Anorexia nervosa (AN) is a subtype of eating disorder characterized by excessive and purposeful restriction of food intake, which leads to substantial weight loss and physiological function decline below normal ranges. The longest duration and greatest fatality rate among mental diseases make AN one of the deadliest and most disabling ailments. While the actual etiology of AN is still unknown, a plethora of data suggests a complicated genesis that involves both environmental and genetic factors. In AN research, epigenetic mechanisms—particularly those influencing gene expression—have acquired relevance as important intermediates in the interaction between genetic predisposition and environmental variables. DNA methylation, in which methyl groups bond to cytosine inside cytosine-guanine dinucleotides (5'-CpG-3' sites), is a well-researched epigenetic modification. Evidence shows that methylation may have a direct influence on transcription factors(TFs).

The investigative stage is presently concentrating on DNA methylation in connection to anorexia nervosa (AN). A recent study, however, reveals a potential relationship between gene methylation and the clinical signs of AN. Notably, GHSR (ghrelin receptor), SNCA (α-synuclein), DAT (dopamine transporter), DRD2 (dopamine receptor D2), OXTR (oxytocin receptor), and LEP (leptin) are among the genes with altered methylation patterns found in AN. These genes are critical for modulating hunger and reward circuits. Moreover, two comprehensive whole-genome sequencing studies (GWAS) on patients with AN have shown a drop in their genome-wide methylation levels.

Anorexia nervosa (AN) appears to be largely caused by dysregulation of appetite control, both in its onset and persistence. The neuropeptide ghrelin, which is released by the gastrointestinal tract, is implicated in the pathophysiology of the central nervous system, cardiovascular system, gastrointestinal tract, reproductive system, and immunological system, in addition to modulating hormones and appetite. P/D1 cells are the key stomach mucosal endocrine cells responsible for generating and releasing the precursor protein ghrelin. It becomes functional ghrelin in the circulation upon acylation. Ghrelin may directly bind to certain receptors in the arcuate nucleus of the hypothalamus (ARC), demonstrating its capacity to traverse the blood-brain barrier. Agouti-Related Protein (AgRP) and Neuropeptide Y (NPY) neurons become more excitable as a result of this interaction, prompting appetite.

Leptin is a peptide hormone produced by white adipose tissue. It impacts several organs, such as the liver, kidneys, skeletal muscles, and hypothalamus, which contain leptin receptors. Leptin stimulates glucose absorption and utilization in white adipose tissue. It controls the intake and release of blood glucose in organs such as the small intestine, liver, and skeletal muscles to ensure glucose balance. Leptin activates the vagus nerve to trigger the secretion of hormones such as glucagon-like peptide-1 (GLP-1) and cholecystokinin (CCK) after eating, which reduces hunger. Leptin has a direct impact on certain receptors in the arcuate nucleus, which in turn affects the transcription of proopiomelanocortin (POMC). The produced transcription product attaches to melanocortin receptors (MCR), stimulating neurons linked to satiety and hence reducing appetite. Leptin suppresses the production of Neuropeptide Y (NPY) and Agouti-Related Protein (AgRP) in neurons, which decreases the stimulating impact of AgRP on the central appetite center, resulting in reduced hunger. Leptin influences excitability in reward circuits, decreasing pleasure after eating and suppressing the urge to consume. Leptin boosts sympathetic nervous system activity, which helps break down fat tissue to stabilize lipid metabolism. The dynamic balance between leptin and ghrelin plays a critical role in regulating eating behavior and internal homeostasis, underscoring its relevance to maintaining physiological equilibrium.

Environmental factors can influence the methylation of specific cytosine-phosphate-guanine (CpG) sites, leading to abnormal transcription factor binding and subsequent alterations in gene expression, resulting in the manifestation of clinical symptoms. In recent years, there has been a growing body of research on the relationship between environmental factors and diseases.

A study in 2013 found that the duration of breastfeeding during childhood was negatively correlated with the methylation levels of the Leptin (LEP) gene. Specifically, children who received sufficient breastfeeding exhibited lower methylation of the LEP gene. Breast milk contains higher nutritional content and essential growth factors compared to formula milk. Adequate breastfeeding was associated with a decrease in LEP gene methylation levels and an increase in plasma LEP levels, serving as a significant preventive factor against obesity. The study also identified a lower obesity risk in children who received adequate nutritional support during childhood.

The methylation levels of the Growth Hormone Secretagogue Receptor (GHSR) gene are also influenced by maternal factors. Previous research indicated that maternal exposure to a cold environment during pregnancy led to increased GHSR expression in offspring, which gradually decreased after birth. A study in 2015 demonstrated that the stress levels of lactating mothers during the postpartum period affected the methylation levels of their offspring's GHSR gene. Postpartum depression and low attention to offspring were associated with an increase in GHSR gene methylation levels, resulting in reduced offspring feeding. Abnormal regulation of emotions also impacts the methylation levels of the GHSR gene. A recent study in adolescents with depression found that negative life events during adolescence led to an increase in GHSR gene methylation levels, positively correlating with the severity of depressive symptoms.

Current research on the impact of environmental factors on DNA methylation has been confirmed in various diseases, including tumors, obesity, and diabetes. This suggests that environmental factors play a crucial role in gene methylation, providing valuable insights for further investigating the correlation between the environment and symptoms of anorexia nervosa (AN).

In order to distinguish between the derivative effects of malnutrition (state markers) and the biological processes that may lead and cause in the physiology of anorexia (properties markers), we investigated the differences between the methylation levels of DNA in acute aneurysm patients and in the GHS-R1a and LEP genes in healthy people, alongside the impact of traumatic experiences and family environments on the condition, while controlling the extent of possible age and cultural impact.

Method

2.1. Study participants

This research involved female volunteers diagnosed with acute anorexia nervosa (AN) and healthy controls (HC). 101 critically underweight anorexia nervosa patients fulfilling DSM-IV criteria were recruited from the Eating Disorder Programs at a University Child and Adolescent Psychiatry Department. The control group contained 52 normal-weight, eumenorrheic, healthy female participants (HC) recruited by advertising among middle school, high school, and university students.

Through structured interviews, information was gathered from all participants on exclusion criteria and potential contributing variables, including name, gender, profession, marriage, cultural status, height, weight, etc., and semi-structured interviews and medical records were supplemented.

HC individuals were disqualified if they had a history of any mental disorder. Participants in the AN groups were excluded if they had a lifetime history of psychiatric diagnoses such as organic brain syndrome, schizophrenia, drug dependency, bipolar illness, neurosis, or eating disorders. Additional exclusion criteria for all participants included people with an IQ of less than 85 and with severe physical complications (such as nervous system disease, heart rate abnormalities, severe electrolyte disorders, etc.), with serious negative suicidal thoughts or behavior; a family history of mental illness; taking psychotropic drugs, hormonal drugs, etc., for a month. Through structured interviews, information was gathered from all participants on exclusion criteria and potential contributing variables, including name, gender, profession, marriage, cultural status, height, weight, etc., and semi-structured interviews and medical records were supplemented.

The design of the research was approved by the Shanghai Mental Health Center Institutional Review Board. All participants (or guardians of children) submitted written informed consent after describing the research procedure.

2.3. Blood Collection, Biochemical Assessments, and Bisulfite Sequencing

After a nighttime fast, blood is collected from the arteries with EDTA between 7:30 and 9:30 in the morning. In the AN group, blood collection happened during the first week following the commencement of enhancing medication. Wait for further examination; the plasma sample is kept at -80°C. DNA methylation of the GHS-R1a gene promoter and the LEPR gene promotor was studied, and genomic DNA isolated from external blood mononucleic cells was evaluated using sulfur dioxide transformation and Sanger sequencing procedures.

2.4. Quality Control (QC)

All sequences underwent scrutiny in the Sequence Scanner, and those with a low Quality Value (QV20) were subjected to repeat sequencing. Only samples that could be technically sequenced adequately were retained for analysis. In the analysis, only CpG positions with 95% valid values were included. Similarly, only subjects with 95% valid CpG values were considered for inclusion. CpG positions with a variance of less than 0.001 were excluded from the analysis.

Ultimately, the study included a total of 115 subjects and a collective of 64 CpG positions (24 from GHS-R1a and 40 from LEP) for subsequent analysis.

**3. Results**

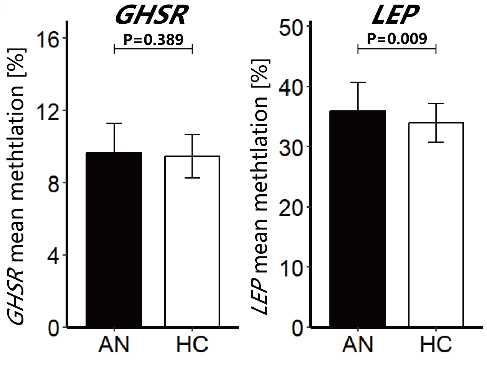
3.1. Sample characteristics

The clinical features of all patients are presented in table 1. As expected, people with acute anorexia nervosa (AN) drastically lowered their BMI and psychiatric levels (measured by EDI-2, EDE-Q).In addition, AN patients were slightly younger than HC patients. In the AN group, 60 patients were classified as restricted type (AN-R) and 41 patients were categorized as/or eliminated type (AN-BP). It is interesting that there were no significant changes in clinical parameters across these subtypes, including body mass index, EDI and EDE-Q.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| *变量* | *AN* | *HC* | *t/Z* | *p* |
| Age | 18 [ 3 ] | 21 [ 6 ] | -1.912 † | 0.055 |
| education years | 12 [ 5 ] | 16 [ 5 ] | -3.609 † | <0.001 \*\*\* |
| years | 17 [ 4.5 ] | 21 [ 6 ] | -3.513 † | <0.001 \*\*\* |
| duration | 10 [ 19 ] | 0 [ 0 ] | 10.115 † | <0.001 \*\*\* |
| Weight | 43 [ 12 ] | 46 [ 12 ] | -3.168 † | 0.002 \*\* |
| high | 1.6 [ 0.06 ] | 1.6 [ 0.05 ] | -0.803 † | 0.422 |
| BMI | 16 ± 2.5 | 18 ± 3.5 | -3.774 ‡ | <0.001 \*\*\* |
| Restraint | 1.8 [ 2.2 ] | 0.2 [ 0.8 ] | 5.801 † | <0.001 \*\*\* |
| Eating concern | 1.6 [ 2.6 ] | 0.2 [ 0.2 ] | 7.265 † | <0.001 \*\*\* |
| Shape concern | 2.2 [ 2.4 ] | 0.56 [ 1.7 ] | 5.344 † | <0.001 \*\*\* |
| Weight concern | 2 [ 2.4 ] | 0.2 [ 1.2 ] | 5.972 † | <0.001 \*\*\* |
| Global score | 2 [ 2 ] | 0.33 [ 0.84 ] | 6.758 † | <0.001 \*\*\* |
| EDI | 174 [ 83 ] | 153 [ 32 ] | 2.691 † | 0.007 \*\* |

3.2. Main Analysis - Mean Methylation

The study of the outcomes of the t-test indicates there is no significant difference in the mean methylation level of the GHS-R1a gene promoter among groups (t = 0.647, p = 0.389). However, a significant difference is seen in the average methylation level of the LEP gene promoter among groups (t = 2.615, p = 0.009).

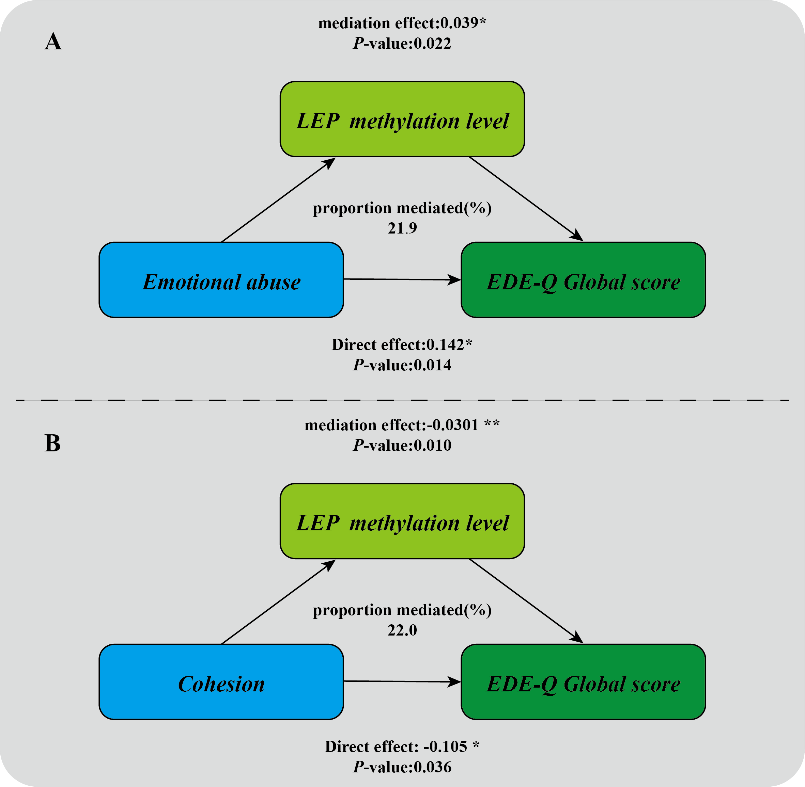


3.3 Relationship between DNA methylation levels and clinical variables

A partial correlation study in the AN group between the mean amount of LEP promoter methylation and the total EDEQ score indicated substantial bilateral correlations (r=0.338,p=0.0006).In addition, the total EDEQ score is substantially connected with the coherence of the FES-CV scale and the emotional abuse of the ETI scale.

3.4.mediating effect of LEP gene methylation

Furthermore, mediation analyses were conducted to explore the mediating effect of LEP methylation(Figure 3).The direct effect between cohesion and EDE-Q Global score is 0.142，P-value was 0.014,LEP methylation level significantly mediated the association with 21% of the association (P < 0.05). The direct effect between emotional abuse and EDE-Q Global score is 0.14,with P-value of 0.014,and the proportion mediatied of LEP methylation is 22.0% .



**Figure 3** Path diagram of the mediation analysis of LEP methylation level. The graphs in (A、B) represented the mediating role of LEP methylation level for emotional abuse and cohesion.

Discussion

This study for the first time reported the mediation effect of blood-cell-based inflammatory biomarkers between sedentary exposure on the risk of sleep disturbance. In the present study of a nationally representative adult sample, we observed that severe sedentary behavior was associated with increased risk of sleep disturbance, while exercise can mitigate this situation to some extent. Sleep disturbance was associated with increased levels of WBC, NEU, NLR, and SII, indicating that sleep disturbance was associated with increased inflammatory states, and high blood-cell-based markers were independent risk factors for sleep disturbance. Of particular importance, there was a novel finding that all four inflammatory biomarkers significantly mediated the association between sedentary behavior and sleep disturbance. However, although a negative trend was found between exercise and sleep disturbance, the mediating role of these inflammatory biomarkers was not significant in this case.

Significant relationships were observed between inflammatory markers and sleep disturbance patients. It has been established that sleep regulated the immune system and there was evidence linking the disruption of sleep rhythm with increased inflammation ([41](https://www.frontiersin.org/journals/immunology/articles/10.3389/fimmu.2022.1080782/full#B41)). Assessment of blood-cell-based markers may help us predict the severity of night sleep disorder, as well as the presence of comorbidities. These blood-count-based parameters, such as WBC, NEU, and SSI, are not only inexpensive and handy tests performed in most areas but also can offer us accurate and reproducible information on systemic inflammation. In recent years, by using the novel index SII, an integrated inflammation marker developed by Hu et al. ([31](https://www.frontiersin.org/journals/immunology/articles/10.3389/fimmu.2022.1080782/full#B31)), we can better grasp the extensive immune and inflammatory state of the body. Previous meta-analyses have detected SII as a strong and independent predictor in patients with several malignancies ([42](https://www.frontiersin.org/journals/immunology/articles/10.3389/fimmu.2022.1080782/full#B42), [43](https://www.frontiersin.org/journals/immunology/articles/10.3389/fimmu.2022.1080782/full#B43)). However, there was no current study on the correlation between sleep disturbance and SII, and this population-based study further confirmed that a high level of SII can be used to reflect an elevated inflammation in sleep disturbance status.

A positive relationship between sedentary behavior and sleep disturbance was found in this study, which was consistent with several previous reports ([44](https://www.frontiersin.org/journals/immunology/articles/10.3389/fimmu.2022.1080782/full#B44)–[46](https://www.frontiersin.org/journals/immunology/articles/10.3389/fimmu.2022.1080782/full#B46)). Considering that sedentary behavior was characterized by waking behavior with less than an energy expenditure of 1.5 metabolic equivalents (METs) ([12](https://www.frontiersin.org/journals/immunology/articles/10.3389/fimmu.2022.1080782/full#B12)), excessive electronic product use and unhealthy lifestyles might aggravate this condition in modern society. Former studies have found that sedentary behavior was strongly associated with increasing levels of cytokines, which also were involved in a number of regulatory and inflammatory processes ([15](https://www.frontiersin.org/journals/immunology/articles/10.3389/fimmu.2022.1080782/full#B15), [47](https://www.frontiersin.org/journals/immunology/articles/10.3389/fimmu.2022.1080782/full#B47), [48](https://www.frontiersin.org/journals/immunology/articles/10.3389/fimmu.2022.1080782/full#B48)). These findings led us to propose whether there was a mediating role of inflammatory markers in the association between sedentary behavior and sleep disturbance. Strikingly, by conducting the mediation analysis, we found that all four blood-cell-based inflammatory biomarkers significantly regulated this process. Indeed, these inflammatory biomarkers provided us a clue to examine the underlying mechanism between the relationship of sedentary behavior and sleep disturbance.

Fortunately, a sedentary population suffering from sleep disturbance can be managed and improved with non-pharmacological treatment including exercise and physical activity ([49](https://www.frontiersin.org/journals/immunology/articles/10.3389/fimmu.2022.1080782/full#B49)–[51](https://www.frontiersin.org/journals/immunology/articles/10.3389/fimmu.2022.1080782/full#B51)). It was generally accepted that exercise exerted a beneficial effect on the quality of sleep. In accordance with previous findings ([52](https://www.frontiersin.org/journals/immunology/articles/10.3389/fimmu.2022.1080782/full#B52)–[54](https://www.frontiersin.org/journals/immunology/articles/10.3389/fimmu.2022.1080782/full#B54)), our research also found that moderate to vigorous physical exercise can significantly reduce the risk of sleep disturbance in this group. One population-based study reallocated 30 min of sedentary time with exercise and found that this can lead to a more favorable inflammatory profile characterized by higher adiponectin and decreased levels of complement component C3, leptin, interleukin 6 (IL-6), and WBC concentrations ([55](https://www.frontiersin.org/journals/immunology/articles/10.3389/fimmu.2022.1080782/full#B55)). Although our mediation analysis did not significantly detect these findings on the basis of the blood-cell inflammatory biomarkers, it complemented existing literature on the short window of potential benefits. Exercise itself was also linked to oxidative stress. Oxidative stress, a process caused by an imbalance between the production and accumulation of reactive oxygen species (ROS) in cells and tissues, may also regulate the relationship between exercise and sleep disturbance. Although a one-bout exercise can elevate ROS, systematic and regular training can prompt the adaption of organisms by increasing mitochondria biogenesis and antioxidant capacity ([56](https://www.frontiersin.org/journals/immunology/articles/10.3389/fimmu.2022.1080782/full#B56)). In addition, there might also be other physiological pathways. Taking melatonin as an example, being physically active rather than being sedentary can result in a shift of the onset of nocturnal melatonin and make potential alterations in sleep quality ([57](https://www.frontiersin.org/journals/immunology/articles/10.3389/fimmu.2022.1080782/full#B57)).

The greatest strength of our study was the use of the nationally representative NHANES population. Secondly, we adjusted for confounding factors including sociodemographic and lifestyle factors to produce more reliable results. By introducing blood-cell-based inflammatory biomarkers as mediators, this study first attempted to establish the relationship between sedentary behavior, exercise, and sleep disturbance. Importantly, realizing the magnitude and specificity of blood-cell-based inflammatory biomarkers on sleep disturbance had further health implications, considering that inflammation status appeared to be amenable to modification by reducing sedentary time and increasing physical activity. Although evidence on the relationship between physical exercise, sedentary behavior, and sleep efficiency is emerging, the mechanism is far from conclusive. Based on our works, we encouraged further studies to examine the effects of specific types of exercise and sedentary behavior on inflammation to better characterize the association with sleep disturbance.

However, the results of this research should be interpreted with caution for several limitations. Firstly, the outcome sleep disturbance was assessed by self-report in NHANES design, which tended to be imprecise compared with an objectively measured test, although the design of a large population sample and a complex multistage sampling made up for the deficiency of this result to some extent. Moreover, another limitation was that this study only analyzed the independent effects of sedentary behavior and exercise on sleep disturbance. Emerging statistical strategies like the 24-h activity model and the functional principal component model were conducive to better explore the relationship between sedentary behavior and exercise in future research ([58](https://www.frontiersin.org/journals/immunology/articles/10.3389/fimmu.2022.1080782/full#B58), [59](https://www.frontiersin.org/journals/immunology/articles/10.3389/fimmu.2022.1080782/full#B59)). Additionally, blood samples were not necessarily obtained temporally proximal to the survey information in NHANES settings. Last but not least, no measures of inflammatory proteins such as CRP or IL-6 were used in the analysis, and clinical conditions such as hypertension and type 2 diabetes should be further explored.

Conclusion

In conclusion, firstly, our study found that, as inexpensive and handy tests, blood-cell-based inflammatory biomarkers can be used to predict the prevalence of sleep disturbance from a national representative sample. Secondly, the mediation effect of WBC, NEU, NLR, and SII was confirmed in the association between sedentary behavior and sleep disturbance. Thirdly, we detected the mitigation role of exercise on sleep disorders in severe sedentary groups, although the mediation analysis did not examine the significant effect of the four inflammatory biomarkers included in this study. Future studies should focus on understanding the additional biology of inflammatory conditions between sedentary behavior, exercise, and sleep disturbance, testing specific interventions targeting at sleep quality through reducing sedentary time and increasing physical activity.