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ORIGINAL ARTICLE

Overweight is associated with lower NR3C1 DNA methylation in adults

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Abstract

Introduction: epigenetic alterations in glucocorticoid receptor gene (NR3C1) have been associated with psychosocial stress, however, their potential role in obesity remains unclear.

Objective: given the complex interplay between psychosocial stress, HPA axis dysregulation, and obesity, the aim of this study was to investigate the association between overweight status and NR3C1 DNA methylation in adults.

Methods: this study was characterized as a cross-sectional analysis. Two hundred and eighty-two Brazilian adults aged between 20 and 59 years were recruited from a public primary health care service. Participants underwent an anthropometric assessment. Venous blood samples were collected and NR3C1 DNA methylation was quantified using pyrosequencing. The association between overweight and NR3C1 DNA methylation was assessed using Mann-Whitney U test, Spearman correlation test and multivariable Poisson regression with robust variance (p<0,05).

Results: the overweight group had lower DNA methylation levels than the non-overweight group for both total and CpG site-specific methylation (p <0.05 and p corrected= 0.037). Through factor analysis, two bins of CpGs were extracted, which were intercorrelated. The low methylation in these bins and in the total segment was explained by overweight status, controlled by confounding variables.

Conclusion: this is one of the first studies to suggest an involvement between weight accumulation and methylation changes in NR3C1, a complex gene that encodes the glucocorticoid receptor and regulates stress responsiveness.

Keywords: obesity, stress, NR3C1, DNA methylation, epigenetics.

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Authors summary

Why was this study done?
This study was conducted to investigate the association between overweight status and NR3C1 DNA methylation in adults. While epigenetic modifications in NR3C1 have been linked to psychosocial stress, their potential role in obesity remains unclear. Given the complex interplay between psychosocial stress, HPA axis dysregulation, and obesity, the researchers aimed to determine whether weight accumulation is associated with NR3C1 DNA methylation changes.

What did the researchers do and find?
This cross-sectional study of 282 adults recruited from a public primary care service setting found an association between overweight status and low levels of NR3C1 DNA methylation, analyzed by pyrosequencing.

What do these findings mean?
This study suggests evidence that weight accumulation is associated with epigenetic changes in NR3C1, a gene that is important for glucocorticoid receptor function and regulation of the stress response.

INTRODUCTION

Overweight and obesity are defined as the abnormal or excessive accumulation of body fat, posing substantial health risks. A body mass index (BMI) greater than 25 indicates overweight status, and a BMI exceeding 30 is classified as obese[1]. Excess weight accumulation is a global epidemic, driven by a complex interplay of biological factors, including intricate genetic predisposition, as well as environmental factors: historical, economic, social, and cultural influences, all of which contribute to significant health risks[2].

Abdominal obesity has been associated with dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis, often resulting in paradoxically altered or normal plasma cortisol levels[3,4]. Glucocorticoids are the primary stress hormones, and their effects are modulated by the mineralocorticoid receptor during physiological and acute stress responses. In cases of chronic HPA axis activation, the glucocorticoid receptor (GR) increases its affinity, playing a crucial role in stress regulation[3,5].

GR is a member of the nuclear receptor superfamily of ligand-dependent transcription factors. In humans, GR is encoded by the NR3C1 gene (nuclear receptor subfamily 3, group C, member 1), situated on chromosome 5 at locus q31-q32, spanning approximately 140,000 base pairs[6,7]. This gene contains seventeen exons, including eight coding exons (numbered 2 to 9) and nine non-coding exons located within the gene promoter[6]. The GR promoter region is rich in cytosine-phosphate-guanine (CpG) sites and lacks TATA or CCAAT sequences, components of the transcription initiation complex. This feature reflects the necessity for constitutive expression of this gene[8]. The initial seven non-coding exons span 3 kb of the proximal promoter region, forming a CpG island, which has been the focal point of epigenetic investigations[7].

GR expression is influenced by epigenetic mechanisms, particularly DNA methylation in CpG islands, which can alter chromatin structure and prevent transcription factor binding, leading to changes in gene expression[7]. In line with this, genes like NR3C1, which are highly expressed, typically exhibit low levels of promoter methylation[9].

However, the potential role of NR3C1 epigenetic alterations in obesity remains unclear. Given the complex bidirectional interplay between psychosocial stress, HPA axis dysregulation, and obesity[10-12], this study hypothesizes that changes in weight may be associated with methylation alterations in the NR3C1 exon 1F region. Thus, the objective of this study was to assess the relationship between overweight status and methylation of the NR3C1 promoter region in adults.

METHODS

Study Design

This cross-sectional study is part of a health research project (PPSUS) with users of public primary care services in Brazil.

Study Location and Period

The study was contucted in 2017 in the city of Alegre-ES, Brazil.

Study Population and Eligibility Criteria

Users of Brazilian public primary care services were selected based on the following criteria: age between 20 to 59 years, not pregnant, no cognitive conditions that would interfere with answering questionnaires. Initially, 382 individuals participated, however, a sub-sample of 282 individuals participated in our biomolecular study of the NR3C1 DNA methylation.

The exclusion criteria for the initial sample group were inconsistent with anthropometric data, use of glucocorticoid medications, and insufficient biological material for analysis after DNA extraction. The 282 participants were categorized into two groups: non-overweight and overweight (94 vs. 188 subjects, respectively).

Data Collection

The anthropometric evaluation was performed by qualified professionals in the morning, after participants had fasted for a minimum of eight hours, following the technical standards of the Food and Nutritional Surveillance System (SISVAN)[13].

Height was measured using an Alturexata® (Belo Horizonte, Brazil) stadiometer, with a maximum capacity of 2.10 m and an accuracy of 0.5 cm. Body weight was measured using an electronic balance (Tanita®, BC601 model; East Kowloon, Hong Kong). Body mass index (BMI) was calculated by the quotient between body weight and square height (kg/m)1and classified according to World Health Organization (WHO) reference for adults1, in which low weight individuals present BMI values <

18.5 kg/m2, eutrophic range from 18.5 to 24.9 kg/m2, overweight from 25.0 to 29.9 kg/m2, and obese individuals BMI being ≥ 30.0 kg/m2. For purposes of dichotomization of the data, we classified participants into two groups: non-overweight (BMI < 25.0 kg/m2) and overweight (BMI ≥ 25.0 kg/m2).

All participants were asked about the continued use of the following types of drugs: hypoglycemic agents, antihypertensives, antidepressants, anxiolytics, sleep regulation, contraceptive hormones or thyroid treatment. These drugs are often used by the general population and could interfere with the regulation of the HPA-axis and/or in metabolism related to the weight gain. The drugs were subsequently grouped into the variable 'continuous medication', maintaining the dichotomization of response: no/yes.

Blood collection was performed by vein puncture in the morning, between 7:00 and 9:00 a.m., following protocols for biochemical analysis of cortisol, fasting glucose and lipidemic profile. Cortisol analysis was performed using the chemiluminescence method[14], while the glucose, total cholesterol, high-density lipoprotein cholesterol (HDL-C), and triglycerides analyzes were by enzymatic colorimetry, using specific colorimetric kits (Bioclin®, Belo Horizonte, Brazil) in an automated biochemical analyzer (Bioclin® BS-120). The Friedewald equation[15] was used to calculate low-density lipoprotein cholesterol (LDL-c).

DNA was extracted from peripheral blood collected in EDTA tubes per the manufacturer's instructions (QIAamp DNA Mini Kit, Qiagen®, Düsseldorf, Germany)[16]. DNA quantity and quality were measured by calculating the DNA concentration and absorbance ratio at 260/280 nm using a NanoDrop®. A ratio between 1.8-2.0 indicated high quality DNA and was the criteria for proceeding to bisulfite conversion.

Sodium bisulfite conversion of 1 µg of DNA from each participant was carried out using the EZ DNA Methylation™ kit (Zymo Research) according to the manufacturer's instructions. This treatment with bisulfite converts unmethylated cytosines into uracil while the methylated cytosines remain intact.

Amplification by Polymerase Chain Reaction (PCR) and pyrosequencing were adapted from previous studies[17-19]. The NR3C1 1F region, which has 47 CpGs sites was amplified using forward and reverse primers, which spanned the gene region: 961 to 1371 (sequence data submitted to the GenBank database with accession number AY436590.1), fragment with 410 base pairs. PCR was performed using HotStart Taq DNA Polymerase (Qiagen®) with 20 ng of bisulfite-treated DNA template per reaction. The quality of the PCR product and lack of contamination were confirmed on 2% agarose gels using GelRedTM (Uniscience).

The PCR products were purified and sequenced using the pyrochemical PSQ96ID (Qiagen®, Valencia, CA) with the reagent kit PyroMark Gold Q96 (Qiagen®, Valencia, CA) according to the manufacturer's protocol. The amplification primers, PCR conditions, and sequencing primers were detailed in previous studies[3,20,21]. Within the 1F region it was possible to sequence and analyze the methylation of six CpGs sites 40 to 45 (Supplementary material).

Data Analysis

The data was then tabulated and submitted for consistency analysis. The study was performed with 282 participants, who were categorized into two groups: non-overweight (94 individuals) and overweight (188 individuals). This subsample exhibited no significant disparities from original sample regarding to the prevalence of overweight or other pertinent covariates, including sex, age, lifestyle, cortisol levels, glycemia and lipid profile.

Data were examined for normality using the Kolmogorov-Smirnov normality test. To characterize the samples by overweight, the categorical data was presented at relative and absolute frequencies and compared by chi-square test. Continuous variables were presented in median and interquartile ranges and compared by the Mann-Whitney U test. Spearman correlation was also tested to verify correlation between NR3C1 DNA methylation and BMI.

Total methylation of the segment (CpG 40 to 45), as well as the median percentage of methylation of each specific CpG site were analyzed. In this case, we used the 5% significance, corrected by the Benjamini-Hochberg method[22]to control the false discovery rate (FDR), with a corrected p-value equal to or less than 0.037, in the case of comparisons between groups, and corrected p-value equal to or less than 0.017, in the case of Spearman correlation analysis.

Subsequently, the methylation data of the six CpGs specific sites was subject to factor analysis to verify the inter-relationship between the CpGs of the analyzed segment. The method of main components extraction was employed, given the non-normality assumption of the variables involved. The model fit quality analysis was performed using Kaiser-Meyer-Olkin criterion (KMO) and, in Bartlett's Sphericity Test, a significance of 5% ($p < 0.05$) was considered[23]. The matrix of factor loads was estimated and, after that, the orthogonal rotation varimax was performed.

After extracting the components, CpGs bins were defined to proceed with the analysis. The utilization of CpG bins in methylation DNA evaluation is a common practice in investigation studies, as evidenced by Bustamante *et al.*[18], WHO[24] and Yehuda *et al.*[24]. In our study, however, the CpGs bins represent the primary components extracted through factor analysis, thereby reflecting the inter-relationships of the CpGs. Subsequently, we proceeded with the analysis by investigating the NR3C1 1F region methylation with comparison tests between non-overweight and overweight groups, Spearman correlations between BMI and NR3C1methylation and, later, Poisson regression with robust variance from the categorization of methylation data dichotomously in: unmethylated (<0.1%) and methylated (≥ 0.1%).

Statistical analyzes were performed using SPSS® software, version 15.0 for Windows (IBM®) and, in the case of Poisson regression, the STATA® software, version 9.0 (StataCorp® LP, College Station) was used. For graphical presentation of the results, GraphPad Prism®,

version 7.0 (GraphPad® Software Inc.) was used, and for the best visualization, we constructed graphs from the values of mean and standard errors of mean.

Multivariate Poisson regression analyzes with robust variance were performed to test if overweight was associated with the methylation of NR3C1 1F region, and if covariables also had predictive methylation ability.

Three different multivariate models were tested, with the following outcome variables: total methylation (Model 1), methylation of CpGs bin 1 (Model 2) and methylation of CpGs bin 2 (Model 3). In addition to the main variable of interest (overweight), all covariates were included: sex, age, alcohol, smoker, continuous medication, altered cortisol, glycemia, and lipid profile, due to their direct or indirect association with obesity and, or with the activation of the HPA axis[2,5,9,10]. To verify the final adherence of the model, an adjustment was made using the Hosmer &Lemeshow test. The measure of effect was given by the prevalence ratio with a 95% confidence interval[26]. The level of significance considered in the model analysis was 5%, and after Benjamini-Hochberg's correction[27], the variables that had explanatory capacity were those that presented p-value equal to or less than the FDR correction, being 0.015, 0.019 and 0.046 for the respective models 1, 2 and 3.

All covariates were dichotomized, except age. Total cholesterol, its fractions and triglycerides were grouped into lipid profile, with the variable also dichotomized as normal or altered.

Ethical and Legal Aspects of the Research

This study was carried out according to the principles of the Helsinki Declaration. Each participant signed a written informed consent form after a clear explanation of the study protocol. The study was approved by the Ethics Committee on Human Research, the Health Sciences Center, Federal University of Espirito Santo, under number 1,574,160/2016.

RESULTS

The prevalence of overweight status, defined as a BMI ≥ 25.0 kg/m2, was observed to be 66.7%. The variables age, biochemical profile, glycemia, total cholesterol and fractions level, and triglycerides exhibited significant differences between the non-overweight and overweight groups, as detailed in Table 1.

Table 1: Characteristics of the study population according to weight

Characteristic	Overweight (BMI ≥ 25 kg/m2)		
Sex - % (n)	No	Yes	p
Male	35.0 (21)	65.0 (39)	0.758
Female	32.9 (73)	67.1 (149)	
Age (years) - median (IR)	39.5 (19.0)	44.0 (17.0)	0.017*
Drink alcohol - % (n)			
No	34.8 (71)	65.2 (133)	0.474
Yes	30.3 (23)	69.7 (53)	
Smoker - % (n)			
No	32.8 (84)	67.2 (172)	0.380
Yes	41.7 (10)	58.3 (14)	
Continuous medication - % (n)			
No	37.3 (57)	62.7 (96)	0.128
Yes	28.7 (37)	71.3 (92)	
Biochemical profile – median (IR)			
Serum cortisol	12.9 (6.7)	11.5 (6.2)	0.151
Glycemia	90.5 (15.0)	95.0 (23.0)	0.016*
Total cholesterol	174.0 (52.0)	187.0 (44.9)	0.047*
HDL_cholesterol	68.0 (28.0)	61.0 (28.)	0.007*
LDL_cholesterol	80.0 (35.0)	89.0 (44.0)	0.036*
VLDL_cholesterol	21.0 (17.0)	28.0 (20.0)	<0.001*
Triglycerides	103.0 (84.0)	140.0 (97.0)	<0.001*

Source: Written by the author BMI: (IR); categorical variables presented in relative (%) and absolute (n) frequencies. * p-value for the tests: Chi-square or Mann-Whitney U, at 5% significance (p <0.05).

CpG site specific methylation levels are graphically represented in figure on supplementary material and the comparison between non-overweight (n = 94) and overweight (n = 188) groups can be seen in Figure 1. Although the NR3C1 DNA methylation profile is low, the overweight group had a significantly lower percentage of DNA methylation than the non-overweight group in the following specific CpG sites: 41, 42, 44 and 45, as shown in Figure 1.

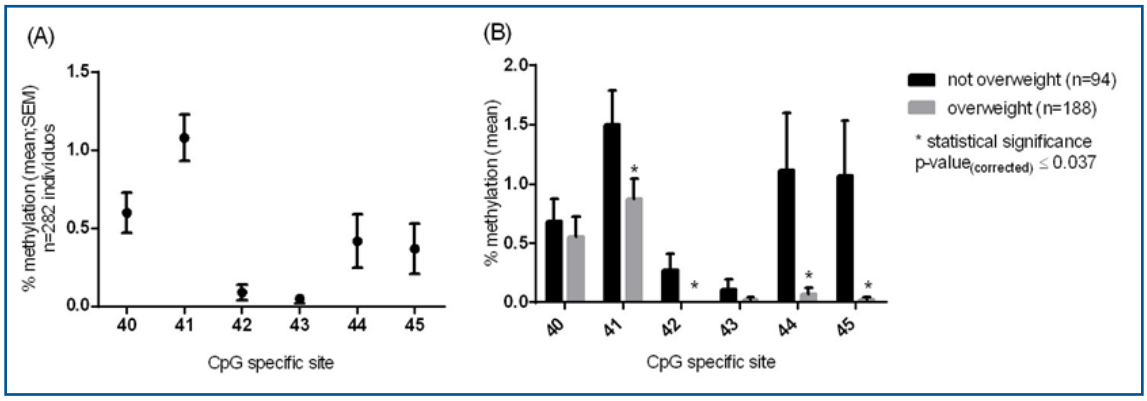

Figure 1: (A) Methylation levels across six CpGs site-specific for the NR3C1 1F region (all study participants). (B) Comparison between non-overweight vs. overweight groups. *Mann-Whitney test.Data are presented in means and standard error of means (SEM) in A and B.

Table 2: CpG interrelationship by Factor Analysis

	CpGs					
Components	40	41	42	43	44	45
1*	-0.420*	0.636*	-0.045	0.720*	0.047	0.070
2**	0.345	0.287	0.811**	-0.046	0.931**	0.934**

*1: CpG40-41-43; ** 2: CpG 42-44-45; Kaiser-Meyer-Olkin (KMO): 0.661; Total cumulative variance: 61.8%; Statistical significance: p <0.001 by Bartlett's Test of Sphericity.

To analyze the interaction between the CpGs, factor analysis was carried out, and a model was generated by extraction of two main components, called "bin 1": CpG40-41-43 and "bin 2": CpG42-44-45, accounting for 61.8% of the total variation in the segment analyzed, as can be seen in Table 2.

The comparison of total segment methylation (the sum of methylation percentages of the CpG 40 to 45) and the methylation of bins 1 and 2, between groups showed that the overweight group had significantly lower percentages of NR3C1 DNA methylation, independent of the analyzed segment (Figure 2A and 2B. Mann Whitney test, $p < 0.05$).

The prevalence of methylation in the NR3C1 1F region was 26.6% of individuals in the total segment (CpG 40 to 45), 26.2% in bin 1 (CpG 40-41-43) and 3.9% in bin 2 (CpG 42-44-45), as shown in Figure 2C.

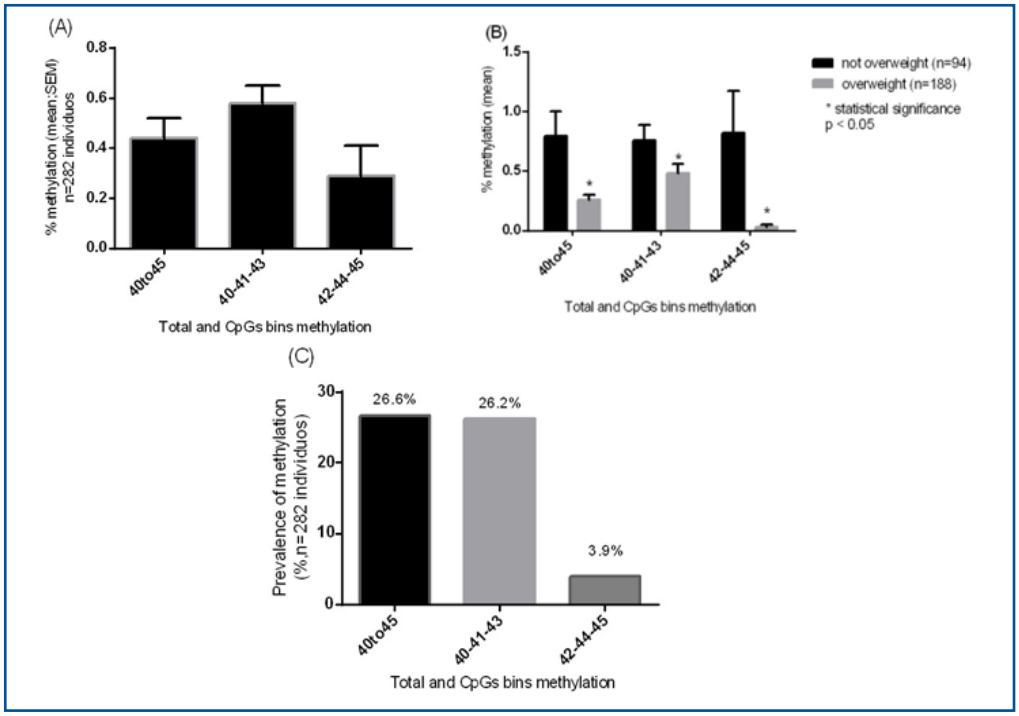

Figure 2: (A) Level of methylation of total segment (the sum of methylation percentages of the CpG 40 to 45) and CpGs bins methylation. (B) Level of total segment and CpGs bins methylation compared between non overweight and overweight adults. Mann Whitney test. (C) Prevalence of total segment and CpGs bins methylation. Quantitative methylation (non-parametric data) presented as mean and standard error of mean (SEM) in A and B for improved graphical representation. Qualitative methylation (categorical data) presented as relative frequency (%) in C

The Spearman correlation analysis (Figure 3) shows an inverse correlation between overweight status and methylation at CpGs 42 (r = -0.148, p = 0.013), 44 (r = -0.142, p = 0.017), and 45 (r = -0.153, p = 0.010), as well as within bin 2 (r = -0.137, p = 0.021).

Poisson regression analysis with robust variance showed that overweight reduces the prevalence of total segment of NR3C1 methylation by about 40%, and of bin 1 (CpG40-41-43) methylation by about 38%, when controlled by the effect of alcoholic beverage use, which was also significant in reducing the prevalence of methylation.

Overweight status reduces the prevalence of DNA methylation in bin 2 (CpG42-44-45), by approximately 77%, when controlled by alteration of the lipid profile. Overweight status was also associated with the lower prevalence ratio. All models were controlled by the confounded variables were statistically significant, but only the first two models exhibited a strong adherence to the Hosmer & Lemeshow adjustment, as illustrated in Table 3.

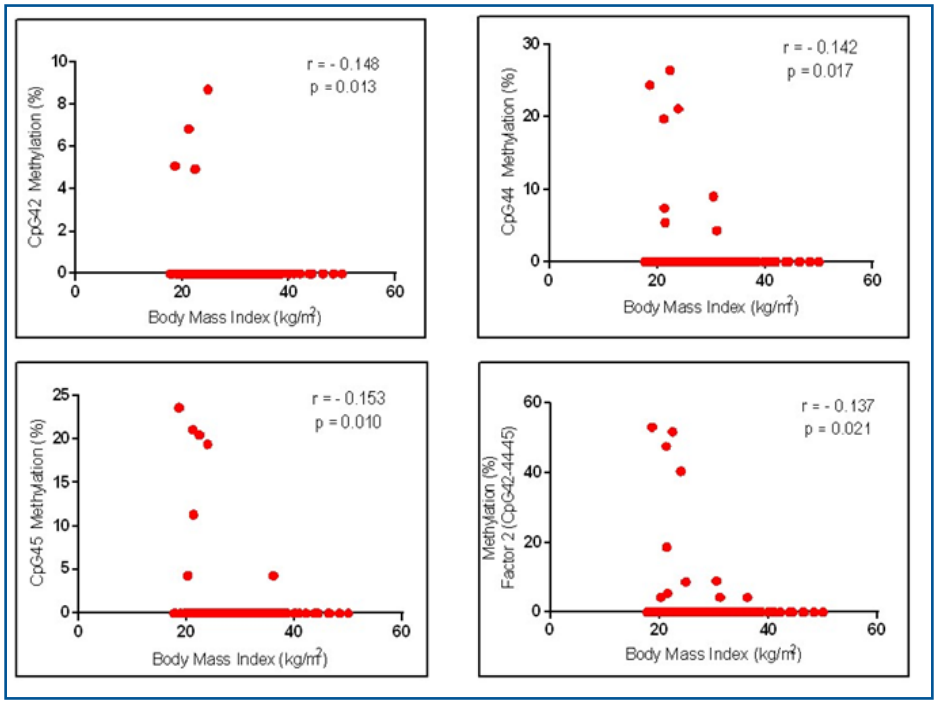

Figure 3: Spearman correlation analysis between Body Mass Index (BMI) and methylation CpGs site-specific for the NR3C1 1F region (all study participants).

Table 3: Multivariate Poisson regression analysis with robust variance for methylation of NR3C1 1F region

Variables	Methylation								
	Total (CpG40to45)			Bin 1 (CpG40-41-43)			Bin 2 (CpG42-44-45)		
	PR	95% CI	p	PR	95% CI	p	PR	95% CI	p
Overweight									
No		1			1			1	
Yes	0.60	0.40-0.90	0.012	0.62	0.41-0.92	0.019	0.23	0.07-0.75	0.015
Sex									
Male		1			1			1	
Female	1.34	0.70-2.55	0.382	1.32	0.69-2.53	0.402	0.88	0.25-3.15	0.847
Age (years)	1.00	0.99-1.02	0.647	1.01	0.99-1.03	0.511	0.99	0.92-1.06	0.718
Drink alcohol									
No		1			1			1	
Yes	0.43	0.22-0.85	0.015	0.43	0.22-0.86	0.017	0.35	0.05-2.68	0.314
Smoker									
No		1			1			1	
Yes	0.89	0.63-1.25	0.485	0.89	0.63-1.25	0.487	0.42	0.16-1.06	0.067

Continuation - Table 3: Multivariate Poisson regression analysis with robust variance for methylation of NR3C1 1F region

Variables	Methylation								
	Total (CpG40to45)			Bin 1 (CpG40-41-43)			Bin 2 (CpG42-44-45)		
	PR	95% CI	p	PR	95% CI	p	PR	95% CI	p
Continuous medication									
No		1			1			1	
Yes	0.91	0.61-1.35	0.632	0.87	0.58-1.30	0.493	0.34	0.06-2.01	0.233
Altered cortisol									
No		1			1			1	
Yes	0.99	0.52-1.90	0.982	1.00	0.52-1.92	0.995	1.26	0.17-9.47	0.821
Altered glycemia									
No		1			1			1	
Yes	1.24	0.82-1.88	0.313	1.24	0.82-1.89	0.308	3.28	0.70-15.34	0.131
Altered lipid profile									
No		1			1			1	
Yes	1.00	0.66-1.51	0.987	1.03	0.67-1.56	0.905	0.20	0.04-0.97	0.046
Log pseudolikelihood		-159,23544			-158,25925			-36,294318	
Pseudo R2		0.046			0.044			0.210	
Prob > Chi2		0.025			0.034			0.040	
Adjustment of models: Hosmer & Lemeshow		0.99			0.99			0.00	

* PR: prevalence ratio; 95% CI: confidence interval; p: p-value.

DISCUSSION

DNA methylation is an epigenetic adaptative mechanism capable of regulating gene expression and can be influenced by environmental pressure, such as diet and stress. Previous studies have demonstrated that chronic psychosocial stress epigenetically modulates the NR3C1 gene, resulting in alterations in GR expression, protein expression, and changes in stress responsiveness[17,28]. Prior research has indicated an association between epigenetic changes associated with the HPA axis and stress, obesity[21], depression[29], and metabolic diseases[20,4].

Dysregulation of the HPA axis has been observed in individuals experiencing psychosocial stress and excessive weight gain[4,31,32], suggesting a potential relationship between obesity and chronic stress[4,30]. A correlation has been reported between abdominal adiposity and diminished stress responsiveness, which may be indicative of an attenuated response of the HPA axis.

In this regard, this investigation observed a relationship between overweight and low-methylated of the NR3C1 1F promoter region. We found that overweight adults had statistically lower levels of NR3C1 DNA methylation when compared to non-overweight group at almost all individual CpG sites, as well as in the interrelations of clustered CpGs. Our findings also point to an inverse correlation of BMI with methylation at three specific CpGs sites and across the interrelation of these sites.

To the best of our knowledge, this is one of the first studies to verify low levels of NR3C1 methylation and excessive weight accumulation. At first glance, the association between lower NR3C1 DNA methylation levels and excessive weight accumulation may seem contradictory, as conditions related to chronic stress are typically associated with HPA axis hyperactivity[17,28,33]. However, emerging evidence suggests that the decreased stress reactivity resulting from prolonged HPA axis activity due to chronic exposure to stressors is also linked to reduced levels of NR3C1 DNA methylation, increased GR expression, and HPA axis hypoactivity[34-37]. This suggests a possible association between chronic exposure to psychosocial stress and obesity as a long-term condition, potentially influenced by epigenetic modulation through the NR3C1 gene.

The relationship between chronic stress and obesity appears to be complex. It involves changes in food intake triggered by reward mechanisms, particularly dopaminergic stimulus of the nucleus accumbens[3,38], as well as the leptin system and problems with satiety control[10,39,40], in response to HPA axis dysregulation and chronic stress.

The NR3C1 gene is involved in HPA axis regulation. The HPA axis is recognized to be important for physical and psychological balance as such it is considered integral to the Psycho-Neuro-Endocrine-Immune system[41-43]. In normal physiological situations, the cortisol-GR complex

regulates HPA axis activation and stress response by negative feedback. Furthermore, the activated GR-ligand also performs the transactivation of genes regulated by the axis[44], and transrepression of inflammation[45] by binding to the transcription factor NFκB, preventing its pro-inflammatory activity[8,46,47].

Chronic stress can lead to the HPA axis dysregulation and negative crosstalk between GR-linkers and NFkB pathways, contributing to the installation of a state of low-grade chronic inflammation, characteristic of the obesogenic state[3,8,48]. This mechanism appears to be epigenetically modulated and changes in NR3C1 methylation may be involved[21].

our results demonstrated that there was an intercorrelation among CpGs within the assessed segment, resulting in the identification of two "bins" within the assessed segment where CpGs exhibited similar methylation patterns, indicating an interrelationship among these CpGs. Tyrka *et al.,* (2016)[9] analyzed DNA methylation within the same region of the NR3C1 gene in adults and also observed a coordinated intercorrelation among CpGs, which has been associated with childhood maltreatment[9]. This is a noteworthy discovery, as most studies typically examine the segment as a whole or focus on individual CpGs at specific sites. However, our findings reveal that CpGs exhibit distinct behaviors, underscoring the importance of investigating these clusters as well.

Consistent to previous studies[9,18,25], we observed very low DNA methylation levels, even when analyzing the total segment. Low DNA methylation are typical in regions with high CpG density, known to be more open to regulation by DNA methylation[49]. However, despite the low overall methylation, small changes within theNR3C1 DNA methylation of the 1F promoter may be functionally relevant, as demonstrated by associations with endocrine outcomes[25]. Prior findings suggest that low methylation levels in this region may influence transcription factor binding and gene expression[17,25,50].

Although NRC31 is a large-scale constitutive gene expressed in the hippocampus, expression in peripheral blood is seen in B lymphocytes and innate immune cells, and is not expressed in T lymphocytes and monocytes[9,6,51]. Peripheral tissues are readily accessible in human disease and studies suggest that the DNA methylation patterns in some loci may be the same in the brain and periphery, revealing epigenetic reprogramming related to pathological conditions[28,51].

Given the relatively weak correlations observed in the Spearman analyses ($r < 0.3$), to strengthen our findings, we explored the association between DNA methylation and overweight status using Poisson multivariate regression with robust variance estimation. This approach confirmed a significant association between overweight and lower methylation levels in the NR3C1 promoter region, controlled by confounding factors.

66% of the sample consisted of overweight individuals. It is possible to surmise that the high prevalence of overweight individuals was expected as the study focuses on users of the public health system[52]. In addition, in both developed and developing countries with low mortality, overweight status has been listed as a serious major risk factor for noncommunicable disease[24,53].

As mentioned previously, this study is among the first to explore the connection between nutritional status, weight accumulation, and NR3C1 DNA methylation in the 1F promoter region. Vieira *et al.*, (2024)[54] shows the association between NR3C1methylation and industrialized food consumption. This and other studies suggest that food can be regarded as a stressor agent[55] capable of altering epigenetics.

One systematic review with meta-analysis[56] examined the relationship between prenatal stress and BMI, uncovering evidence that exposure to stress among pregnant women was linked to an increase in their children's BMI[57].They found an association between childhood and adolescent body mass index (BMI) and early-life adversities such as exposure to material deprivation, loss, or threat of loss, and high adversity, albeit with a small effect size. In a previous study, the relationship between DNA methylation in the NR3C1 1F region and birth weight, in conjunction with placental development, was assessed. This study revealed a significant correlation between birth weight and the average extent of methylation, as well as a significant association between GR methylation and the large size for gestational age of fetuses[58]. Another group of researchers found hypermethylation of certain CpG sites of the GR gene promoter in women with bulimia, however these findings were in exon 1C7.

Although a cross-sectional study does not allow causality to be inferred, our results suggest a relationship between chronic stress, epigenetic alterations and excessive weight gain. More research is needed to further elucidate the epigenetic mechanisms involved in this relationship.

CONCLUSION

The significance of these discoveries extends beyond the scope of this study, establishing itself as a pioneering study in field of nutritional status, weight accumulation, and NR3C1 gene methylation within the 1F promoter region. The study's findings are of paramount importance, as they not only enhance our understanding of this pivotal domain but also substantiate and fortify the preliminary results disseminated by our research group.

This validation serves to reinforce the robustness of our findings and highlights the significant contribution of this study to the broader research landscape.

Furthermore, the results underscore the critical importance of the obesity issue.In light of these findings, it is imperative to direct new research efforts toward a more detailed understanding of the epigenetic mechanisms involved. This intricate and multifaceted scenario encompasses psychosocial stress and excessive weight gain.

Author Contributions

Flávia Vitorino Freitas: Conceptualization, Methodology, Analysis, Visualization, Writing - Original Draft Writing - Review and Editing. Tamires dos Santos Vieira: Writing - Original Draft, Visualization. Aline Ribeiro Borçoi: Writing - Original Draft, Visualization. Review and Editing. Suzanny Oliveira Mendes: Writing - Original Draft, Visualization. Wagner Miranda Barbosa:

Formal analysis, Data curation. Mayra Vitorino Freitas Flávio: Formal analysis, Data curation. Júlia de Assis Pinheiro: Analysis, Data curation, molecular analyses. Juliana Krüger Arpini: Analysis, Data curation, molecular analyses. Joaquim Gasparini dos Santos: Analysis, Data curation, molecular analyses. Ivana Alece Arantes Moreno: Analysis, Data curation, molecular analyses. Marcele Lorentz Mattos de Souza: Data curation, Analysis. Bárbara Risse Quaioto: Data curation, Analysis. Amanda Sgrancio Olinda: Data curation, Analysis. Lidia Maria Rebolho Batista Arantes: Resources, materials and molecular analyses. Bruna Pereira Sorroche: Resources, materials and molecular analyses. José Claudio Casali-da-Rocha: Writing - Original Draft. Adriana Madeira Alvares-da-Silva: Methodology, Resources, Acquisition of financing, Supervision. All authors critically reviewed the content and approved the final version for publication.

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Conflicts of Interest

All authors disclose any actual or potential conflict of interest including any financial, personal or other relationships with other people or organizations within 3 years of beginning the work that could inappropriately influence (bias) the present work.

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Resumo

Introdução: as alterações epigenéticas no gene do receptor de glicocorticoide (NR3C1) têm sido associadas ao estresse psicossocial; no entanto, sua possível função na etiologia da obesidade ainda não está clara.

Objetivo: considerando a complexa interação entre o estresse psicossocial, a desregulação do eixo HPA e a obesidade, o objetivo deste estudo foi investigar a associação entre o status de sobrepeso e a metilação do gene NR3C1 em adultos.

Método: estudo transversal onde duzentos e oitenta e dois adultos foram recrutados com idade entre 20 e 59 anos, usuários de serviço público de atenção primária à saúde. Os participantes foram submetidos a uma avaliação antropométrica. Amostras de sangue venoso foram coletadas e a metilação do gene NR3C1 foi quantificada por pirosequenciamento. Foi utilizado o teste U de Mann-Whitney, o teste de correlação de Spearman e a regressão multivariável de Poisson com variância robusta para análise estatística ($p<0,05$).

Resultado: o grupo com sobrepeso apresentou níveis mais baixos de metilação do gene NR3C1, quando comparado ao grupo sem sobrepeso ($p <0,05$ e p corrigido = 0,037). A baixa metilação no segmento total e em compartimentos de CpGs intercorrelacionados foi explicada pelo status de sobrepeso controlado por variáveis de confusão.

Conclusão: este estudo sugere um envolvimento entre acúmulo de peso e alterações de metilação no gene NR3C1, um gene complexo que regula a resposta ao estresse, relacionado ao estresse psicossocial.

Palavras-chave: obesidade, estresse, NR3C1, DNA, epigenética.