estadual do Centro Oeste |
| City, State/Province, Country | Guarapuava, Paraná, Brazil |

**Sixth author**

|  |  |
| --- | --- |
| Given / First name and middle initial | Emerson |
| Family / Last name | Carraro |
| Degree(s). Only highest academic and professional degrees. Do not include certifications. Please refer to AMA style guide for more information | PhD. |
| Name of department(s) and institution(s) to which work should be attributed for this author (eg, Kinesiology Department, University of Georgia) | Laboratory of Neuroanatomy and Neurophysiology, Universidade Estadual do Centro Oeste |
| City, State/Province, Country | Guarapuava, Paraná, Brazil |

**Seventh author**

|  |  |
| --- | --- |
| Given / First name and middle initial | Andressa |
| Family / Last name | Hosni |
| Degree(s). Only highest academic and professional degrees. Do not include certifications. Please refer to AMA style guide for more information | Physiotherapy student |
| Name of department(s) and institution(s) to which work should be attributed for this author (eg, Kinesiology Department, University of Georgia) | Laboratory of Neuroanatomy and Neurophysiology, Universidade Estadual do Centro Oeste |
| City, State/Province, Country | Guarapuava, Paraná, Brazil |

**Eighth author**

|  |  |
| --- | --- |
| Given / First name and middle initial | Andressa L. |
| Family / Last name | Miri |
| Degree(s). Only highest academic and professional degrees. Do not include certifications. Please refer to AMA style guide for more information | Physiotherapy student |
| Name of department(s) and institution(s) to which work should be attributed for this author (eg, Kinesiology Department, University of Georgia) | Laboratory of Neuroanatomy and Neurophysiology, Universidade Estadual do Centro Oeste |
| City, State/Province, Country | Guarapuava, Paraná, Brazil |

**Nineth author**

|  |  |
| --- | --- |
| Given / First name and middle initial | Jossinelma C. |
| Family / Last name | Gomes |
| Degree(s). Only highest academic and professional degrees. Do not include certifications. Please refer to AMA style guide for more information | Physiotherapy student |
| Name of department(s) and institution(s) to which work should be attributed for this author (eg, Kinesiology Department, University of Georgia) | Laboratory of Neuroanatomy and Neurophysiology, Universidade Estadual do Centro Oeste |
| City, State/Province, Country | Guarapuava, Paraná, Brazil |

(for additional authors, please copy, paste the author information box, then fill in) JMPT/titlepageform/2016 – 7

**A NEW APPROACH TO MANUAL THERAPY FOR THE IMMUNE SYSTEM: AN EXPERIEMTNAL STUDY**

Daniel Grosjean1, Afonso Shiguemi Inoue Salgado2, Rodolfo Borges Parreira2, Lisandro Antônio Ceci2, Ivo Ilvan Kerppers3, Emerson Carraro4, Andressa Hosni5, Andressa Leticia Miri5, Jossinelma Camargo Gomes5

1 Grosjean

2School of Postural and Manual Therapy, Salgado Institute of Integral Health, Londrina-PR, Brazil. Zip code: 86055-240; Telephone: +55 43 33754712, fax: +55 43 33754709; E-mail (Salgado, ASI) – afonsosisalgado@yahoo.com.br; (Parreira, RB) – dolfo23@yahoo.com

3 Laboratory of Neuroanatomy and Neurophysiology, *Universidade Estadual do Centro Oeste*, Guarapuava, Paraná, Brazil, [ikerppers@gmail.com](mailto:ikerppers@gmail.com)

4 Laboratory of Virology and Molecular Biology, *Universidade Estadual do Centro Oeste*, Guarapuava, Paraná, Brazil, [emersoncarraro@bol.com.br](mailto:emersoncarraro@bol.com.br)

5 Physiotherapy students, *Universidade Estadual do Centro Oeste*, Guarapuava, Paraná, Brazil, [andhosni@outlook.com](mailto:andhosni@outlook.com), [andressamiri@hotmail.com](mailto:andressamiri@hotmail.com), [jossinelma@hotmail.com](mailto:jossinelma@hotmail.com)

**A NEW APPROACH TO MANUAL THERAPY FOR THE IMMUNE SYSTEM: AN EXPERIEMTNAL STUDY**

**Abstract**

**Objective**: The aim of the present study was to analyze the effects of micro-physiotherapy on the acute stress induced in rats by analyzing the cytokines Th1 and Th2.

**Methods**: Forty-five Wistar rats (weighing approximately 200 grams) were divided into three groups (3, 14 and 21 days) and then sub-divided into groups of five (control group, placebo group and treated group). The animals were deprived of sleep for a period of four days. The treatment applied involved soft touches on determined points of the referred organs and tissues. Analysis of the Th1 and Th2 systems was performed using flow cytometry.

**Results**: Upon analysis of the anti-inflammatory cytokine, interleukin 4 (IL-4), statistical analysis showed significant reduction in concentration of IL-4 in the treated group at 21-day after treatment comparing to the control group at 21-day (p = 0.008); and the treated group, tumor necrosis factor had significant lowest values comparing to the control group (p = 0.008) and the placebo group (p = 0.014) at 21-days after treatment.

**Conclusion**: Based on the analysis of rats submitted to micro-physiotherapy, the immune system was influenced in terms of treating the mechanism of acute stress by modulating the pro and anti-inflammatory cytokines.

Keywords: Stress; Immune System; Interleukins; rats; manual therapy

INTRODUCTION

All of the functions of a living organism depend on adequate equilibrium with the environment. This balance is maintained by an adaptive response that consists of a series of physical and emotional reactions that limit the deleterious effects of stressors in an attempt to maintain homeostasis in the body. Homeostasis is constantly challenged or threatened by intrinsic and extrinsic factors.1 In order to maintain this equilibrium, an adaptive response is necessary. According to Selye,2 this depends on the quality (physical or emotional), intensity and duration of the stimulus, in which any disturbance that alters homeostasis is considered a stressor. This response to stress is necessary for survival. When the intensity of any stressor goes above the body's threshold, a “stress syndrome” is the result.1

Therefore, stress can be defined as any situation capable of disturbing our physiological or psychological homeostasis and can lead to, depending on the intensity and duration of the stressor, repercussions that affect behavioral, endocrine and immunological properties.3 Nowadays, we are exposed to a wide range of adverse situations that have a significant impact on many aspects of our day-to-day lives, depending on how the body reacts to a stressor. Homeostasis in the immune system is completely dependent on the adequate interaction between regulator cells and costimulatory molecules.4 The main mediators involved in adaptive responses to stressors are glucocorticoids and catecholamines, while the equilibrium between the cytokines Th1 (pro-inflammatory) and Th2 (anti-inflammatory) is also significant.5

The Th1/Th2 equilibrium is fundamental to the protection of the body against external agents. For example, Th1 cells become active in response to intracellular bacterial and viral attacks, while also playing a role in the activation of macrophages and in the appearance of antigens through the liberation of interferon-γ (IFN- γ), interleukin-2 (IL-2) and tumor necrosis factor-α (TNF-α). Conversely, the immune cytokines that are characteristic of Th2, such as IL-4, IL-5, IL-10 and IL-13, promote humoral defense by stimulating mastoids, eosinophils and B cells against extracellular pathogens.6 Several previous studies have shown that adults submitted to acute stress exhibit increased production of Th1 cytokines,4 as well as greater Th2 activity, with more production of pro-inflammatory agents, such as TNF-α, which are notable in Th1/Th2 imbalances. According to Xiang et al.,7 this imbalance may persist for up to a week after the end of a stressful event. These mechanisms are partly responsible for abnormalities in the immune system, with an increase in pro-inflammatory activity leading to a significant risk of developing a disease or disorder if it is not duly regulated.

A number of treatment options exist, including the use of drugs to reestablish the Th1/Th2 equilibrium.8-11 However, conservative and non-pharmacological treatment protocols represent a good alternative for patients, given that they have little or no side effects. Manual therapy is one of these options and involves a wide range of techniques in which the therapist uses their hands to make contact with the patient´s skin in order to assess, identify and treat a variety of clinical conditions and reestablish the body´s normal function.12 The literature contains little information about the effects of manual therapy on the immune system, particularly in relation to Th1 and Th2 cytokines.13,14

Micro-physiotherapy involves manual therapy that acts directly on the surface of the body to identify and treat clinical conditions, with global results.15,16 The technique involves identifying the primary cause of the disease and/or symptoms and allowing the body to “heal itself”. Thus, the body will recognize the aggressor (antigen) and begin the elimination through cellular and tissue reprogramming.17 Assuming that micro-physiotherapy is an holistic approach, and since stress affects the body as a whole, including several different systems, we hypothesize that micro-physiotherapy would act on the protective systems, in this case the immune system, to promote a rebalance of the components such as the cytokines Th1 and Th2. These cytokines respond to an acute or chronic stressful event by deregulating the immune system. Therefore, the aim of the present study was to analyze the effects of micro-physiotherapy on the acute stress induced in rats by analyzing the cytokines Th1 and Th2.

METHODS

**Samples**

In total, 45 Wistar rats (weighing approximately 200 grams) were used in the present study. Before the induction of acute stress, the rats had free access to food and water and were kept in a light/dark cycle of 12 hours. They were randomly divided into three groups as follows: Control Group (CG), which involved stress without treatment (n=15); the Placebo Group (PG), which involved stress and caressing the animals back (n=15); and the Treated Group (TG), in which the animals were treated using micro-physiotherapy (n=15). These groups were sub-divided into CG3, CG7 and CG21; PG3, PG7 and PG21; TG3, TG7 and TG21; where 3, 7 and 21 means the days of the analysis made after the treatment. The present study received approval from the Animal Use Ethics Committee of Midwest State University (Unicentro) under protocol number 034/2014.

**Induction of acute stress**

A sleep deprivation model was used to induce acute stress.18 The animals were caged individually and alternatively submitted to the following conditions: 16 hours without water; two nights of continuous illumination; two periods (7 and 17 h) of 45° inclination of the cage; one period of 17 hours in a dirty cage (100 ml of water in a sawdust bedding); one period (8 h) without food; and a period of 17 h paired with another animal in a cage (the animals were always paired with the same partner).

**Manual therapy procedure**

Treated group

The application of the micro-physiotherapy begun with palpation assessment and the micro-physiotherapy treatment were performed at the same time by one of the researchers (DG), who had prior experience of this technique. The assessment involved a palpation examination in which was carried out on the animals’ skin in an attempt to find a decrease in cutaneous tissue mobility. Such method could be demonstrated by viscerosomatic reflex where this reflex is initiated by afferent impulse from a visceral disorder to a somatic tissues resulting in a sensory and motor changes in skeletal muscle, and skin, for example19. This was shown in a study where the authors20 found consistent findings of viscerosomatic reflex where osteopaths throughout palpation detected changes in somatic tissues such as, decreased mobility of the skin.

The researcher used their two hands or their middle and index fingers, depending on the size of the body area under investigation. Locations of the decreased mobility of the skin found in the animals are shown in figure 1. After these restrictions were encountered, the aim of the therapy was to decrease the tension or restriction in the mobility of skin tissue. This was conducted by bringing both hands together on the hypomobile points found and maintaining this position until the tension was relieved.

INSERT FIGURE 1 OVER HERE

Placebo and Control Group

Each animal from the placebo group was removed from its cage, placed on the researcher´s hand (AH), and with other hand had its neck and back stroked gently during 10 minutes in a 1 Hz cycle approximately. This researcher (AH) was not aware about the micro-physiotherapy technique, to exclude any possible effects of this technique. In this manner, we were trying to show two distinct effects. The control group was left undisturbed throughout the entire protocol, except for regular cage cleaning, which was done for all animals by the same researcher.

**Flow Cytometry**

In total, 1 ml of blood was collected from each rat for the flow cytometry analysis (BD Accuri C6 - Becton Dickinson, USA). This content was deposited in microtubes of 1.5 ml and kept in a water bath for 15 minutes, before being centrifuged at 1500 RPM for 5 minutes at 18 degrees Celsius. After being centrifuged, the supernatant (serum) was separated for later use. The BD™ Cytometric Bead Array (CBA) mouse th1/th2 Cytokine kit was used to detect IL-2, IL-4, IL-6, TNFα and IFN, following the manufacturer’s instructions. Initially, we added 50ul of the serum sample from each rat to 50ul of the mix containing an equal proportion of the specific beads for each cytokine, with 50ul of detection reagent and 50ul of standard diluent. After incubation for two hours in the dark, 1 ml of water was added and centrifuged for 2000 RPM for 5 minutes at 4 degrees Celsius. Subsequently, the supernatant was discarded and resuspended in 300ul of water.

The theoretical limit of detection for each cytokine using the BD™ Cytometric Bead Array (CBA) mouse th1/th2 Cytokine Kit was defined as the concentration corresponding to two standard deviations above the mean fluorescence of 30 repetitions of the negative control (0pg/ml). The following limits were applied: IL-2 = 0.1pg/ml; IL-4 = 0.03pg/ml and TNF = 0.9pg/ml. The cytometry reading was conducted manually, with the acquisition of 10,000 events from each sample.

**Analysis of the cytometry data**

FCap 3.0 Array software was used to analyze the samples after the performance of the flow cytometry. The results were displayed in graphs, including the mean and standard deviations values.

**Euthanasia**

All rats were euthanized 1 day after the procedures. The animals were anesthetized with 80 mg/kg ketamine and 15 mg/kg xylazine and euthanized with an intraperitoneal injection of a lethal dose of thiopental. The procedure was performed in an experimental room without the presence of other animals.

**Statistical Analyses**

The Shapiro-Wilk normality test was used and the sample was analyzed using the Kruskal-Wallis test, with Dunn´s post-test.

**RESULTS**

Figure 2 displays the mean and standard deviation values for interleukin 2 in the control, placebo and treated (micro-physiotherapy) groups. Note that these values were 648.8±144,2 pg/ml in CG3, 863.9±33.19 pg/ml in CG7 and 978.8±12.01 pg/ml in CG21. There was an increase of interleukin 2 (pg/ml) in the CG. The mean values were 1191.0±591.8 pg/ml in PG3, 635.6±284.3 pg/ml in PG7 and 1484.0±71,92 pg/ml in PG21, with a notable variance between the interleukin values in PG3 and PG21. The mean values were 907.8±1156 pg/ml for TG3, 156.4±140.3 pg/ml for TG7 and 50.35±49.07 pg/ml for TG21. No statistically significant values were found in the Kruskal Wallis test (*P*=0.1483).

INSERT FIGURE 2 OVER HERE

Figure 3 displays the mean and standard deviation values for IL-4: CG3 = 845.5±219.4; CG7 = 1204.0±381.0; and CG21 = 1718.0±197.8. Note that the presence of this interleukin increased in the CG during the study period. The mean and standard deviation values in PG3 were 929.2±266.7, while those in PG7 were 755.4±70.9 and those in PG21 were 953.9±474.9. In the PG, there was a clear maintenance of the pg/ml of interleukin 4. In TG3, the mean values were 49.15±36.23, while in TG7 they were 74.79±70.09 and in TG21, they were 67.37±27.92. Note that the levels of interleukins increased slightly in TG7 and remained low in the other two periods. A statistically significant difference was recorded in the statistical analysis (p=0.0008). Dunn´s post-test confirmed significant differences between the groups, the largest of which occurred between CG21 and TG21 (*P*=0.0078).

INSERT FIGURE 3 OVER HERE

The pg/ml levels for TNF-α were 1788.0±85.50 pg/ml in CG3, 2464.0±295.3 pg/ml in CG7 and 2657.0±7.6 pg/ml in CG21. The TNF-α levels were found to increase in the CG and the increase was continuous. The mean and standard deviation values were 3052.0±1496.0 pg/ml in PG3, 2536.0±88.71 pg/ml in PG7 and 2636.0±593.8 pg/ml in PG21. In this case, for the PG, the TNF-α levels exhibited a slight decrease in the concentration. The mean and standard deviation values were 62.28±72.87 pg/ml in TG3, 1039.0±670.7 pg/ml in TG7 and 74.85±68.41 pg/ml in TG21. Notably, the concentration of TNF-α was higher in TG7 than in TG3 and TG21. TG3 and TG21 exhibited similar levels. In the statistical analysis, the values exhibited significant data, with a p-value of p=0.0033. A statistically significant difference was recorded in Dunn´s test in CG21 ≠ TG21 and PG21 ≠ TG21, with p-values of 0.0078 and 0.0144, respectively (Fig 4).

INSERT FIGURE 4 OVER HERE

DISCUSSION

Chennaoui et al.21 analyzed the presence of TNF-α during sleep deprivation and reported that this cytokine plays a role in this situation. The same authors confirmed that the concentrations of TNF-α and IL-6 were significantly higher among sedentary sleep-deprived rats than among sedentary control rats. In addition, the IL-6 concentration was statistically lower in sleep-deprived rats that performed exercise than among sedentary sleep-deprived rats. In the present study, the TNF values were high in the CG and PG groups (3, 7 and 21 days), when compared with the TG groups. This suggests that the technique used to treat the animals was effective in terms of controlling the liberation of TNF by the cells. No significant correlation was found between the CG and the TG. The present study confirmed that TNF-α (pro-inflammatory cytokine) can activate immune-mediated inflammation in the brain, thereby increasing the laboratory evidence that TNF-α activates the molecular inflammatory mechanisms that induce neurotoxicity, for example.

The technique of micro-physiotherapy promoted immediate and delayed activity of the immune system (see Figs 2,3, and 4). It is possible to confirm the influence of pro- and anti-inflammatories by comparing TNF-α and IL-2 with IL-4, which increased in the 21-day period in the treated group. Subtle tactile stimulation is capable of maintaining the homeostasis of the metabolism of the skin, thereby demonstrating the interaction between the neuroendocrine system and cutaneous stimulation.22,23 This subtle contact (promoted in micro-physiotherapy) alters the HPA axis through variations in the immune system, given that the skin and its appendages can generate the same mediators used during the responses to systemic stress.24 Through the HPA axis of the skin, the active stress of the mastoid cells leads to a selective suppression of the TH1 response, altering humoral immunity.25 A number of authors have demonstrated that the skin and its appendages are richly innervated and their efferent signals are well represented by the sensory cortex,24,26 which may explain the improvement in the regulation of cytokines in the present study. Treatment protocols that involve subtle tactile stimulation have provided favorable results among humans and animals in relation to stress,27 the autonomous nervous system28 and neuro immunomodulatory systems.29

Meltzer et al.30 and MacNeil et al.31 reported that the high SNS tone is a response to the inflammatory alterations to the system. High circulating concentrations of TNF-α, IL-1 and IL-6 act on the hypothalamus to stimulate the pathways of the central nervous system that drive the sympathetic system to expend energy in the relevant target tissues, including secondary lymphoid tissues and the inflammation sites. In the present study, the subtle contact involved in the micro-physiotherapy identified restrictions in tissue mobility in areas corresponding to the adrenal glands, pituitary gland, and thymus. After the intervention involving light touch (approximately 5 to 10 grams), there was a significant reduction in the quantity of the cytokines IL-4 and TNF-α. These responses to stress may occur as a result of the affective functions that these subtle touches promote, when compared with discriminative tactile functions. Type-C afferent fibers (Cf) respond with minimal contact in a pleasant manner.32 Furthermore, Milne et al.33 have previously demonstrated that spinothalamic neurons converge these nociceptive inputs towards the skin when faced with a visceral stimulus. According to Craig,34 the Cf can be considered as a large extension of the afferent system that is involved in the monitoring of tissues (in skin, muscles and viscera). This system integrates several internal signals (of the body) and the cutaneous tissue, which is vital for the maintenance of homeostatic balance.

In addition, psychological stress can impair several aspects of the immune system. Reiche et al.35 reported that communication between the CNS, the endocrine system and the immune system involves chemical messengers, soluble mediators which are secreted by nerve cells, cells from the endocrine organs or immune cells, and psychological stressors may disturb this communication network. Palumbo et al.18 found that the concentration of IL-8 was significantly lower among patients with post-traumatic stress disorder. In a second assessment, they confirmed higher concentrations of IL-2 and IL-6. In the present study, it was found that the level of IL-2 remained high in CG3, PG3 and TG3, when compared with the level found in CG7, PG7 and most notably, TG7. It was notable that the level of IL-2 was low in TG21. Based on these results, it is possible to hypothesize that the micro-physiotherapy affected the liberation of IL-2 during the study period. This inhibition related to the technique decreased over the 21-day period.

Some limitations may be raised in this study. The lack of another researcher to check the validity and reliability of palpation in areas of poor skin mobility. Regarding to the placebo group, a same researcher who performed the technique in the experimental group could have performed on the placebo, however, he would not be "blind" to the applied technique and this could interfere with the results. In relation to analyzes, histochemical data from the supra renal and pituitary gland would be interesting to see the effects of the micro-physiotherapy technique on cortisol and acetylcholine respectively. Future studies should address these issues to better understand the mechanisms behind the micro-physiotherapy technique, as well as research in humans where the effect of psychological stress is closest to reality.

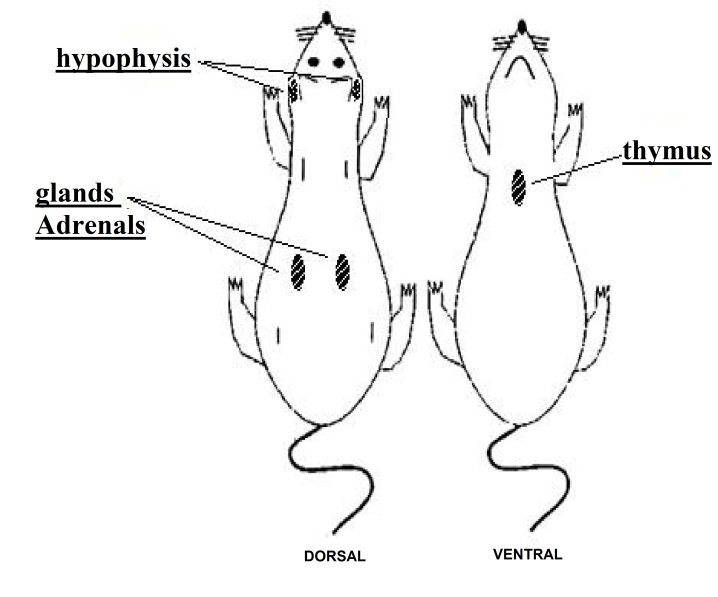
CONCLUSION

According to our data, we found that the rats in the treated group, pro and anti-inflammatory cytokines were modulated after 21 days of a single treatment when comparing to control group (IL-4) and to placebo group (TNF-α). Thus, we could infer that micro-physiotherapy would help in regulate the immune system in such situation.

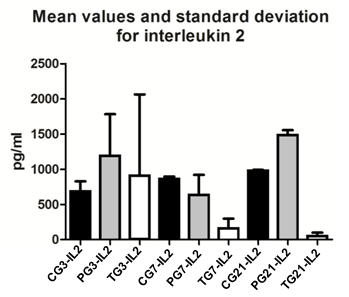
REFERENCES

1. Chrousos GP, Gold PW. The Concepts of Stress and Stress System Disorders Overview of. Jama. 1992;267:1244–52.
2. Selye H. A syndrome produced by diverse nocuous agents. 1936. J Neuropsychiatry Clin Neurosci. 1998;10(2):230–1.
3. McEwen BS, Seeman T. Protective and Damaging Effects of Mediators of Stress: Elaborating and Testing the Concepts of Allostasis and Allostatic Load. Ann N Y Acad. 1999;896(1):30–47.
4. Xiang L, Marshall GD. Immunomodulatory effects of in vitro stress hormones on FoxP3, Th1/Th2 cytokine and costimulatory molecule mRNA expression in human peripheral blood mononuclear cells. Neuroimmunomodulation. 2011 Jan;18(1):1–10.
5. McEwen BS. Central effects of stress hormones in health and disease: Understanding the protective and damaging effects of stress and stress mediators. Eur J Pharmacol. 2008;583(2-3):174–85.
6. Ginaldi L, De Martinis M, D’Ostilio A, Marini L, Loreto MF, Corsi MP, et al. The immune system in the elderly: I. Specific humoral immunity. Immunol Res. 1999;20(2):101–8.
7. Xiang L, Rehm KE, Marshall GD. Effects of strenuous exercise on Th1/Th2 gene expression from human peripheral blood mononuclear cells of marathon participants. Mol Immunol. 2014;60(2):129–34.
8. Martino M, Rocchi G, Escelsior A, Fornaro M. Immunomodulation Mechanism of Antidepressants: Interactions between Serotonin/Norepinephrine Balance and Th1/Th2 Balance. Curr Neuropharmacol. 2012;10(2):97–123.
9. Palumbo ML, Trinchero MF, Zorrilla-Zubilete MA, Schinder AF, Genaro AM. Glatiramer acetate reverts stress-induced alterations on adult neurogenesis and behavior. Involvement of Th1/Th2 balance. Brain Behav Immun. 2012 Mar;26(3):429–38.
10. Ouyang Q, Huang Z, Wang Z, Chen X, Ni J, Lin L. Effects of pristane alone or combined with chloroquine on macrophage activation, oxidative stress, and TH1/TH2 skewness. J Immunol Res. 2014;2014:613136.
11. Ahmad SF, Zoheir KMA, Ansari MA, Korashy HM, Bakheet SA, Ashour AE, et al. Stimulation of the histamine 4 receptor with 4-methylhistamine modulates the effects of chronic stress on the Th1/Th2 cytokine balance. Immunobiology. 2015;220(3):341–9.
12. Fitzgerald GK, McClure PW, Beattie P, Riddle DL. Issues in determining treatment effectiveness of manual therapy. Phys Ther. 1994;74(3):227–33.
13. Krohn M, Listing M, Tjahjono G, Reisshauer A, Peters E, Klapp BF, et al. Depression, mood, stress, and Th1/Th2 immune balance in primary breast cancer patients undergoing classical massage therapy. Support Care Cancer. 2011;19(9):1303–11.
14. Rapaport MH, Schettler P, Bresee C. A preliminary study of the effects of repeated massage on hypothalamic-pituitary-adrenal and immune function in healthy individuals: a study of mechanisms of action and dosage. J Altern Complement Med. 2012;18(8):789–97.
15. Salgado ASI, Parreira RB, Santos I, Urbano J, Foncêca N, Bénini P, et al. Effects of microkinesitherapie on heart rate variability. Man Ther Posturology & Rehabil J. 2013;11:488–93.
16. Pereira A, Carvalho E, Kerppers II, Furmann M, Pires J, Ribeiro L, et al. Assessment of heart rate variability in fibromialgia after micro-physiotherapy. Man Ther Posturology Rehabil J. 2014;12:191–5.
17. Menezes J. Inteligência quantica - por um mundo melhor. Rio de Janeiro: Novo ser; 2006. 110 p.
18. Palumbo ML, Canzobre MC, Pascuan CG, Ríos H, Wald M, Genaro AM. Stress induced cognitive deficit is differentially modulated in BALB/c and C57Bl/6 mice: correlation with Th1/Th2 balance after stress exposure. J Neuroimmunol. 2010;218(1-2):12–20.
19. Bael MC. Viscerosomatic reflex: a review. Am J Osteophat Assoc. 1985;85:786-801.
20. Licciardone JC, Fulda KG, Stoll ST, Gamber RG, Cage AC. A case-control study of osteopathic palpatory findings in type 2 diabetes mellitus. Osteopath Med Prim Care. 2007;1:6.
21. Chennaoui M, Gomez-Merino D, Drogou C, Geoffroy H, Dispersyn G, Langrume C, et al. Effects of exercise on brain and peripheral inflammatory biomarkers induced by total sleep deprivation in rats. J Inflamm (Lond). 2015;12:56.
22. Kuhn CM, Schanberg SM, Field T, Symanski R, Zimmerman E, Scafidi F, et al. Tactile-kinesthetic stimulation effects on sympathetic and adrenocortical function in preterm infants. J Pediatr. 1991;119(3):434–40.
23. Mathai S, Fernandez A, Mondkar J, Kanbur W. Effects of tactile-kinesthetic stimulation in preterms: a controlled trial. Indian Pediatr. 2001;38(10):1091–8.
24. Ito N, Ito T, Kromminga A, Bettermann A, Takigawa M, Kees F, et al. Human hair follicles display a functional equivalent of the hypothalamic-pituitary-adrenal axis and synthesize cortisol. FASEB J. 2005;19(10):1332–4.
25. Marshall JS, Gomi K, Blennerhassett MG, Bienenstock J. Nerve growth factor modifies the expression of inflammatory cytokines by mast cells via a prostanoid-dependent mechanism. J Immunol. 1999;162(7):4271–6.
26. Arck PC, Slominski A, Theoharides TC, Peters EMJ, Paus R. Neuroimmunology of stress: skin takes center stage. J Invest Dermatol. 2006;126(8):1697–704.
27. Henricson M, Ersson A, Määttä S, Segesten K, Berglund A-L. The outcome of tactile touch on stress parameters in intensive care: a randomized controlled trial. Complement Ther Clin Pract. 2008;14(4):244–54.
28. Diego MA, Field T. Moderate Pressure Massage Elicits a Parasympathetic Nervous System Response. Int J Neurosci. 2009;119(5):630–8.
29. Waters-Banker C, Butterfield TA, Dupont-Versteegden EE. Immunomodulatory effects of massage on nonperturbed skeletal muscle in rats. J Appl Physiol. 2014;116(2):164–75.
30. Meltzer JC, MacNeil BJ, Sanders V, Pylypas S, Jansen AH, Greenberg AH, et al. Contribution of the adrenal glands and splenic nerve to LPS-induced splenic cytokine production in the rat. Brain Behav Immun. 2003;17(6):482–97
31. MacNeil BJ, Jansen AH, Greenberg AH, Nance DM. Activation and selectivity of splenic sympathetic nerve electrical activity response to bacterial endotoxin. Am J Physiol. 1996;270(1 Pt 2):R264–70.
32. Olausson H, Cole J, Rylander K, McGlone F, Lamarre Y, Wallin BG, et al. Functional role of unmyelinated tactile afferents in human hairy skin: sympathetic response and perceptual localization. Exp brain Res. 2008;184(1):135–40.
33. Milne RJ, Foreman RD, Giesler GJ, Willis WD. Convergence of cutaneous and pelvic visceral nociceptive inputs onto primate spinothalamic neurons. Pain. 1981;11(2):163–83.
34. Craig AD. How do you feel? Interoception: the sense of the physiological condition of the body. Nat Rev Neurosci. 2002;3(8):655–66.
35. Reiche EMV, Nunes SOV, Morimoto HK. Stress, depression, the immune system, and cancer. Lancet Oncol. 2004;5(10):617–25.

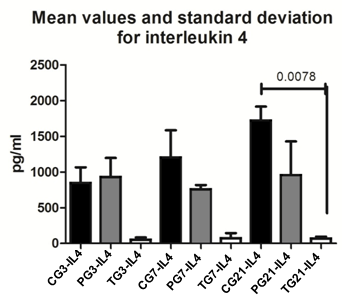
**Figures**

****

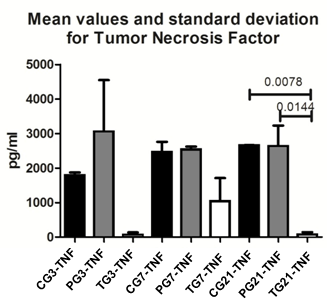
**Fig 1.** *Points that were confirmed and later treated using the technique.*

****

**Fig 2.** *Analysis of the flow cytometry for Interleukin 2 (IL2) in the control group at 3 (CG3-IL2),7 (CG7-IL2), and 21 (CG21-IL2) days after trratment; placebo group at 3 (PG3-IL2), 7 (PG7-IL2), and 21 (PG21-IL2) days after treatment; and treated group at 3 (TG3-IL2), 7 (TG7-IL2), and 21 (TG21-IL2) days after treatment. No statistically significant differences were found.*



**Fig 3.** *Analysis of interleukin 4 (anti-inflammatory) in the groups studied based on serum analysis and flow cytometry. Note: CG3-IL4, CG7-IL4, and CG21-IL4 – control group at 3, 7, and 21 days after trratment; PG3-IL4, PG7-IL4, and PG21-IL4 – placebo group at 3, 7, and 21 days after treatment; and TG3-IL4, TG7-IL4, and TG21-IL4 – treated group at 3, 7, and 21 days after treatment. A significant p-value was found between the 21-day control group and the 21-day treated group.*

**

**Fig 4.** *Flow cytometery analysis for the tumor necrosis factor (TNF). Note: CG3-TNF, CG7- TNF, and CG21- TNF – control group at 3, 7, and 21 days after trratment; PG3- TNF, PG7- TNF, and PG21- TNF – placebo group at 3, 7, and 21 days after treatment; and TG3- TNF, TG7- TNF, and TG21- TNF – treated group at 3, 7, and 21 days after treatment. A significant p-value was found between the 21-day treated group and 21-day placebo group, and 21-day treated group and 21-day control group.*