**Title**

**Evaluation of sympathetic adrenergic branch of cutaneous neural control throughout thermography and its relationship to nitric oxide levels in patients with Fibromyalgia**

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**1. Introduction**

Fibromyalgia syndrome (FMS) is defined as a complex disease, characterized by chronic widespread musculoskeletal pain and other symptoms such as hyperalgesia, allodynia, fatigue, joint stiffness, anxiety, depression, headache, sleep disorders and cognitive problems (Giacomelli et al., 2013; Häuser et al., 2015; Wolfe et al., 2016). FMS affects, on average, 2.10% of the world's population. Its prevalence is higher in women (4.2%) than in men (0.2%), with a female-to-male ratio of 21:1 and it is most frequent in middle aged, between 40 and 49 years old (Branco et al., 2010; Cabo-Meseguer et al., 2017).

The aetiology of FMS remains uncertain, however, the prevailing hypothesis shows that a Central Nervous System (CNS) disorder could explain the diffused musculoskeletal pain in this population (Chinn et al., 2016; Russell and Larson, 2009). These studies have reported that FMS patients present an increased pronociception and a decreased antinociception of the CNS, showing a sympathetic hyperactivity and a hyper-excitability of the nociceptive peripheral inputs (Chinn et al., 2016; Russell and Larson, 2009).

The latest researches highlight the existence of blood microcirculation alterations and capillarity disorders in patients with FMS (Choi and Kim, 2015; Morf et al., 2005). These abnormalities could be explained by changes in the innervation to the arteriovenous anastomoses (AVAs) at the skin of the FMS patients (Albrecht et al., 2013). AVAs are direct connections between small arteries and veins which are innervated by sympathetic fibres and are abundant in the glabrous skin of the hands, feet, nose and ears (Albrecht et al., 2013; Walløe, 2016). Albrecht et al. (2013) examined hypothenar skin biopsies of FMS patients in order to study the innervation of AVAs and their connection with the peripheral nervous system. They discovered an excessive innervation of AVAs, characterized by an increase of peptidergic sensory innervation over the sympathetic innervations in FM patients. Probably, the increased sensory innervation of hand´s AVAs promotes vasodilation, given that the stimulation of peptidergic sensory branches in the skin can activate the local “axon-reflex” and liberate a large amount of substance P and calcitonin gene-related peptide (CGRP) (Albrecht et al., 2013). Both substances are potent vasodilators which are able to modify the sensory feedback mechanisms among capillaries and precapillary arterioles, influencing the peripheral blood circulation (Burnstock and Ralevic, 1994; Holzer, 1998). However, although the vasodilation of the peripheral arterioles is considered as a consequence of an altered neural vasoregulation of the AVAs, it would, be also caused by molecules liberated from vascular endothelial cells such as nitric oxide (NO) (Johnson et al., 1995).

NO is a molecule involved in the vasodilation process, causing local hyperperfusion of the tissues and local hyperthermia (Lewis et al., 1993; Schulman, 1997). The scientific literature has reported conflicting data regarding NO levels in FMS patients. While some studies have found higher serum NO levels in FMS patients (Çimen et al., 2009; Koca et al., 2018), other researchers did not find significant differences in NO levels in comparison to healthy people (Akkuş et al., 2009; Alaşehirli et al., 2007). However, in our concerns, there are no previous studies that evaluated NO levels and its relationship with the vasodilatory peripheral response and general thermogenesis in FMS patients.

AVAs and NO play also a crucial role in the process of thermogenesis that is defined as heat production related to the physiological process necessary for maintaining general body thermoregulation and basal metabolism (Busbridge and Rothwell, 1993; Cheung, 2015; Scholander et al., 1950). In a recent study, Elmas et al. (2016) reported that FMS patients had an increased core body temperature due to an Autonomic Nervous System (ANS) disorder which might affect the blood microcirculation and sweating functions, thereby modifying the core body temperature (Jones, 1998; Jones and Plassmann, 2002).

On the other hand, thermography is a method that it provides a good relationship between peripheral blood flow variations and skin thermal properties of the cutaneous tissue (Fujimasa et al., 2000; Sagaidachnyi et al., 2014). Thermography uses the “heat transfer theory” for evaluating the blood circulation in different parts of the body. This theory proposes that a major heat in a region of the body is related to major blood affluence. By contrast, the cold regions of the body are related to smaller blood affluence (Sagaidachnyi et al., 2017).

In view of the reported findings, the aim of the present study was to analyse the vasodilatory peripheral response mediated by sympathetic adrenergic system at dorsal and palm sites of the glabrous skin of the hands, the core body temperature, and the relationship between microvasculature abnormalities, general thermogenesis and NO levels in a population of women diagnosed with FMS in comparison with healthy people.

**2. Methods**

2.1. Study Design and Participants

Forty-two women diagnosed with FMS and recruited from two Spanish Fibromyalgia Associations (AGRAFIM & AFIXA) participated in this observational case-control study. Fifty-two healthy women were recruited from relatives of the patients and volunteers by local advertisement from University of Granada (Spain). The recruitment of the patients was performed between January 2019 and June 2019. Telephone contact was made in order to set the visit throughout these six months. Only one study visit was required for each patient. Each patient was evaluated on the same day, over a total time of 60 minutes. The study was conducted in accordance with the Helsinki Declaration 2013 and was approved by the Ethics and Research Committee of the CEI Andalusia Heath Service of Granada (Granada, Spain), with Approval Number 1797-N-17. All participants signed a written informed consent form prior to their inclusion in the study.

Inclusion criteria for FMS patients were: 1) diagnosed in accordance to the American College of Rheumatology criteria for classification of FMS (revision in 2016) (Wolfe et al., 2016) by a rheumatologist of the Public Health System of Andalucía (Spain); 2) aged from 18 to 70 years; and 3) lack of any other rheumatic diseases. Inclusion criteria for control group were: 1) aged from 18 to 70 years and 2) lack of any other rheumatic diseases. Exclusion criteria for both groups were: 1) male sex; 2) presence of cardiac, renal or hepatic insufficiency; 3) severe physical disability; 4) fever after infection in the pass two week; 5) hypotension/hypertension; 6) psychiatric illness; 7) neurological disorders; 8) cancer; 9) previous history of surgery; 10) treatment with vasoactive drugs or anticoagulants or drug history; and 11) skin alterations.

2.2. Measures

Firstly, participants completed questionnaires regarding the medical history in order to obtain the demographic data. These demographic data comprised age, sex, height, weight, body mass index, and hand dominance. Besides, the Spanish version of the Revised Fibromyalgia Impact Questionnaire (FIQ-R) was used to assess the degree of FMS symptoms (Salgueiro et al., 2013). Subsequently, main outcomes measures were evaluated in the same day. Before participation in the study, patients had been informed to wear comfortable clothing such as sport shirt and sweat pants and not to wear any accessories such as watches, bracelets and rings. All participants were only asked to avoid from ingesting any vasoactive substance (alcohol, caffeine, nicotine) and food in the two hours prior to the evaluation.

2.2.1. Peripheral vascular response

The infrared thermography (IT) is a non-invasive technique that measures local changes in skin temperature. IT is a useful method that providing physiological information about the microvasculature and skin surface temperature (Clauw, 2014; Gaskell, 1956; Kulshreshtha et al., 2012). IT has demonstrated a sensitivity of 90% and a specificity of 86% (Mirbod and Sugiura, 2017). Thermographic images of dorsal and palm site of both hands were acquired using a FLIR B335 infrared thermography camera (FLIR Systems, INC., USA). Atmospheric temperature of the camera was set at 20ºC and spectral emissivity was set at 0.98, since human skin behaves as a blackbody with an emissivity of 0.96-0.99 (Ring and Ammer, 2012; Sanchez-Marin et al., 2009). All thermal images were conducted with compliance to the recommendations of the European Association of Thermology (Ring and Ammer, 2012). All thermograms were obtained in the same conditions. The participants stayed in a sitting position in a room with a constant temperature of 20ºC, following an acclimatization period of 20 minutes. After acclimatization, IT was firstly performed at the dorsal site and then at the palm site of both hands, images were captured from the distal phalanx to the wrist (Lim et al., 2014). The maximum, minimum and average temperature from each point of both hands was calculated using the camera Software (**Fig. 1.**). IT was conducted in the afternoon for both groups in order to control changes in the circadian rhythm (Neves, 2017).

2.2.2. Core body temperature

The core body temperature was measured in the external auditory canal with an infrared thermometer (Infrared Dermal Thermometers, Exergen). This technique reflects an accurate measurement of the core temperature due to the relationship of the tympanic artery with the hypothalamus showing a sensitivity of 91% and a specificity of 90% (Bijur et al., 2016; Gasim et al., 2013). Moreover, axillary temperature was taken, since it also reflects the body temperature (Gasim et al., 2013; Lodha et al., 2000).

2.2.3. NO Levels Measurement

NO production was indirectly quantified using an ozone chemiluminiscence-based method (MacArthur et al., 2007). The total amount of NO was determined by a modification of the procedure described by Braman and Hendrix (López-Ramos et al., 2005) using the purge system of Sievers Instruments, model NOA 280i (GE, Analytical Instruments, Colorado (CO), USA). The final NO values were referred to the total protein concentration in the initial extracts (MacArthur et al., 2007).

2.3. Statistical analysis

Statistical analyses were performed using SPSS Statistics Version 24 for Windows (IBM Corporation, Armonk, NY, USA). The Kolmogorov-Smirnov test was used to analyse the normality and the distribution of the variables (P>0.05).Unpaired Student t-test with 95% confidence interval (95% CI; α-value=0.05) was used for continuous data to compare differences of means between the groups for sociodemographic and clinical data. A two-way analysis of covariance (ANCOVA) was performed in order to assess the main objective of the study. The groups were women diagnosed with FMS (cases) and healthy women (controls). The key variables were the maximum, minimum and average temperature values at dorsal and palm sites of both hands (dominant: D and non-dominant: ND) at each point, while age and body mass index (BMI) were used as covariates. Linear regression was used to test interactions between serum NO levels and the temperature of the skin surface of both hands and the tympanic and axillary temperature. The results are reported as percentage change (β) with 95% CI. Statistical significance was set at P<0.05.

Sample size calculation was performed by using NCSS-PASS software. According to the previous study of Brusselmans et al. (2015) a sample size of 40 FMS patients and 40 healthy controls was estimated in order to provide a 95% CI, a power of 80%, and an alpha level (α) of 0.05. Sample size was increased until 225 subjects taking account an expected percentage of losses around 65%.

**3. Results**

3.1. Demographic and Clinical Data

Demographic and clinical data for participants are shown in **Table 1**. A total of 42 women diagnosed with FMS with mean age of 56.45±6.58 years and 52 healthy women with mean age of 57.15±10.52 years met the inclusion criteria. Women diagnosed with FMS showed a significant higher weight and BMI than healthy women (P≤0.003). There were no significant differences for age, height or serum NO levels between groups (P≥0.248). The results of ANCOVA showed significant differences between the groups for the tympanic temperature (F=10.706, P=0.002). FMS participants showed higher core body temperature (Table 1). Note that the mean of the total score of FIQ-R was 72.48±12.73 in FMS participants.

3.2. Temperature of dorsal fingertips and dorsal centre of the hands

ANCOVA showed statistically significant differences in the temperature of the dorsal site of both hands between the groups for the variables maximum, minimum and average of thumb fingertip (D: F ≥ 97.787, *P* ≤ 0.001; ND: F ≥ 114.285, P ≤ 0.001); maximum, minimum and average of index fingertip (D: F ≥ 110.460, P ≤ 0.001; ND: F ≥ 122.228, P ≤ 0.001); maximum, minimum and average of middle fingertip (D: F ≥ 128.550, P ≤ 0.001; ND: F ≥ 129.516, P ≤ 0.001); maximum, minimum and average of ring fingertip (D: F ≥ 135.768, P ≤ 0.001; ND: F ≥ 111.077, P ≤ 0.001); maximum, minimum and average of pinkie fingertip (D: F ≥ 130.445, P ≤ 0.001; ND: F ≥ 112.445, P ≤ 0.001); maximum, minimum and average of dorsal centre (D: F ≥ 64.851, P ≤ 0.001; ND: F ≥ 67.330, P ≤ 0.001). ANCOVA also revealed a significant effect of the covariate age for the temperature at minimum dorsal thumb fingertip (ND: F=4.003, P=0.048). **Fig. 2.** Show the average temperature of the dorsal site dominant and non-dominant of both hands in women with Fibromyalgia and healthy controls.

3.3. Temperature of palm fingertips and palm centre of the hands

ANCOVA showed statistically significant differences in the temperature of the palm site of both hands between the groups for the variables maximum, minimum and average of thumb fingertip (D: F ≥ 114.536, P ≤ 0.001; ND: F ≥ 95.807, P ≤ 0.001); maximum, minimum and average of index fingertip (D: F ≥ 113.901, P ≤ 0.001; ND: F ≥ 113.147, P ≤ 0.001); maximum, minimum and average of middle fingertip (D: F ≥ 150.888, P ≤ 0.001; ND: F ≥ 122.583, P ≤ 0.001); maximum, minimum and average of ring fingertip (D: F ≥ 125.304, P ≤ 0.001; ND: F ≥ 128.308, P ≤ 0.001); maximum, minimum and average of pinkie fingertip (D: F ≥ 78.065, P ≤ 0.001; ND: F ≥ 96.556, P ≤ 0.001); maximum, minimum and average of palm centre (D: F ≥ 107.449, P ≤ 0.001; ND: F ≥ 128.583, P ≤ 0.001); maximum, minimum and average of thenar eminence (D: F ≥ 84.179, P ≤ 0.001; ND: F ≥ 118.286, P ≤ 0.001); maximum, minimum and average of hypothenar eminence (D: F ≥ 133.310, P ≤ 0.001; ND: F ≥ 90.660, P ≤ 0.001). ANCOVA also revealed a significant effect of the covariate age for the temperature at maximum palm thumb fingertip (D: F=4.192, *P*=0.044; ND: F=4.502, *P*=0.037; minimum palm thumb fingertip (D: F=5.722, *P*=0.019; ND: F=7.024, *P*=0.009); average palm thumb fingertip (D: F=4.670, *P*=0.033; ND: F=4.674, *P*=0.033); maximum palm centre (D: F=5.986, *P*=0.016; ND: F=7.007, *P*=0.010) and average palm centre (ND:F=4.771, P=0.032). **Fig. 3.** Show the average temperature of the palm site dominant and non-dominant of both hands in women with Fibromyalgia and healthy controls.

3.4. Association between thermography image analysis and serum NO levels

Linear regression analysis indicated that minimum temperature of dorsal centre of D hand (β=-3.501, 95% CI= -6.805, -0.198, P=0.038); maximum temperature of palm centre of ND hand (β=-5.594, 95% CI= -10.106, -1.081, P=0.016); minimum temperature of palm centre of ND hand (β=-4.090, 95% CI= -7.905, -0.275, P=0.036); average temperature of palm centre of ND hand (β=-5.519, 95% CI= -9.933, -1.106, P=0.015), and the maximum temperature of the thenar eminence of D hand (β=-5.800, 95% CI=-10.508, -1.092, P=0.017) were significantly associated with NOx levels after adjustment for age, menopause state and BMI in controls but not in women diagnosed with FMS. No significant differences were found between serum NO levels and the rest of temperature variables between cases and controls (**Table 2**).

3.5. Association between tympanic and axillary temperature and serum NO levels

Finally, linear regression analysis for the tympanic and axillary temperature and serum NO levels, showed that only tympanic temperature (β=-9.321, 95%CI= -17.974, -0.669, P=0.035) was significantly associated with NO levels after adjustment for age, menopause state and BMI in the control group (**Table 3**).

**4. Discussion**

The results of this study show that FMS patients presented higher maximum, minimum and average temperature at each point of the dorsal and palm sites of both hands measured than the controls. Also, FMS patients showed higher core body temperature in the tympanic artery, than healthy women. Finally, we did not found a significantly association between serum NO levels and the temperature in FMS, however some interactions were achieved at the dorsal and palm sites of the hands in controls,

Our results indicate that tympanic temperature was higher in FMS group, suggesting an increased thermogenesis. Previous studies have shown that FMS patients have an increased core body temperature, related to an imbalance of the ANS (Brusselmans et al., 2015; Elmas et al., 2016; Kulshreshtha et al., 2012). Elmas et al. (2016) measured the body temperature with a skin temperature probe that operated with a range of 0-50 ºC to the inside of the right arm of the patients and observed that temperature in FMS patients was higher than in controls. In other study, (Brusselmans et al., 2015) showed that FMS patients presented an increased axillary temperature compared with the controls, but without changes for the tympanic temperature. These results are in contrast with our findings, since differences between groups were only achieved at tympanic level. Discrepancies may be explained by the different devices used for measuring core body temperature. However, as Abdi et al. (2016) previously reported, the tympanic method have a better accuracy and precision for detecting this variable. Supporting our results, scientific literature evidences that AVAs vasodilation is related to release of substance P and CGRP at blood flow (Charkoudian, 2010; Holzer, 1992; Johnson and Kellogg, 2010a; Minson, 2010). The substance P is delivered at blood circulation after physical or stress conditions (cold or heat), stimulating the mast cells that released vasoactive substances (interleukin 6) and pro-inflammatory mediators (tumor necrosis factor) at blood flow (Theoharides et al., 2015). Several works have reported that these molecules are elevated in FMS patients (Paus et al., 2006; Theoharides et al., 2015, 2010; Tsilioni et al., 2016). Therefore, the increase in these vasoactive markers might be related to the elevated core body temperature that we have recorded in our FMS participants.

Our findings highlight an excessive peripheral vasodilation of the microvasculature of the hands in FMS. Researchers have previously reported that ANS disorders are associated with an alteration of the innervation of the AVAs at glabrous skin of the hands of FMS patients (Albrecht et al., 2013). The thermoregulatory activity in this area is regulated by the adrenergic vasoconstrictor system that can be activated during cold stress (Gibbins et al., 2003; Johnson and Kellogg, 2010b; Kellogg, 2006). Facing cold conditions, such as cold stress test, FMS patients show similar characteristics of Raynaud’s phenomenon, including cyanosis and vasospasm on the fingers of the hands (Scolnik et al., 2016). The adrenergic axons of the AVAs are stimulated by cold conditions, they can detect a reduction of skin surface temperature and transmit this information to the CNS in order to stimulate the sympathetic vasoconstrictor system. This process impedes the blood supply to the peripheral capillaries of the superficial plexus with the objective of delivering the blood to the deep venous system, thereby maintaining tissue temperature and preserving tissue viability (Benzinger, 1963; Daanen, 2003). In line with our results regarding higher temperature observed in the palmar site of the digits (33.36±1.06) and dorsal centre temperature (33.40±0.92) of both hands in the FMS group, Scolnik et al. (2016) assessed Raynaud and FMS digits and dorsal hands temperature by infrared thermography and showed that patients diagnosed with FMS had significant higher baseline temperature of the digits (32.1±7.3 versus 29.0±7.3 ºC) and dorsum (31.9±4.3 versus 30.2±4.4 ºC) of the hands compared with Primary Raynaud’s patients, despite that there were no differences in symptom characteristics between Primary Raynaud’s and FMS patients (Scolnik et al., 2016). By contrast, a recent study has demonstrated a lower cold detection pain threshold in hands of patients with FMS (29.9±0.7ºC) compared with healthy controls (31.0±0.6ºC) throughout an electrochemical skin conductance (Pickering et al., 2020). This could due to an impaired sudomotor function at the dominant hands in FMS patients, being necessary explored more studies regarding to the nociception-autonomic system intertwining (Pickering et al., 2020). Further longitudinal studies are necessary to clarify the contribution of AVAs to the capillary circulation regarding changes in the temperature of the glabrous skin of the hands before and after ice-water test in FMS.

The dilation of peripheral capillaries could also be caused by vasoactive compounds such as NO which plays an important role in cutaneous vasoactive vasodilation, eliciting hyperperfusion and local hyperthermia (Johnson et al., 1995). Gratt and Anbar. (2005) reported elevated NO levels in patients with chronic orofacial pain associated with an excessive vasodilation and hyperthermia in this region. Our data showed no differences in NO levels between FMS patients and controls and no significant associations between NO and hand temperature. However, some correlations were found in the control group. Previous works have informed that NO is not involved in reflex vasodilation at peripheral cutaneous of forearm skin (Crandall and MacLean, 2001; Dietz et al., 1994). However, other studies reported that NO plays an important role in cutaneous vasodilation after a whole body hyperthermia in healthy subjects (Kellogg et al., 2008). Consequently, the role that NO plays in vasodilation and peripheral blood flow is still unclear and suggests the presence of other vasodilator mechanisms that may contribute to NO effects (Fujii et al., 2017; Kellogg et al., 2008). Taking into account the contradictory results in the literature, more research is needed to validate our preliminary findings.

We should recognize some limitations of the current study. Firstly, the small sample size. Therefore, additional researches in a larger sample are needed to confirm our results. Secondly, no causal conclusions can be drawn due to the cross-sectional design of the present study. Thirdly, it is know that there is considerable intra-subject variability in thermography quantification (Clark et al., 1999). This variability can be minimized by incorporating as the protocol that we have done in our study. Fourthly, we did not control the menstrual cycle phase of the participants, which could affect the reflex vascular responses (Lafferty et al., 1985). Fifthly, the existence and symptoms of neurological disorders were examined by questionnaire, which might have led to undetected possible underlying neurological disturbs of the hands. Besides, we have not recorded the eye temperature as a reliable and reproducible method for estimating the body core temperature (Tan et al., 2009; Vardasca et al., 2019). Finally, only women were included in the study due to the higher prevalence of FMS among women, and to avoid a possible confounder (Arout et al., 2018; Buskila et al., 2000). However, to the best of our knowledge, this is the first study to investigate the thermographic pattern of the hands as expression of sympathetic neural control activity and the relationship with NO in patients diagnosed with FMS.

**5. Conclusions**

In conclusion, we have found an increased tympanic and hand temperature at all points assessed by IT in FMS participants compared with healthy people. These results suggest a dysfunction of sympathetic cutaneous neural control and therefore in blood microcirculation and thermogenesis in these patients.Future studies should investigate the relationship between thermal images of the hands and NO levels for validating our data as well as the relationship with FMS clinical symptoms and others biomarkers.

**CRediT authorship contribution statement**

**María Encarnación Aguilar Ferrándiz**: Investigation, Conceptualization, Writing - Review & Editing; **Antonio Casas Barragán**: Investigation, Methodology; **Rosa María Tapia Haro**: Resources, Writing - Original Draft; **Alma Rus**: Resources, Data Curation; **Francisco Molina**: Formal analysis, Data Curation, Writing - Original Draft. **María Correa Rodríguez**: Formal analysis; Methodology, Writing - Review & Editing.

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**Figure Legends:**

**Fig. 1.** Thermography image of the hands of a patient diagnosed with Fibromyalgiaand healthy control.

The analysis of the temperature of the skin surface was conducted through a circle at the centre of each dorsal and palm fingertip (diameter 10×10 mm), at dorsal and palm centre of each hand (diameter 20×20 mm), at the thenar eminence of each hand, (diameter 38×72 mm), and at the hypothenar eminence of each hand (diameter 31×75 mm). (A) Image of the dorsal and palmar thermography of the hands from the same Fibromyalgia participant. (B) Image of the dorsal and palmar thermography of the hands from the same healthy control participant.

**Fig. 2.** Box plots of average temperature of the dorsal site of the hands in cases of women with Fibromyalgia and healthy controls.

In the box plots are shown the average temperature (ºC) from each point (thumb fingertip, index fingertip, middle fingertip, ring fingertip, pinkie fingertip, dorsal centre) of the dominant and non-dominant dorsal site of both hands between women diagnosed with Fibromyalgia and healthy controls. In the box plots, the boundary of the box closest to zero indicates the 25th percentile, the black line within the box marks the median, and the boundary of the box farthest from zero indicates the 75th percentile. Whiskers above and below the box indicate the 10th and 90th percentiles.

**Fig. 3.** Box plots of average temperature of the palm site of the hands in cases of women with Fibromyalgia and healthy controls.

In the box plots are shown the average temperature (ºC) from each point (thumb fingertip, index fingertip, middle fingertip, ring fingertip, pinkie fingertip, thenar eminence, hypothenar eminence, palm centre) of the dominant and non-dominant palm site of both hands between women diagnosed with Fibromyalgia and healthy controls. In the box plots, the boundary of the box closest to zero indicates the 25th percentile, the black line within the box marks the median, and the boundary of the box farthest from zero indicates the 75th percentile. Whiskers above and below the box indicate the 10th and 90th percentiles.

**Table Legends:**

**Table 1**. Demographic and clinical characteristics of women with FMS and healthy women.

**Table 2**. Associations between nitric oxide (NOx) levels and temperature of dorsal site of both hands among cases (women with FMS) and controls (healthy women).

**Table 3**. Beta estimates and confidence intervals for the association between nitric oxide (NOx) and tympanic and axillary core temperature among cases (women with FMS) and controls (healthy women).

**Table 1**. Demographic and clinical characteristics of women with FMS and healthy women.

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Women with FMS**  **(n=42)** | **Healthy women**  **(n=52)** | ***P*-value** |
| **Age (years)** | 56.45±6.58 | 57.15±10.52 | 0.707 |
| **Height (cm)** | 159.12±5.67 | 157.63±6.51 | 0.248 |
| **Weight (kg)** | 72.76±12.48 | 65.58±10.34 | 0.003\* |
| **BMI (kg/m2)** | 28.88±5.70 | 26.29±4.04 | 0.012\* |
| **NOx (μmol/mg protein)** | 27.88±21.44 | 29.81±18.13 | 0.637 |
| **Tympanic temperature ºC** | 36.03±0.68 | 35.62±0.58 | 0.002\* |
| **Axillary temperature ºC** | 35.65±0.53 | 35.58±0.63 | 0.341 |
| **FIQ-R** |  |  |  |
| **FIQ-R.1** | 20.00±4.79 | - | - |
| **FIQ-R.2** | 13.79±4.46 | - | - |
| **FIQ-R.3** | 38.44±5.75 | - | - |
| **Total score** | 72.48±12.73 | - | - |

\* Significance level *P*<0.05.

Note. Data are expressed as mean ± standard deviation (SD). FMS: Fibromyalgia Syndrome; BMI: body mass index; NOx: nitric oxide metabolites; FIQ-R= revised Fibromyalgia Impact Questionnaire; FIQ-R.1= activity level of the FIQ; FIQ-R.2= overall impact of the FIQ-R; FIQ-R.3= intensity of symptoms of the FIQ-R.

**Table 2**. Associations between nitric oxide (NOx) levels and temperature of dorsal site of both hands among cases (women with FMS) and controls (healthy women).

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  |  |  | | **NOx Levels** | |  |  |
|  |  |  |  | **FMS Women (n=42)** | |  | **Healthy Women (n=52)** | |
|  |  |  | **β** | **95 % CI** | ***P*-value** | **β** | **95 % CI** | ***P*-value** |
| **Dorsal site of the hand** |  |  |  |  |  |  |  |  |
|  | **Maximum ºC** | **D** | 7.313 | (-6.641, 21.266) | 0.295 | -0.827 | (-2.857, 1.203) | 0.416 |
|  |  | **ND** | 7.676 | (-5.765, 21.117) | 0.255 | -1.350 | (-3.457, 0.757) | 0.204 |
| **Thumb Fingertip** | **Minimum ºC** | **D** | 3.479 | (-3.845, 10.803) | 0.342 | -0.656 | (-2.680, 1.367) | 0.517 |
|  |  | **ND** | 4.671 | (-4.889, 14.232) | 0.329 | -1.291 | (-3.518, 0.936) | 0.249 |
|  | **Average ºC** | **D** | 6.240 | (-5.247, 17.728) | 0.278 | -0.799 | (-2.802, 1.204) | 0.427 |
|  |  | **ND** | 6.073 | (-5.584, 17.729) | 0.298 | -1.339 | (-3.498, 0.820) | 0.218 |
|  | **Maximum ºC** | **D** | 4.653 | (-5.737, 15.044) | 0.370 | -1.154 | (-2.913, 0.605) | 0.193 |
|  |  | **ND** | 2.604 | (-6.444, 11.652) | 0.563 | -1.172 | (-3.110, 0.767) | 0.230 |
| **Index Fingertip** | **Minimum ºC** | **D** | 3.271 | (-5.709, 12.250) | 0.465 | -1.044 | (-2.948, 0.860) | 0.276 |
|  |  | **ND** | 5.156 | (-1.674, 11.985) | 0.135 | -0.851 | (-2.890, 1.187) | 0.405 |
|  | **Average ºC** | **D** | 3.966 | (-6.758, 14.690) | 0.458 | -1.156 | (-2.960, 0.648) | 0.204 |
|  |  | **ND** | 2.018 | (-7.133, 11.168) | 0.658 | -1.171 | (-3.165, 0.823) | 0.243 |
|  | **Maximum ºC** | **D** | 3.690 | (-7.362, 14.741) | 0.503 | -1.577 | (-3.395, 0.240) | 0.087 |
|  |  | **ND** | 3.676 | (-7.393, 14.745) | 0.505 | -1.617 | (-3.500, 0.266) | 0.091 |
| **Middle Fingertip** | **Minimum ºC** | **D** | 3.314 | (-5.427, 12.054) | 0.447 | -1.418 | (-3.272, 0.435) | 0.130 |
|  |  | **ND** | 1.533 | (-7.991, 11.056) | 0.746 | -1.653 | (-3.550, 0.245) | 0.086 |
|  | **Average ºC** | **D** | 3.390 | (-7.686, 14.466) | 0.539 | -1.535 | (-3.368, 0.297) | 0.099 |
|  |  | **ND** | 4.154 | (-7.144, 15.453) | 0.461 | -1.654 | (-3.551, 0.244) | 0.086 |

**(Continued) Table 2**. Associations between nitric oxide (NOx) levels and temperature of dorsal site of both hands among cases (women with FMS) and controls (healthy women).

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  |  |  | | **NOx Levels** | |  |  |
|  |  |  |  | **FMS Women (n=42)** | |  | **Healthy Women (n=52)** | |
|  |  |  | **β** | **95 % CI** | ***P*-value** | **β** | **95 % CI** | ***P*-value** |
| **Dorsal site of the hand** |  |  |  |  |  |  |  |  |
|  | **Maximum ºC** | **D** | 2.912 | (-8.256, 14.080) | 0.600 | -0.775 | (-2.572, 1.021) | 0.390 |
|  |  | **ND** | 2.855 | (-7.320, 13.030) | 0.573 | -1.268 | (-3.179, 0.644) | 0.189 |
| **Ring Fingertip** | **Minimum ºC** | **D** | -2.268 | (-11.418, 6882) | 0.618 | -0.529 | (-2.413, 1.355) | 0.575 |
|  |  | **ND** | -4.608 | (-10.990, 1.775) | 0.152 | -1.154 | (-3.082, 0.773) | 0.234 |
|  | **Average ºC** | **D** | 1.431 | (-10.952, 13.813) | 0.816 | -0.724 | (-2.570, 1.122) | 0.434 |
|  |  | **ND** | 0.102 | (-11.926, 12.129) | 0.986 | -1.277 | (-3.212, 0.658) | 0.191 |
|  | **Maximum ºC** | **D** | 3.002 | (-6.970, 12.974) | 0.546 | -0.348 | (-2.176, 1.481) | 0.704 |
|  |  | **ND** | 1.046 | (-8.133, 10.225) | 0.819 | -1.181 | (-3.111, 0.749) | 0.224 |
| **Pinkie Fingertip** | **Minimum ºC** | **D** | 4.444 | (-1.927, 10.815) | 0.166 | -0.134 | (-2.026, 1.758) | 0.887 |
|  |  | **ND** | -0.380 | (-4.702, 3.942) | 0.860 | -0.930 | (-3.251, 1.390) | 0.424 |
|  | **Average ºC** | **D** | 3.318 | (-6.904, 13.541) | 0.515 | -0.318 | (-2.168, 1.532) | 0.731 |
|  |  | **ND** | -0.081 | (-9.090, 8.928) | 0.986 | -1.107 | (-3.073, 0.860) | 0.263 |
|  | **Maximum ºC** | **D** | 3.295 | (-6.200, 12.791) | 0.486 | -3.301 | (-7.185, 0.583) | 0.094 |
|  |  | **ND** | 3.559 | (-5.381, 12.500) | 0.425 | -2.386 | (-6.744, 1.972) | 0.276 |
| **Dorsal Centre** | **Minimum ºC** | **D** | 1.334 | (-5.942, 8.611) | 0.712 | -3.501 | (-6.805, -0.198) | 0.038\* |
|  |  | **ND** | 1.875 | (-5.507, 9.257) | 0.610 | -2.881 | (-6.561, 0.799) | 0.122 |
|  | **Average ºC** | **D** | 1.350 | (-6.876, 9.577) | 0.741 | -3.405 | (-7.028, 0.219) | 0.065 |
|  |  | **ND** | 2.645 | (-5.327, 10.618) | 0.506 | -2.898 | (-7.031, 1.235) | 0.165 |

**(Continued) Table 2**. Associations between nitric oxide (NOx) levels and temperature of dorsal site of both hands among cases (women with FMS) and controls (healthy women).

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  |  |  | | **NOx Levels** | |  |  |
|  |  |  |  | **FMS Women (n=42)** | |  | **Healthy Women (n=52)** | |
|  |  |  | **β** | **95 % CI** | ***P*-value** | **β** | **95 % CI** | ***P*-value** |
| **Palm site of the hand** |  |  |  |  |  |  |  |  |
|  | **Maximum ºC** | **D** | 3.004 | (-3.876, 9.883) | 0.382 | -0.941 | (-3.007, 1.124) | 0.364 |
|  |  | **ND** | 0.541 | (-6.986, 8.067) | 0.885 | -1.198 | (-3.386, 0.990) | 0.276 |
| **Thumb Fingertip** | **Minimum ºC** | **D** | -2.727 | (-8.409, 2.955) | 0.337 | -0.849 | (-3.036, 1.338) | 0.439 |
|  |  | **ND** | -0.385 | (-5.782, 5.011) | 0.886 | -1.250 | (-3.681, 1.182) | 0.307 |
|  | **Average ºC** | **D** | 1.477 | (-5.288, 8.242) | 0.661 | -0.836 | (-2.955, 1.283) | 0.431 |
|  |  | **ND** | -0.053 | (-7.365, 7.259) | 0.988 | -1.261 | (-3.512, 0.991) | 0.266 |
|  | **Maximum ºC** | **D** | 3.599 | (-3.178, 10.375) | 0.289 | -1.276 | (-3.088, 0.537) | 0.163 |
|  |  | **ND** | 0.453 | (-5.772, 6.678) | 0.884 | -1.046 | (-2.997, 0.905) | 0.286 |
| **Index Fingertip** | **Minimum ºC** | **D** | 2.171 | (-3.889, 8.231) | 0.473 | -1.244 | (-3.073, 0.586) | 0.178 |
|  |  | **ND** | -4.070 | (-10.036, 1.896) | 0.175 | -0.794 | (-2.814, 1.226) | 0.433 |
|  | **Average ºC** | **D** | 2.629 | (-4.069, 9.327) | 0.432 | -1.248 | (-3.077, 0.581) | 0.176 |
|  |  | **ND** | -0.501 | (-6.577, 5.576) | 0.868 | -0.958 | (-2.928, 1.011) | 0.333 |
|  | **Maximum ºC** | **D** | 2.027 | (-5.221, 9.274) | 0.574 | -1.698 | (-3.606, 0.210) | 0.080 |
|  |  | **ND** | 2.288 | (-4.459, 9.034) | 0.496 | -1.378 | (-3.303, 0.546) | 0.156 |
| **Middle Fingertip** | **Minimum ºC** | **D** | 0.231 | (-6.455, 6.917) | 0.945 | -1.541 | (-3.494, 0.412) | 0.119 |
|  |  | **ND** | 1.415 | (-4.549, 7.379) | 0.634 | -1.255 | (-3.178, 0.667) | 0.195 |
|  | **Average ºC** | **D** | 0.665 | (-6.287, 7.617) | 0.847 | -1.597 | (-3.533, 0.339) | 0.104 |
|  |  | **ND** | 1.220 | (-5.033, 7.473) | 0.695 | -1.361 | (-3.289, 0.567) | 0.162 |

**(Continued) Table 2**. Associations between nitric oxide (NOx) levels and temperature of dorsal site of both hands among cases (women with FMS) and controls (healthy women).

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  |  |  | | **NOx Levels** | |  |  |
|  |  |  |  | **FMS Women (n=42)** | |  | **Healthy Women (n=52)** | |
|  |  |  | **β** | **95 % CI** | ***P*-value** | **β** | **95 % CI** | ***P*-value** |
| **Palm site of the hand** |  |  |  |  |  |  |  |  |
|  | **Maximum ºC** | **D** | 1.301 | (-6.179, 8.780) | 0.727 | -0.559 | (-2.404, 1.286) | 0.545 |
|  |  | **ND** | -0.625 | (-6.514, 5.264) | 0.831 | -1.237 | (-3.074, 0.599) | 0.182 |
| **Ring Fingertip** | **Minimum ºC** | **D** | 0.207 | (-4.840, 5.255) | 0.934 | -0.256 | (-2.172, 1.660) | 0.790 |
|  |  | **ND** | -0.915 | (-6.393, 4.563) | 0.737 | -0.942 | (-2.881, 0.996) | 0.333 |
|  | **Average ºC** | **D** | -0.452 | (-7.614, 6.710) | 0.899 | -0.483 | (-2.349, 1.382) | 0.605 |
|  |  | **ND** | -0.186 | (-1.815, 1.218) | 0.692 | -1.155 | (-3.052, 0.743) | 0.227 |
|  | **Maximum ºC** | **D** | -2.857 | (-9.016, 3.303) | 0.353 | -0.510 | (-2.320, 1.301) | 0.574 |
|  |  | **ND** | -1.212 | (-7.525, 5.102) | 0.700 | -1.061 | (-2.931, 0.808) | 0.259 |
| **Pinkie Fingertip** | **Minimum ºC** | **D** | 0.057 | (-3.264, 3.377) | 0.973 | -0.374 | (-2.258, 1.510) | 0.691 |
|  |  | **ND** | -2.494 | (-5.885, 0.897) | 0.145 | -1.086 | (-3.017, 0.846) | 0.264 |
|  | **Average ºC** | **D** | -2.557 | (-8.620, 3.506) | 0.398 | -0.432 | (-2.289, 1.426) | 0.642 |
|  |  | **ND** | -2.137 | (-7.891, 3.617) | 0.457 | -1.064 | (-2.930, 0.803) | 0.258 |
|  | **Maximum ºC** | **D** | 6.190 | (-7.739, 20.118) | 0.374 | -3.627 | (-7.640, 0.387) | 0.075 |
|  |  | **ND** | 2.826 | (-11.649, 17.301) | 0.695 | -5.594 | (-10.106, -1.081) | 0.016\* |
| **Palm Centre** | **Minimum ºC** | **D** | -1.416 | (-12.151, 9.319) | 0.791 | -3.603 | (-7.370, 0.163) | 0.060 |
|  |  | **ND** | 4.888 | (-5.319, 15.095) | 0.338 | -4.090 | (-7.905, -0.275) | 0.036\* |
|  | **Average ºC** | **D** | 6.359 | (-6.572, 19.290) | 0.326 | -3.295 | (-7.282, 0.691) | 0.103 |
|  |  | **ND** | 3.955 | (-9.300, 17.209) | 0.549 | -5.519 | (-9.933, -1.106) | 0.015\* |

**(Continued) Table 2**. Associations between nitric oxide (NOx) levels and temperature of dorsal site of both hands among cases (women with FMS) and controls (healthy women).

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  |  |  | | **NOx Levels** | |  |  |
|  |  |  |  | **FMS Women (n=42)** | |  | **Healthy Women (n=52)** | |
|  |  |  | **β** | **95 % CI** | ***P*-value** | **β** | **95 % CI** | ***P*-value** |
| **Palm site of the hand** |  |  |  |  |  |  |  |  |
|  | **Maximum ºC** | **D** | 4.236 | (-5.853, 14.324) | 0.400 | -5.800 | (-10.508, -1.092) | 0.017\* |
|  |  | **ND** | 2.842 | (-7.015, 12.700) | 0.563 | -4.318 | (-9.240, 0.605) | 0.084 |
| **Thenar Eminence** | **Minimum ºC** | **D** | 0.468 | (-7.922, 8.858) | 0.911 | -2.490 | (-6.749, 1.769) | 0.245 |
|  |  | **ND** | -2.327 | (-1.736, 1.212) | 0.721 | -4.037 | (-8.512, 0.439) | 0.076 |
|  | **Average ºC** | **D** | 2.192 | (-9.568, 13.953) | 0.708 | -4.328 | (-8.937, 0.281) | 0.065 |
|  |  | **ND** | -1.172 | (-12.391, 10.048) | 0.834 | -4.336 | (-9.248, 0.576) | 0.082 |
|  | **Maximum ºC** | **D** | 9.773 | (-1.055, 20.602) | 0.076 | -3.240 | (-7.575, 1.095) | 0.139 |
|  |  | **ND** | 6.221 | (-5.281, 17.723) | 0.280 | -4.376 | (-8.842, 0.089) | 0.055 |
| **Hypothenar eminence** | **Minimum ºC** | **D** | 1.118 | (-6.363, 8.599) | 0.764 | -0.719 | (-4.507, 3.069) | 0.704 |
|  |  | **ND** | 0.075 | (-4.415, 4.565) | 0.973 | 0.024 | (-3.167, 3.215) | 0.988 |
|  | **Average ºC** | **D** | 0.481 | (-9.737, 10.700) | 0.924 | -1.579 | (-5.553, 0.428) | 0.428 |
|  |  | **ND** | -1.300 | (-9.652, 7.053) | 0.754 | -4.087 | (-8.443, 0.269) | 0.065 |

\*Significance level *P*<0.05

Note. a) FMS: Fibromyalgia Syndrome; b) Beta (**β)**: regression coefficient, adjusted for age, menopause status and body mass index; c) 95% CI: 95% confidence interval; d) NOx: nitric oxide metabolites; e) ºC: Celsius degree; f) D: dominant; g) ND: non dominant.

**Table 3**. Beta estimates and confidence intervals for the association between nitric oxide (NOx) and tympanic and axillary core temperature among cases (women with FMS) and controls (healthy women).

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | |  | | **NOx** | |  |  |
|  | | **Cases**  **(n=42)** | |  |  | **Controls**  **(n=52)** |  |
|  | | **β** | **95 % CI** | **p-value** | **β** | **95 % CI** | **p-value** |
| **Tympanic temperature ºC** |  | -1.303 | (-12.056, 9.450) | 0.807 | -9.321 | (-17.974, -0.669) | 0.035\* |
| **Axillary temperature ºC** |  | 8.631 | (-5.826, 23.087) | 0.234 | -7.625 | (-16.432, 1.182) | 0.088 |
| **Difference tympanic temperature ºC** | **D** | -0.969 | (-8.781, 6.842) | 0.803 | -0.215 | (-4.064, 3.634) | 0.911 |
| **ND** | 0.369 | (-6.726, 7.464) | 0.917 | 1.620 | (-2.669, 5.910) | 0.451 |
| **Difference axillary temperature ºC** | **D** | 3.320 | (-5.480, 12.121) | 0.449 | -0.608 | (-4.735, 3.519) | 0.768 |
| **ND** | 4.381 | (-3.164, 11.926) | 0.247 | 1.609 | (-2.677, 5.896) | 0.454 |

\*Significance level P<.05

Note. FMS: Fibromyalgia Syndrome; Beta (β): regression coefficient, adjusted for age, menopause status and body mass index; 95% CI: 95% confidence interval; NOx: nitric oxide metabolites; ºC: Celsius degree; D: dominant; ND: non dominant.