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LEPTIN LEVELS IN PATIENTS WITH FIBROMYALGIA AND RELATIONSHIP WITH THE FEATURES OF THE DISEASE

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Leptin levels in patients with fibromyalgia and relationship with the features of the disease

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Summary

RESUMO 4

ABSTRACT 6

Introduction 8

Materials and methods 11

Study population 11

Interview and HADS Questionnaire 12

Physical Examination 13

Laboratory Evaluation 13

Statistical Analysis 14

Results 15

Study population 15

Pain threshold 17

HADS score 18

Serum leptin levels 19

Relationship between leptin and BMI and with BFM 19

Relationship between leptin concentrations and pain threshold and with HADS 20

Body fat distribution 22

Discussion 23

Leptin levels and relationship with BMI and BFM 23

Correlation of leptin and HADS questionnaire 24

Leptin and pain threshold 26

Body fat distribution 27

Limitations and Conclusion 28

Aknowledgments 30

References 31

Appendix 35

Appendix 1 – Socio-demographic Interview 35

Appendix 2 – Physical Examination 37

Appendix 3 – Hospital Anxiety and Depression Scale (versão portuguesa) 39

RESUMO

Introdução: A fibromialgia é uma doença caracterizada por dor generalizada e pela presença de sensibilidade dolorosa aumentada à pressão em zonas anatómicas bem definidas. Além disso, os doentes referem com frequência sintomas associados, tais como perturbações do humor como depressão, ansiedade, sono não repousante e cansaço, entre outras. A hipótese de que a leptina, uma hormona derivada do tecido adiposo, possa estar implicada na fisiopatologia da fibromialgia, é reforçada pela observação de que afecta dois dos sintomas mais frequentes desta doença: aumento da sensibilidade à dor e perturbações do humor. Contudo, os resultados dos estudos nestas matérias são algo contraditórios.

Objectivos: O nosso estudo teve o intuito de comparar os níveis séricos de leptina num grupo de pacientes com fibromialgia e num grupo controlo. Além disto, pretendeu-se averiguar se os níveis de leptina estavam relacionados com depressão/ansiedade e com o limiar de dor. O nosso estudo pretendeu ainda comparar a distribuição de massa gorda corporal em doentes fibromiálgicos e em controlos saudáveis.

Métodos: Doseámos os níveis séricos de leptina de 19 doentes do sexo feminino com o diagnóstico estabelecido de fibromialgia e de 19 controlos saudáveis. Todos os participantes foram submetidos a um exame físico (que incluiu a avaliação dos perímetros abdominal e da coxa, das pregas cutâneas em 4 locais específicos, dos limiares dolorosos em 5 localizações e ainda um estudo da bioimpedância) e responderam a um breve inquérito demográfico, assim como à versão portuguesa do Hospital Anxiety and Depression Scale.

Resultados: O nosso estudo mostrou que não havia diferenças significativas nos níveis de leptina entre os dois grupos. Os doentes com fibromialgia apresentaram menor limiar de dor e maior prevalência de depressão e ansiedade. Verificámos que os níveis séricos de leptina não estavam associados nem com a depressão/ansiedade, nem com o limiar de dor. O nosso trabalho também revelou que não havia diferenças entre os dois grupos estudados, no que concerne à distribuição da massa gorda.

Conclusões: Os doentes com fibromialgia e os indivíduos saudáveis têm níveis similares de leptina. As concentrações de leptina não se associaram nem à depressão/ansiedade, nem ao limiar doloroso. Não se encontrou um padrão de distribuição preferencial de massa gorda corporal que se relacionasse com a fibromialgia.

Palavras-chave: fibromialgia, leptina, limiar de dor, ansiedade, depressão

ABSTRACT

Background: Fibromyalgia is a condition characterized by chronic widespread pain and the presence of tenderness on pressure in specific anatomic sites. Moreover, patients commonly report associated features, such as mood disorders like depression and anxiety, non-restorative sleep and fatigue, among others. The hypothesis that leptin, an adipocyte-derived hormone, may be involved in the pathophysiology of fibromyalgia is supported by observations that it affects two of the most outstanding manifestations of this condition: enhanced pain sensitivity and mood disturbances. However, results are still controversial.

Objective: The aim of this study was to compare serum leptin levels in patients with fibromyalgia and healthy controls. Moreover, we tried to ascertain if leptin levels correlate with depression/anxiety and with pain threshold. In addition, we compared body fat distribution between patients with fibromyalgia and healthy controls.

Methods: We measured serum leptin levels in 19 female patients with a diagnosis of fibromyalgia and 19 matched healthy controls. All participants underwent a physical examination (which included evaluation of waist and hip circumferences, skinfolds thickness in 4 specific sites, pain threshold in 5 locations and bioimpedance analysis) and answered to a brief demographic questionnaire, as well as a portuguese translation of Hospital Anxiety and Depression Scale.

Results: In our study, we found no significant differences in leptin levels between both groups. Patients with fibromyalgia presented lower pain threshold and higher prevalence of depression and anxiety than controls. Also, we found no significant correlations between leptin with either depression/anxiety disorders, or with pain threshold. Finally, we didn't observe statistically significant differences regarding body fat distribution between the studied groups.

Conclusions: Patients with fibromyalgia had similar serum leptin levels as healthy subjects. Leptin levels were not associated with depressive/anxiety disorders, or with pain threshold. There was not a preferred body fat distributing related to fibromyalgia.

Keywords: fibromyalgia, leptin, pain threshold, anxiety, depression

Introduction

Fibromyalgia (FM) is a debilitating and persistent condition characterized by widespread pain that affects all 4 body quadrants, experienced for at least 3 months, and the presence of tenderness on pressure in at least 11 of 18 well defined anatomic sites ("tender points") [1].

In addition to pain, associated features of FM often include chronic fatigue, non-restorative sleep and mood disturbances including depression, among many others [1].

Prevalence of FM in Portugal has been estimated at around 3,6%, affecting more women than men [2]. Although FM is not a fatal disease, patients report severe disability and the disease is associated with high consumption of healthcare resources [3], resulting from its great impact upon patients' quality of life [4].

There are multiples theories regarding the pathophysiology of FM, such as hormonal dysregulation [5], immunological vulnerability [6], aberrant central pain mechanisms with peripheral modulation [7], but to date there are no fully satisfactory explanations. At the same time, the absence of laboratory tests to support the diagnosis of FM creates a pressing need for further investigations, for both clinical and research purposes.

In this paper, we will focus on leptin and its possible relationship with FM.

Leptin is a pleiotropic adipocyte-derived hormone which acts centrally through receptors in the hypothalamic arcuate nucleus, leading to satiation and reduction of food intake. This is mediated by stimulation of neurons expressing anorexigenic neuropeptides such as proopiomelanocortin and inhibition of those expressing orexigenic peptides such as neuropeptide Y [8, 9].

Leptin receptors are also distributed in other brain areas, including limbic structures implicated in the control of mood and emotions, such as hippocampus, cortex and amygdale [10].

Leptin's role in the pathophysiology of depression is currently being explored, but evidence supports the concept that leptin insufficiency and/or leptin resistance may contribute to vulnerability to depression [11].

Animal studies have demonstrated low levels of circulating leptin in rat models of depression [11]. Acute administration of leptin in rats improved the performance of 3 tests considered as highly predictive of efficacy in the treatment of human depression and anxiety [12]. Taken together, these data suggest leptin has antidepressant and anxiolytic properties.

In addition, there's a link between leptin and inflammation, since leptin is involved in modulating immune response and has been associated with an increased production of proinflammatory molecules, like TNF-α and IL-6 [13]. It is now recognized that depression is strongly associated with a proinflammatory serum cytokine profile and dysregulation of hypothalamic-pituitary-adrenal axis [14]. These observations offer a mechanism for a potential role of leptin in the pathophysiology of depression.

However, available information about the role of leptin in human depression is limited and controversial. Studies have reported increased [15-17], decreased [18, 19] or unchanged leptin levels in people with depression [20, 21].

It has also been suggested that leptin may be involved in the regulation of pain sensitivity and threshold. In animal models both the increase of endogenous leptin or administration of exogenous leptin leads to exacerbated nociceptive response and decrease of pain threshold [22, 23]. The underlying mechanisms are poorly understood.

Leptin has been reported to enhance NMDA receptors and to produce cytokines such as IL-1β, through activation of JAK/STAT pathways, sensitizing nociceptors. Leptin also promotes the synthesis of α-MSH, which is known to increase pain sensitivity [23, 24].

These findings provide potential biological pathways through which leptin may modulate nociception and pain perception.

Such observations led us to hypothesis that leptin may be related to FM, as it affects two of the most outstanding manifestations of this condition: enhanced pain sensitivity and mood disturbances.

Thus, the aim of this study is i) to compare leptin levels between FM and control group; ii) to ascertain if leptin levels correlate with depression/anxiety and with pain threshold; iii) to compare body fat distribution between FM and control groups.

Materials and methods

Study population

This study was conducted in partnership with other colleagues, as part of a project called FMScan. Although each of the investigators worked towards specific objectives, all data was gathered from the same sample to maximize the efficiency of the whole investigation.

Due to the overall design of the studies being conducted, we decided to study female patients with fibromyalgia paired with an unaffected sister.

Participants were drawn from a list of 712 patients with an established diagnosis of FM from a single site (all diagnosed and followed by Prof. J.A.P. da Silva).

The following screening criteria were used for selection: Female gender, age between 18 and 55 years old, absence of any other chronic pain condition and residence within a radius of 100 km from the study centre.

Selected patients were contacted by phone and invited to participate if 1. they had at least one unaffected sister; 2 the mother of both was the same person, still alive and capable of participating and providing reliable information; 3. all the family members were willing to travel to the research site and participate in the study, which involved signing an informed consent, responding to questionnaires, providing a blood sample and undergoing physical examination. Participants were reimbursed for transportation costs but no other compensations were offered.

The study was approved by Ethical Committee of the Faculty of Medicine of the Universidade de Coimbra.

All research proceedings were performed in the morning. After receiving and explanation of the study procedures and having an opportunity to present any questions and discuss all issues, participants signed an informed consent form. This was followed by a fasting blood sample collection. Breakfast was offered to participants before the other procedures were started.

Interview and HADS Questionnaire

The sociodemographic features and clinical findings of the participants were recorded. This included their age, marital status, educational level, medication use, previous surgeries, and other comorbidities, among others.

We also made use of a validated portuguese translation of Hospital Anxiety and Depression Scale (HADS) [25]. HADS is a brief questionnaire used to identify the presence of depression and anxiety disorders. It consists of two independent subscales (measuring depression and anxiety, respectively), both with seven items, which are scored separately. Each item is answered by participants on a 4-point (0-3) response category, and the possible score for each subscale ranges from 0 to 21. According to the authors, the way that HADS is currently issued includes the division of each mood state into 4 ranges: a score between 0 and 7 is “normal”, between 8 and 10 “mild”, between 11 and 14 “moderate” and between 15 and 21 “severe” [26].

Physical Examination

Participants underwent physical examination, and anthropometric measures were assessed: height (in m) and weight (in kg) were measured, and Body Mass Index (BMI) was calculated dividing weight by the squared value of the height (kg/m^2).

The waist and the hip circumferences were measured, and recorded as the mean of 3 measurements, using a flexible measuring tape (in cm). Biccipital, tricipital, subscapular and thigh skinfolds thickness were recorded as the mean of 3 measurements, using a Harpenden® skinfold caliper (in mm). Body composition was determined by bioimpedance analysis (InBody® 720 Body Composition Analysis), and the values of Body Fat Mass (BFM), Waist/Hip Ratio (WHR) and Body Fat Mass in Upper Limbs (UL), Trunk (T) and Lower Limbs (LL) were recorded in kg.

Pressure Pain Threshold (PT) was evaluated in each subject using a dolorimeter, in 5 specific body sites: the sternal manubrium, bilaterally, the middle point of the tibia; and the nail bed of the forefinger of each hand. Participants were told to expect an increasing sensation of pressure, and to indicate when it became painful. The pain threshold was calculated as the mean of 2 measurements, and the result was expressed in kg/cm^2.

Laboratory Evaluation

Fasting venous blood samples were drawn in the morning from each subject. Serum was extracted by centrifugation at 5000 rpm for 10 minutes and stored frozen at -20ºC until analysis, which was performed less than a month after sample collection.

Serum leptin concentrations were determined by radioimmunoassay using reagents supplied by Linco® Research Inc, in the Hormonology Laboratory of Hospitais da Universidade de Coimbra, and leptin levels were recorded in ng/mL.

Statistical Analysis

All the data were stored in a database created in Microsoft® Office Excel® 2010, and statistical analysis was carried out using PASW Statistics® 18.0.

The normality of the distribution was tested using Kolmogorov-Smirnov and Shapiro-Wilk tests, depending on whether we were analyzing the whole sample (N>30), or analyzing pairwised subjects (N<30 in each group). Comparisons between groups were performed by t-tests for paired samples and by Wilcoxon test (depending of the existence of normality of the variables). Nominal variables were comparable using χ^2 test. Relationships between variables were assessed using Pearson or Spearman correlations, as appropriate. A p value of < 0.05 was considered as statistically significant in all tests.

Results

Study population

The screening criteria described above, reduced the potential population to 317 individuals, which were contacted by phone. Of these, 278 were excluded for the following reasons: 121 did not have an unaffected sister, 27 their sister lived too far away to attend, 73 were already orphans or their mother was not capable of participating, 57 were not reachable through the phone. Of the remaining 39 families satisfying inclusion criteria, 11 refused to participate and 6 never made themselves available to attend the research centre.

Of the remaining 22 families that were included in the overall project, in this paper we excluded 3 of them (in 1 family both patient and sister had FM criteria; the leptin assays from the other 2 families weren't ready by the time we wrote this paper). Also, the mothers were not included in this study.

To resume, this study's population consisted in 38 subjects: 19 FM patients and 19 healthy sisters as controls.

Their demographic characteristics are presented in Table I.

Table I – Demographic **c**haracteristics of the study participants (n=38)

	FM Group (n=19)	**Control Group** (n=19)	*p* value
Age (years)	41,05 ± 10,07	40,84 ± 9,75	0,948
Marital Status			
Single	4	6	
Married	15	12	0,401 *
Divorced	0	1	
Years of Education (years)	12,16 ± 4,18	11,42 ± 4,86	0,617
Weight (kg)	64,95 ± 7,75	68,31 ± 10,40	0,266
Height (m)	1,59 ±0,06	1,60 ± 0,05	0,696

Results are expressed as mean ± SD or as number of subjects. Statistical analysis by Student's t test and χ^2 test (*).

Pain threshold

Table II – Comparison of pain threshold between FM group and Control group

	FM Group	**Control Group**	*p* value
PT Right Tibia (kg/cm^2)	2,82 ± 1,84	5,03 ± 1,82	0,002
PT Left Tibia (kg/cm^2)	2,77 ± 1,26	4,67 ± 1,65	0,001
PT Sternal Manubrium (kg/cm^2)	1,68 ± 1,21	3,26 ± 1,20	0,001
PT Right Nail Bed (kg/cm^2)	2,92 ± 1,49	5,13 ± 1,95	0,002
PT Left Nail Bed (kg/cm^2)	2,74 ± 1,63	5,04 ± 1,86	0,002 *

Results are expressed as mean ± SD; Statistical analysis by paired Student's t-test and by Wilcoxon test (*); PT, pain threshold.

We assessed pain threshold in both groups, and the mean values of each measured site are presented in Table II. As expected, FM group had lower thresholds, compared to Control group, with statistically significant differences ($p < 0,05$).

HADS score

Table III – Comparison of HADS score between FM group and Control group

	FM Group	**Control Group**	*p* value
HADS-Depression	8,11 ± 4,33	4,37 ± 2,87	0,012
HADS-Anxiety	12,16 ± 2,79	7,68 ±2,69	0,000

Results are expressed as mean ± SD; Statistical analysis by paired Student's t-test; PT, pain threshold.

In what concerns to HADS questionnaire, we found statistically significant differences between FM group and Control group, in depression (p = 0,012) and anxiety (p = 0,000) subscales. In order to understand if the differences that we found in the mean values of both subscales between groups did really mean differences in presence or absence of depression/anxiety disorder, we went further and analyzed the differences between both groups, considering a score ≥ 8 as positive for the presence of the respective state, as indicated in HADS manual [26].

Once again, we found statistically significant differences between FM and Control groups, for subscales of depression (p = 0,013) and anxiety (p = 0,019), indicating that the prevalence of depressive/anxious symptoms was higher in FM patients.

In FM group, 52,6% of the subjects presented depressive disorder, and 94,7% presented anxious disturbance. In Control group, the percentages were 10,5% and 57,9%, respectively.

Serum leptin levels

Table IV – Comparison of serum leptin levels between FM group and Control group

	FM Group	**Control Group**	*p* value
Leptin (ng/mL)	19,67 ± 8,19	22,34 ± 11,32	0,334

Results are expressed as mean ± SD; *p* value is based in paired Student's t-test;

As demonstrated in Table IV, there were no statistically significance differences in serum leptin levels between FM group and Control group.

Relationship between leptin and BMI and with BFM

Table V – Relationship between serum leptin and BMI and with BFM

	All (n=38)		**FM Group** (n=19)		**Control Group** (n=19)	
	rho	*p*	*r*	*p*	*r*	*p*
BMI (kg/m^2)	0,226 *	0,172 *	0,367	0,123	0,489	0,034
BFM (kg)	0,310 *	0,059 *	0,357	0,133	0,542	0,017

Results are expressed as Pearson correlation coefficients and as Spearman correlation coefficient (*); BMI, body mass index; BFM, body fat mass

Concerning the relationship between serum leptin levels and BMI, no significant relationship was observed, considering the whole sample. When we analysed both groups separately, we only found significant relationship between these 2 variables in Control group.

A similar analysis was made, but considering BFM instead of BMI. Likewise, no significant association was found considering the whole sample. Splitting the sample in FM and Control groups, our results demonstrated that leptin and BFM were related only in Control group.

Relationship between leptin concentrations and pain threshold and with HADS

Table VI – Correlation analysis between leptin concentrations and selected variables

	All (n=38)	**FM group** (n=19)	**Control group** (n=19)
PT Right Tibia (kg/cm^2)	-0,087	-0,079	-0,409
PT Left Tibia (kg/cm^2)	0,003	-0,055	-0,315
PT Sternal Manubrium (kg/cm^2)	-0,130	-0,229	-0,152
PT Right Nail Bed (kg/cm^2)	-0,152	-0,114	-0,417
PT Left Nail Bed (kg/cm^2)	-0,012	0,166 *	-0,333
HADS-Depression	-0,097	-0,166	-0,114
HADS-Anxiety	-0,147	-0,037	-0,436

Results are expressed as Pearson correlation coefficients and as Spearman correlation coefficient (*); all p values > 0,05; PT, pain threshold.

The correlation analysis between leptin levels and pain threshold was performed considering the whole sample (FM + Control groups), and separately for each group. Either way, we found no relationship between pain threshold and serum leptin levels (all *p* values > 0,05).

We also investigated the relationship between leptin concentrations and the HADS's score (divided into its own subscales), for the whole sample. Likewise, we found that there was no correlation between the hormonal levels and the scores of the questionnaire (*p* (D) = 0,564; *p* (A) = 0,378), and those findings were maintained when we performed the analyses separately for FM group (*p* (D) = 0,498; *p* (A) = 0,882) and Control group (*p* (D) = 0,643; *p* (A) = 0,062).

Body fat distribution

Table VII – Comparison of anthropometric parameters between FM group and Control group

	FM Group	**Control Group**	*p* value
BMI (kg/m^2)	25,69 ± 3,05	26,85 ± 4,73	0,308
Waist Circumference (cm)	90,2 ± 6,2	93,2 ± 9,0	0,127
Bicipital Skinfold (mm)	20,1 ± 7,0	24,1 ± 8,1	0,059
Tricipital Skinfold (mm)	29,2 ± 5,7	30,6 ± 7,0	0,448
Subscapular Skinfold (mm)	25,3 ± 9,1	28,2 ± 10,2	0,279
Thigh Skinfold (mm)	43,8 ± 9,6	45,1 ± 7,9	0,537
WHR	0,93 ± 0,05	0,91 ± 0,08	0,906 *
BFM (kg)	21,77 ± 4,67	22,78 ± 8,22	0,598
BF UL (kg)	1,49 ± 0,46	1,64 ± 0,85	0,727 *
BF T (kg)	11,4 ± 2,7	11,6 ± 4,6	0,836
BF LL (kg)	3,11 ± 0,62	3,34 ± 1,35	0,711 *

Results are expressed as mean ± SD; Statistical analysis by paired Student's t-test and by Wilcoxon test (*); BMI, body mass index; WHR, Waist/Hip Ratio; BFM, body fat mass

Anthropometric parameters were similar in FM group and in Control group ($p > 0,05$), which indicated that there were no statistically significant differences regarding body fat distribution. The recorded values are demonstrated in Table VII, as mean ± SD for each parameter.

Discussion

Leptin is an adiposity hormone that regulates food intake and energy balance, providing information on the amount of body fat to the brain: increasing levels of leptin should lead to satiation. Nevertheless, elevated leptin levels have been reported in a large proportion of obese individuals, which implies resistance to endogenous leptin in human obesity [27].

It was also reported that leptin may be involved in the mechanisms of pain threshold and other humour disturbances, such as depression/anxiety, which are common features of FM. Thus, we intended to evaluate leptin levels in FM patients, and to ascertain if the hormonal levels are related to the aforementioned features.

Leptin levels and relationship with BMI and BFM

To our knowledge, there is only data regarding leptin levels in FM patients in a "Letter to the Editor", in which the authors found significantly higher leptin concentrations in FM group, when compared to age-, sex- and BMI-matched controls [28].

In our study, we found similar leptin levels in FM group and in Control group (p = 0,334). Our results were not in accordance with the previous work, although we cannot explain the reasons for this discrepancy.

Leptin concentrations have been associated with BMI [29]. However, BMI takes into account body weight and height instead of body fat content defined as the fat component of body weight. Limited attention has been paid to the relationship of leptin concentrations with body composition measures other than BMI. Given that BMI does not accurately measure adiposity, the relationship between body fatness on leptin concentrations may be more

pronounced when more reliable methods (such as bioelectrical impedance analysis) are used to assess total body fat content, since the degree of adiposity is a key determinant of leptin concentration [30].

Surprisingly, our data showed an odd result when we performed the correlation study between leptin and BMI and between leptin and BFM. Considering all subjects (n = 38), we found that leptin levels were not correlated neither with BMI (*rho* = 0,226; *p* = 0,172), nor with BFM (*rho* = 0,310; *p* = 0,059). However, the *p* value of the correlation between leptin and BFM can be considered as borderline, and we have a strong conviction that this value would be statistically significant if we had a larger sample.

Considering both groups separately, we found that in Control group leptin levels correlated with BMI (*r* = 0,489; *p* = 0,034) and with BFM (*r* = 0,542; *p* = 0,017), but the same didn't happen in FM group for BMI (*r* = 0,367; *p* = 0,123) and for BFM (*r* = 0,357; *p* = 0,133). This situation led us to the hypothesis that an unknown factor that was only present in FM group could interfere in the levels of leptin, and interrupted the well known positive relationship between BFM and leptin, as well as the positive association between BMI and leptin.

Correlation of leptin and HADS questionnaire

In our paper, a correlation study between leptin levels and the scores of depression subscale of HADS questionnaire was performed. The results showed that there was no relationship between hormonal levels and the scores of HADS-Depression, in the whole sample and in both groups separately.

We also performed a comparison between leptin concentrations and HADS-Depression, considering a score ≥ 8 as positive for the presence of depressive symptoms, as previously mentioned. We found no significant differences in serum leptin levels in the presence or absence of depression (p = 0,610) (data not shown).

Our results were concordant with previous publications [20, 21], which demonstrated that leptin levels didn't differ between depressed subjects and healthy controls.

However, we found in the literature a number of studies that have also examined the relationship between leptin and depression, with conflicting results. There are works which documented higher leptin levels in depressed patients [15-17]; by contrast, other reports found that low leptin levels were associated with depression [18, 19].

One possible reason for this seemingly contradictory data may be that leptin levels are influenced by certain factors such as age, body fat mass, sample sizes, and comorbidity with other disorders. Moreover, another possibility that could explain these inconsistent results may lie in the different scientific and methodological approaches.

In what concerns to HADS-Anxiety, we also found no relationship between its scores and leptin levels, considering the whole sample and both groups separately. Once again, we compared leptin concentrations and the scores from HADS-Anxiety, considering a score ≥ 8 as positive for the presence of anxiety symptoms. The results showed no differences between serum leptin in the presence or absence of anxiety (p = 0,117) (data not shown).

There is only one study in which exogenous leptin was given intraperitoneally to rodents, to assess the effects of the hormone on anxiety-related behaviors. It demonstrated that administration of leptin induced anxiolytic-like effects on the experimental animals [12]. However, currently there are no human studies regarding leptin levels and the anxiety status

and as far as we know, this was the first effort to trying to understand if leptin levels are related with anxiety symptoms.

Leptin and pain threshold

Pain perception is a complex and externally inaccessible experience determined by the integration of complex neuronal processes modulated by central factors, including neurotransmitters and neurohormones. Nociception can increase or decrease according to individual differences, such as gender, age, depression, among other non-specific characteristics [29-31].

As expected, in FM group we found lower values for pain threshold when compared to healthy subjects, which means they are more sensitive to pain stimuli.

There is evidence in the literature that leptin might be involved in modulation of pain, as leptin had been reported to enhance NMDA receptor function and to promote the synthesis of molecules that are known to increase pain sensitivity, like IL-1β and α-MSH [23, 24].

In the meantime, studies on pain sensitivity in laboratory animals are also controversial. Kutlee *et al.* found that administration of exogenous leptin caused a decrease in pain threshold in mice [22], although the underlying mechanisms are still unclear. Another work in Zucker rats (which have been reported to have hyperleptinemia [32]) supported the results of the previous study, demonstrating shorter latencies to tail flick tests [33]. On the other hand, pain threshold was found to be higher in dietary-induced obese rats [34]. Thus, it is tempting to suggest that changes in pain threshold associated to obesity may be due to leptin.

A previous publication in FM patients, showed a negatively correlation between BMI and pain threshold (r = -0,238; p = 0,021) [35]. Another study demonstrated that obese individuals revealed more pain sensitivity than non-obese subjects [36]. Considering that obesity is a status of hyperleptinemia, as told before, and that BMI positively correlates with leptin concentrations, we could thus speculate that leptin is capable of producing nociceptive effects.

We addressed the question of whether leptin was related to sensitivity to pressure-induced pain, since there are no previous human studies regarding this issue. Our data showed that there was not a relationship between serum leptin concentrations and the pain threshold, in each of the measured sites. Based on the evidences already presented, the failure to find a relation between leptin and pain threshold may indicate an insufficient sample size to detect such association.

Body fat distribution

Although BFM represents the body fat content, it doesn't necessarily reflect body fat distribution. When we are investigating leptin levels, body fat distribution should be considered, because leptin production was found to be higher in subcutaneous than in visceral fat depots [27, 37]. But, once again, data concerning the previous statement are also ambiguous. Investigators found that BFM had an impact on leptin concentrations, while body fat distribution didn't affect leptin levels in overweight women [38]. Peltz *et al.* concluded that there was no specific body fat distribution pattern determining serum leptin concentrations, based on their study conducted in Mexican-American women [39]. In

summary, the relationship between leptin levels and body fat distribution in different ethnic groups remains unclear.

In order to investigate the body fat distribution in FM patients, which has not been done until now, we assessed anthropometric measures and bioimpedance analysis between FM and Control group.

Our results showed no significantly differences in body fat distribution between both groups, as we can see in Table VII. Likewise, no differences regarding body fat distribution pattern were found, and based on the mean values of WHR (FM group = 0,93 ± 0,05 and Control group = 0,91 ± 0,08), we could conclude that gynoid pattern was the most prevalent in our sample.

Limitations and Conclusion

This study was not without limitations. As a result from the difficulty in recruiting FM patients with unaffected sister and their mother still alive (required in order to get the data needed for all the researchers involved in this project), the sample size wasn't large enough to draw firm conclusions. We also performed multiple tests and didn't control for medication, smoking and phase of menstrual cycle, which have been reported to modulate leptin production [40, 41]. Furthermore, leptin secretion has diurnal variations [42], but leptin levels were measured only once in our study. However, all samples were obtained approximately at the same time, after an overnight fasting. In this study, only one researcher performed the measurement of each skinfold site, waist and hip circumferences. Although these

measurements are subject to considerable intra-observer variations, we attempted to reduce errors by repeating the measures three times and subsequently used the mean values in the analysis. In evaluating pain threshold, physical examination was performed by all researchers, although we tried to standardize the procedure, and used the average of two measures at each site for analysis.

In conclusion, our data showed that leptin levels didn't differ between FM patients and healthy controls. Moreover, our results were not fully consistent with the existing literature, since we demonstrated that leptin didn't relate with depression, anxiety and with pain threshold, although the reasons for this are not easily explained. We also assessed body fat distribution for the first time in FM patients, and concluded that there was not a preferential pattern of body fat distribution related to the disease.

In addition, we suggest that clinical significance of our findings needs to be confirmed by further studies, and future samples sizes should be increased.

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Appendix

Appendix 1 – Socio-demographic Interview

INQUÉRITO DEMOGRÁFICO

Data: ____/____/_____

Nº: _____

Iniciais do nome (1,2 e última): _____

Data de nascimento: _____/___/___

Nacionalidade: _____

Residiu no estrangeiro até aos 18 anos? _____

Desde que idade: _____ anos. Regressou aos: _____ anos.

Número de irmãos: _____

Posição na fratria: _____ (do mais velho para o mais novo)

Sexo dos irmãos (F-feminino, M-masculino): ___; ___; ___; ___; ___; ___; ___; ___
(do mais velho para o mais novo)

Tem filhos? Sim ☐ Não ☐

Nº total de filhos vivos: ____ Abortos espontâneos: _____ Abortos Provocados: ____

Filhos falecidos: ____

1.: idade à data da morte: ____ Ano da morte: _____

2.: idade à data da morte: ____ Ano da morte: _____

3.: idade à data da morte: ____ Ano da morte: _____

Pai: vivo ☐ separado ☐ Ano: _____ Falecido ☐ Ano: _____

Estado civil: _____

Profissão:

Por conta própria ☐ Por conta de outrem ☐

Desempregada ☐ Reformada ☐ Com que idade? _____ anos

Anos de educação formal: _____ Concluídos em: ____ anos

Critérios de Fibromialgia: Sim ☐ Não ☐

Data de início dos sintomas (mês e ano): ____/_____

Data em que primeiro procurou cuidados médicos por esses sintomas (mês e ano): ____/_____

Data do diagnostico (mês e ano): ____/_____

Que tratamentos faz actualmente para a Fibromialgia? (fármacos e outros)

_____
_____
_____
_____
_____

Se tem outras doenças, indique quais:

_____
_____
_____
_____

Que tratamentos faz para estas doenças?

_____
_____
_____
_____

Se já fez cirurgias, indique quais, e o ano da cirurgia:

_____
_____
_____
_____

Quem foi, para si, a principal figura maternal durante a sua infância, até aos sete anos?

_____

Appendix 2 – Physical Examination

EXAME FÍSICO

Peso: _____ kg Altura: _____ m IMC: _____ kg/m^2

Nº de pontos dolorosos: ____

Limiar de dor:		**A**	**B**	**Média**
a. Ponto médio da tíbia:	Dta:	_____Kg	_____Kg	_____Kg
	Esq:	_____Kg	_____Kg	_____Kg
b. Ponto médio do manúbrio esternal:		_____Kg	_____Kg	_____Kg
c. Leito ungueal do indicador:	Dta:	_____Kg	_____Kg	_____Kg
	Esq:	_____Kg	_____Kg	_____Kg

Massa gorda:

a. Perímetro abdominal:

(na linha que passa nas cristas ilíacas, numa expiração normal)

A	**B**	**C**	**Média**
_____cm	_____cm	_____ cm	_____ cm

b. Perímetro do braço:

A	**B**	**C**	**Média**
_____cm	_____cm	_____ cm	_____ cm

c. Perímetro da coxa:

A	**B**	**C**	**Média**
_____cm	_____cm	_____ cm	_____ cm

d. Prega bicipital:

(medida três vezes no braço não dominante)

A	**B**	**C**	**Média**
_____mm	_____mm	_____ mm	_____ mm

e. Prega tricipital:

(medida três vezes no braço não dominante)

A	**B**	**C**	**Média**
_____mm	_____mm	_____ mm	_____ mm

f. Prega subescapular:

(medida três vezes no braço não dominante)

A	**B**	**C**	**Média**
_____mm	_____mm	_____ mm	_____ mm

g. Prega da coxa:

A	**B**	**C**	**Média**
_____mm	_____mm	_____ mm	_____ mm

h. Bio-impedância

Appendix 3 – Hospital Anxiety and Depression Scale (versão portuguesa)

Este questionário foi concebido para ajudar a saber como se sente. Pedimos-lhe que leia cada uma das perguntas e faça uma cruz (X) no espaço anterior à resposta que melhor descreve a forma como se tem sentido na última semana.

Não demore muito tempo a pensar nas respostas. A sua reacção imediata a cada questão será provavelmente mais correcta do que uma resposta muito ponderada.
Por favor, faça apenas uma cruz em cada pergunta.

1. Sinto-me tenso/a ou nervoso/a: ___ Quase sempre ___ Muitas vezes ___ Por vezes ___ Nunca	**8. Sinto-me mais lento/a, como se fizesse as coisas mais devagar:** ___ Quase sempre ___ Muitas vezes ___ Por vezes ___ Nunca
2. Ainda sinto prazer nas coisas de que costumava gostar: ___ Tanto como antes ___ Não tanto agora ___ Só um pouco ___ Quase nada	**9. Fico de tal forma apreensivo/a (com medo), que até sinto um aperto no estômago:** ___ Nunca ___ Por vezes ___ Muitas vezes ___ Quase sempre
3. Tenho uma sensação de medo, como se algo terrível estivesse para acontecer: ___ Sim e muito forte ___ Sim, mas não muito forte ___ Um pouco, mas não me aflige ___ De modo algum	**10. Perdi o interesse em cuidar do meu aspecto físico** ___ Completamente ___ Não tenho o cuidado que devia ___ Talvez cuide menos do que antes ___ Tenho o mesmo interesse de sempre
4. Sou capaz de rir e de ver o lado divertido das coisas: ___ Tanto como antes ___ Não tanto como antes ___ Muitos menos agora ___ Nunca	**11. Sinto-me de tal forma inquieto/a que não consigo estar parado/a** ___ Muito ___ Bastante ___ Não muito ___ Nada
5. Tenho a cabeça cheia de preocupações ___ A maior parte do tempo ___ Muitas vezes ___ Por vezes ___ Quase nunca	**12. Penso com prazer nas coisas que podem acontecer no futuro:** ___ Tanto como antes ___ Não tanto como antes ___ Bastante menos agora ___ Quase nunca
6. Sinto-me animado/a ___ Nunca ___ Poucas vezes ___ De vez em quando ___ Quase sempre	**13. De repente tenho sensações de pânico** ___ Muitas vezes ___ Bastantes vezes ___ Por vezes ___ Nunca
7. Sou capaz de estar descontraidamente sentado/a e sentir-me relaxado/a: ___ Quase sempre ___ Muitas vezes ___ Por vezes ___ Nunca	**14. Sou capaz de apreciar um bom livro ou um bom programa de rádio ou televisão:** ___ Muitas vezes ___ De vez em quando ___ Poucas vezes ___ Quase nunca