Research Article

Investigating the Relationships of Adipokines and Cardiovascular Disease Risk Factors with Normal-Weight Obesity Syndrome Among Women

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Abstract

Background: Normal weight obesity (NWO), known as a syndrome, is characterized by normal weight and body mass index (BMI) but high adipose tissue (more than 30%).

Objectives: The present study was conducted to investigate the relationship between adipokines and lipid factors as risk factors in cardiovascular diseases (CVDs) and NWO syndrome in women.

Methods: In this case-control study, anthropometric data were obtained from 20 - 40-year-old women referring to nutrition and diet clinics in Ahvaz, Iran. Then, based on the inclusion criteria, including normal BMI and no physical or mental illness, the subjects were divided into the NWO (body fat percentage (BF%) > 30) group and the non-NWO (BF% < 30) group depending on their BF%. Twenty blood samples were taken from each group, and their chemerin and adipokine serum levels were measured using both the enzyme-linked immunosorbent assay (ELISA) method with high sensitivity and the sandwich and competitive ELISA techniques.

Results: The serum levels of adipokine chemerin and IL-1α, IL-1β, IL-6, and tumor necrosis factor alpha (TNF-α), as well as the serum concentrations of triglyceride (TG), low-density lipoprotein (LDL), and total cholesterol, were significantly higher in the NWO group than in the control group (P < 0.05).

Conclusions: The findings of this study indicated a significant correlation between chemerin, adipokines, and lipid factors, as CVD risk factors, and NWO syndrome and fat tissue percentages in women. Therefore, fat tissue measurement is recommended as a more accurate index than BMI in predicting CVDs.

Keywords: Adipokines, Obesity, Cytokines, Body Mass Index, Adipose Tissue

1. Background

Today, obesity is one of the most common diseases in the world. About 1.2 billion of the world's population is estimated to be overweight (1). In addition, studies conducted in Iran indicate the increased prevalence of obesity in recent years and its further increase with the increased urbanization in the coming years (2). Obese individuals are more susceptible to non-communicable diseases, such as cardiovascular diseases (CVDs), diabetes mellitus (DM), and some types of cancers. Cardiovascular diseases have an increasing trend in humans, and a part of their high prevalence can be attributed to obesity (3).

Obesity represents the development of adipose tissue, and based on the new definitions, obesity, and overweight are referred to the excessive growth of the fat tissue in the body (4). The adipose tissue is active as an endocrine and paracrine organ, producing and secreting a large number of cytokines and biologically active mediators, including leptin, tumor necrosis factor alpha (TNF-α), IL-6, chemerin, resistin, and adiponectin (5). These adipokines affect not only fat metabolism and body weight homeostasis but also insulin resistance and diseases such as type 2 DM, atherosclerosis, and blood coagulation system inflammation (6).

Recent findings indicate that the plasma levels of cytokines exhibit higher levels in humans and animals with excess adipose tissue (4, 7). Studies suggest that proinflammatory cytokines can stimulate and release IL-8 (a member of the CXC family of cytokines involved in the pathogenesis of atherosclerosis) by various types of human adipocyte cells (8).

Central obesity and high rates of abdominal obesity are strongly correlated with hyperinsulinemia, insulin resistance, and the possibility of developing type 2 DM and CVDs in both obese and metabolically obese individuals (9). Population studies indicate the central role of metabolic syndrome in the incidence of CVDs (10, 11). Therefore, weight management can contribute to reducing the number of individuals at risk of DM and CVD (12). The prevalence of metabolic syndrome in individuals with a normal body mass index (BMI) (BMI: 18.5 - 20.9) and individuals with a slight overweight (BMI: 25 - 26.9) has been reported as 0.9 - 3% and 9.6 - 22.5%, respectively, depending on gender and ethnicity (13).

Moreover, in normal-weight individuals, the increased risk of metabolic syndrome and CVD may be of a genetic origin or result from abnormal body composition (14). A syndrome known as normal weight obesity (NWO) has been recently identified, which is characterized by normal weight and BMI but high adipose tissue (over 30%) (15). Individuals with NWO syndrome are distinguished from those with abnormal weight but are metabolically obese (metabolically obese normal-weight (MONW)) because they do not suffer from metabolic syndrome (16).

The pathogenesis of obesity is not well understood yet; however, its pathophysiology has been considered by most researchers due to the discovery of some hormones as the subject of their research. Therefore, the determination of serum levels of adipokines and lipid profiles and their associations with the emerging NWO syndrome will play a critical and practical role in elucidating obesity-induced problems and reducing their complications.

2. Objectives

In the present study, the concentrations of proinflammatory plasma cytokines, including IL-1α, IL-1β, IL-6, TNF-α, and adipokine chemerin, were evaluated in individuals with NWO syndrome. Then, the relationships of these cytokines with lean tissue percentage, fat tissue percentage, and lipid indices (triglyceride (TG), low-density lipoprotein-cholesterol (LDL-C), high-density lipoprotein-cholesterol (HDL-C), total cholesterol) associated with CVDs were compared between subjects with NWO and non-obese subjects.

3. Methods

3.1. Ethical Considerations and Statistical Population

The protocol used in this study was approved by the Ethics Committee of Shiraz University, Shiraz, Iran.

The statistical population of the study included all women referring to nutrition and diet clinics in different regions of Ahvaz, Iran.

3.2. Sampling Method and Sample Size

The minimum sample size was calculated using the following formula (17):

$$n = \frac{2\left(Z_{\frac{\alpha}{2}} + Z_{1-\beta}\right)^2}{\delta^2} \tag{1}$$

where n is the minimum sample size for each group, Z_x is the probability of observing a Z score of x or more, and δ is the effect size. Assuming a maximum acceptable type I error (α) of 0.05, a minimum acceptable study power (1-β) of 0.8, and an effect size of 0.9 (for all measured cytokines), then:

$$\begin{aligned} n &= \frac{2(1.96 + 0.84)^2}{0.9^2} \\ &= \frac{15.68}{0.81} \\ &\approx 20 \end{aligned} \tag{2}$$

We came to a minimum sample size of 20 per study group. The anthropometric data were obtained from the women referring to nutrition clinics. They were divided into two groups, namely, NWO (body fat percentage (BF%) > 30) and non-NWO (BF% < 30), based on their BF%. The inclusion criteria included being in the age range of 20 - 40 years, having a normal BMI, no disease, lack of diet or obesity over the past month, and no use of drugs affecting the fluid balance in the body. The exclusion criteria included no use of alcohol, coffee, caffeine, and tea 12 hours before performing the measurements, no heavy exercise 24 hours before performing the measurements, and no use of drugs causing water loss or retention in the body (diuretics and corticosteroids, such as hydrocortisone and prednisolone). In the next step, blood samples were collected from the subjects.

3.3. Measurement of Anthropometric Indices

In this cross-sectional study, after receiving written informed consent from 20 - 40-year-old women referring to nutrition and diet clinics, a questionnaire containing items related to age, height, weight, and BMI was completed for them. The subjects' weights were measured using a digital weighing scale with a precision of 100 g, and their heights were also measured using a 1 mm precision gauge after they took off their shoes. BMI was calculated by dividing weight (kg) by squared height (m²). Moreover, the BF% was calculated using the bioelectric impedance

method ([18](#)), and resistance was measured using a body composition analyzer (N20, USA).

The measurements were performed for each subject while considering the following points: Twelve hours of fasting, no use of alcohol, coffee, caffeine, and tea 12 hours before performing the measurements, no heavy exercise 24 hours before performing the measurements, and no use of drugs causing water loss or retention in the body (diuretics and corticosteroids, such as hydrocortisone and prednisolone).

The subjects were asked to lie down on the bed for 5 to 10 minutes in minimal clothing, without any jewelry or other metal or electronic stuff, and hold their arms and legs at a 45-degree angle from their bodies so that none of their body parts touch each other. Then, the lead sites were cleaned with alcohol, four leads were attached to the wrists and ankles., and the measurements were performed using the device.

3.4. Measurement of Serum Concentrations of Adipokines and Lipid Profiles

Ten mL of blood samples were taken from the subjects in fasting condition (12 h); then, the blood serum was immediately separated by centrifugation at 3000 rpm and kept at -70°C until the tests were performed.

The serum levels of IL-1β, IL-6, IL-1α, TNF-α, and chemerin were measured with a sensitivity of 0.2, 0.4, 1.6, and 8 pg/mL, respectively, using both the enzyme-linked immunosorbent assay (ELISA) method with high sensitivity and the sandwich and competitive ELISA techniques using the kit manufactured by the Abcam Company, USA.

In addition, the lipid profile, including total cholesterol, TG, HDL, and LDL, was measured by the enzymatic colorimetric assay using the kit manufactured by the Pars Azmun Company, Iran.

3.5. Data Analysis

The data were analyzed using SPSS software (version 17.0, SPSS Inc., Chicago, IL, USA), and the results were reported as mean ± standard deviation (SD).

The independent samples *t*-test was exploited to compare serum levels of adipokines and lipid profiles in the two groups. Moreover, the Pearson correlation coefficient test was used to determine the relationship between the above-mentioned factors and BF%.

A 95% confidence interval was calculated for the mean data values, and P < 0.05 was considered the significance level.

4. Results

Comparing the mean values of the anthropometric indices (Table 1) revealed that the mean age, height, weight, and BMI were not significant between the two groups. However, the mean BF% and the lean tissue percentage were significant between the two groups (P < 0.05).

Comparing the mean biochemical variables in the NWO group and the control (non-obese) group is illustrated in Table 2. The findings showed that the mean serum concentrations of IL-1α, IL-1β, IL-6, TNF-α, and adipokine chemerin in the NWO group were significantly higher than in the control group (P < 0.001). Furthermore, the serum concentrations of TG, LDL, and total cholesterol were significantly higher in the NWO group than in the control group (P < 0.05). However, the HDL concentration was not significantly different between the two groups (Table 2).

The results of the Pearson correlation coefficient indicated a significant positive correlation between the BF% and the serum levels of TG, LDL-C, total cholesterol, IL-1α, IL-1β, IL-6, TNF-α, and adipokine chemerin (Figure 1). Additionally, there was a significantly negative correlation between the lean fat percentages and the above-mentioned parameters. Based on the Pearson correlation coefficients (Figure 1), a significantly positive correlation could be observed between adipokines and lipid profiles (except HDL). There was no significant correlation between adipokines and lipid profiles on the one hand and BMI (BMI in the normal range of 18.5 - 24.9) and weight on the other hand (Figure 1). Furthermore, there was no significant correlation between BF% and weight (r = -0.094) on the one hand and BMI (BMI in the normal range of 18.5 - 24.9) (r = -0.034) on the other hand.

5. Discussion

The present study showed that the mean serum levels of chemerin, as the adipokine secreted from the adipose tissue, were significantly higher in women with NWO than in healthy women and that there was a significantly positive correlation between this adipokine and the lipid profile (except HDL) and adipose tissue.

Since 1997, the World Health Organization (WHO) has declared obesity as one of the major problems in many developed and developing countries ([19](#)). Obesity is a multifactorial chronic disease that has been the focus of public health circles due to its increasing prevalence and is reported as a pandemic complication by the WHO ([20](#)). In epidemiological studies, BMI is used to indicate weight and obesity, and many studies have linked it to the measured BF% ([21](#)). However, evidence suggests that

Table 1. Anthropometric Parameters in the Normal Weight Obesity Group and the Control (Non-obese) Group [a]

Index	Groups	
	Normal Weight Obesity (n = 20)	Non-obese (n = 20)
Age (y)	29.15 ± 6.1	29.45 ± 5.6
Weight (kg)	57.15 ± 5.3	59.37 ± 3.1
Height (cm)	160.1 ± 5.7	160.2 ± 5.1
Body mass index (kg/m^2)	22.28 ± 1.6	23.28 ± 0.9
Body fat percentage [b]	35.9 ± 3.0	26.84 ± 2.1
Lean body mass [b]	64.0 ± 3.0	73.1 ± 2.1

[a] Values are expressed as mean ± SD.
[b] $P < 0.05$

Table 2. Biochemical Parameters in the Normal Weight Obesity Group and the Control (Non-obese) Group [a]

Index	Groups	
	Normal Weight Obesity (n = 20)	Non-obese (n = 20)
Triglyceride (mg/dL) [b]	142.6 ± 21.8	118.6 ± 12.8
LDL (mg/dL) [b]	103.4 ± 7.1	95.3 ± 7.4
HDL (mg/dL)	43.5 ± 7.5	43.6 ± 7.4
Total cholesterol (mg/dL) [b]	175.3 ± 12.1	162.5 ± 9.9
IL-1α (pg/mL) [b]	27.6 ± 2.5	15.5 ± 2.8
IoL-1β (pg/m) [b]	17.0 ± 4.1	7.2 ± 2.3
IL-6 (pg/mL)	13.5 ± 3.3	7.0 ± 2.1
TNF-α (pg/mL) [b]	42.3 ± 3.7	20.6 ± 3.3
Chemerin (pg/m) [b]	57.4 ± 8.5	14.0 ± 3.2

Abbreviations: LDL, low-density lipoprotein; HDL, high-density lipoprotein; TNF-α, tumor necrosis factor alpha
[a] Values are expressed as mean ± SD.
[b] $P < 0.05$

BMI is not a valid indicator for different populations, and the relationship between BMI and BF% varies among different population groups (22). Different methods can be used to measure BF%, but a limited number of these methods, such as BMI and bioelectric resistance, are applicable in epidemiological studies (23). The advantages of the bioelectric resistance method are that it is portable, does not require trained individuals, is non-invasive, and requires the least possible measurement time (24). Also, the safety of this method for all age groups and subjects with specific conditions (pregnancy and illness) has been proven in several studies (25).

Chemerin contributes to the differentiation of the adipocytes and increases glucose uptake (26). The production of chemerin, as a chemotactic protein, has been reported in inflammatory regions; this molecule potentially plays an important role in controlling the immune response in the inflamed area and the injured tissues and is also known to be an anti-inflammatory agent (27).

A study by Buzaoglu et al. also reported similar results regarding the correlation between chemerin and plasma TG levels (26).

Another study by Weigert et al. suggests that blood chemerin levels increase in patients with type 2 DM and obesity (28). Moreover, it was observed that in vitro cultured fat tissue samples, insulin increased chemerin secretion from the fat tissue in a dose- and time-dependent manner. There was also a positive correlation between blood serum chemerin levels and leptin, resistin, C-reactive protein (CRP), TNF-α, and IL-6 (28). Furthermore, another study revealed a significantly positive correlation between the blood chemerin and chemerin content of subcutaneous and abdominal adipose tissue on the one hand and BMI, glucose, insulin, and TG on the other hand (29).

Chemokine-like receptor 1 (CMKLR1) expression in the vascular endothelial cells is regulated by proinflammatory
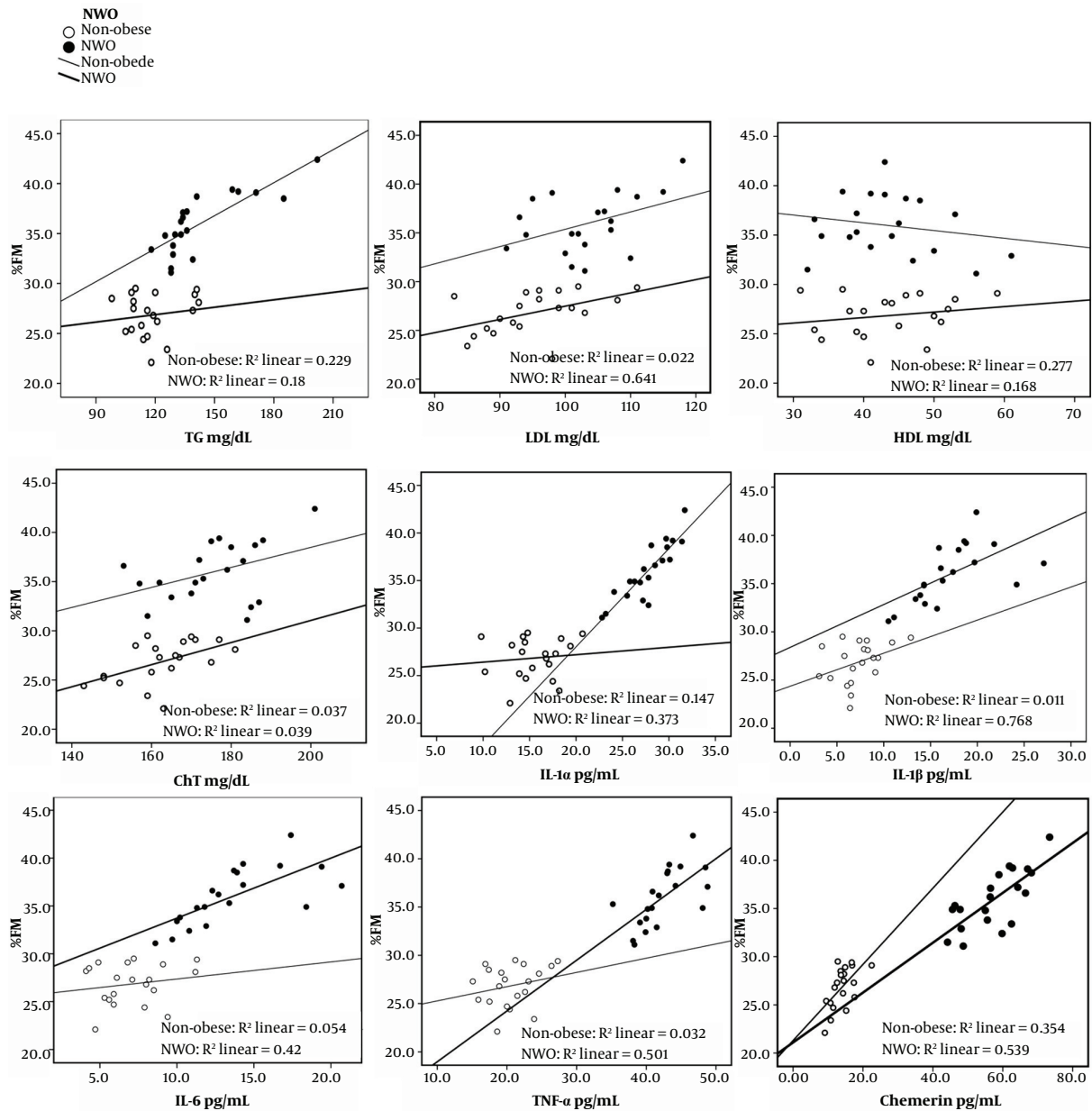
Figure 1. The correlations between biochemical parameters concentration (in the normal weight obesity (NWO) group and the control (non-obese) group) and the body fat percentage (BF%)

cytokines, such as TNF-α, IL-1β, and IL-6 (30). The present study showed a significantly positive relationship between chemerin and TNF-α, IL-1β, IL-1α, and IL-6. The findings of the present study indicated that the mean serum levels of the proinflammatory cytokines of IL-1α, IL-1β, IL-6, and TNF-α were significantly higher in the NWO group than in the healthy group, which is consistent with the results of

Di Renzo et al. on IL-1α and IL-6 (31). In the mentioned study, 60 Italian white women were divided into three groups of 20 people. The groups included the normal (N) group, the NWO group, and the obese (OB) group with normal BMI and BF < 30%, normal BMI and BF > 30%, and BMI > 25 and BF < 30%, respectively. Then, the anthropometric indices, body composition, plasma levels

of some cytokines, glutathione (GSH), lipid hydroperoxide (LOOH), and nitric oxide (NO) metabolites, as well as the lipid and glucose parameters, were measured and compared in the three groups. The results revealed that GSH and NO metabolites were lower in the NWO and OB groups than in the N group. In addition, the LOOH level in the NWO and OB groups was higher than in the N group. Finally, there was a strong correlation between the GSH level and body weight, BF%, waist circumference, lean tissue percentage, IL-1α, IL-6, IL-10, IL-15, and TG ([31]).

The results of another study in Italy investigating 74 women indicated a significant difference in plasma HDL levels between the NWO group and the non-NWO group. There was also a significant correlation between the CVD risk factors, LDL/HDL ratios, lean tissue, and resting metabolic rate (RMR) in women with NWO ([29]), whereas, in the present study, there was no significant difference between the two groups in terms of the HDL serum level. Nevertheless, other results obtained in the aforementioned study were consistent with the results of the present study.

In the United States, 6171 subjects over 20 years of age with a normal BMI were evaluated for body composition, blood factors, and CVD risk factors. The individuals were divided in terms of BF%; BF > 23.1% in men and BF > 33.3% in women were considered the NWO indicators. Compared to the group with a lower BF%, the prevalence of metabolic syndrome in the NWO group was four times higher than that in the non-NWO group. Moreover, individuals with NWO had a higher prevalence of dyslipidemia, hypertension, and CVD ([32]). In another study in Switzerland, 3213 women and 2912 men aged 35 - 75 were examined. The prevalence of NWO in women and men was 5.4% and less than 3%, respectively, so the study was limited to women. Compared to the non-NWO women, the NWO women had a higher lipid profile and a higher prevalence of dyslipidemia and hyperglycemia. However, there was no significant difference between the two groups in terms of CRP, adiponectin, and liver enzyme levels ([33]). The results of the present study also showed a higher mean serum level of lipid profile (except HDL) in the NWO group than in the healthy group.

We also showed a significantly positive correlation between the BF% and the serum levels of TG, LDL-C, total cholesterol, IL-1α, IL-1β, IL-6, TNF-α, and adipokine chemerin. Additionally, there was a significantly negative correlation between the lean tissue percentages and the aforementioned parameters. However, no significant correlation was found between HDL and BF%. These results are in line with the findings of De Lorenzo et al.'s study on the relationship between the lean tissue percentage and lipid profile ([15]). In this study, there was a significantly

positive correlation between adipokines and lipid profile (except HDL). Similarly, in their study, Weigert et al. and Di Renzo et al. observed a significantly positive correlation between chemerin, IL-6, TNF-α levels, and lipid profile ([28], [31]).

Comparing the mean biochemical variables in the NWO group and the control (non-obese) group in the present study indicated higher mean concentrations of chemerin, IL-1α, IL-1β, IL-6, and TNF-α in the NWO group than in the control group. Moreover, the biological effects of the increase in each of the adipokines on IL-1α and IL-1β included the induction of fever, acute-phase proteins (APPs), fibroblast proliferation, smooth muscle cells, the production of antibodies, cytokines, angiogenesis, metastasis, cartilage disintegration., the effects on glucose homeostasis and insulin sensitivity through central and peripheral mechanisms, decreased expression and activity of lipoprotein lipase, increased lipolysis, and the effects on adipocyte differentiation through inhibition of the peroxisome proliferator-activated receptors (PPAR). Moreover, these effects for IL-6 included reduced insulin and leptin signaling, stimulation of the release of APPs, such as the CRP from the liver, induction of hypothalamic fever, stimulation of fatty acid oxidation and lipolysis, and induction of insulin resistance. Furthermore, the biochemical effects of TNF-α included the induction of insulin resistance, increased lipolysis in adipocytes, decreased adiponectin, increased expression of IL-6 and atherogenic role, increased expression of adhesion molecules in the vascular wall, increased expression of scavenger receptor and uptake of the oxidized LDL in macrophages and stimulation of their secretion in the vascular wall ([34]).

The effects of chemerin included the alteration of insulin sensitivity in adipocytes and skeletal muscle, adipocyte differentiation, proliferation, migration, apoptosis of vascular smooth muscle, plaque stability in atheroma injury, proinflammatory and anti-inflammatory activity in the immune cells, and effects on endothelial performance concerning the production of NO and inflammatory cytokines ([35]). Furthermore, these results indicate higher plasma lipid profile concentrations in the NWO group than in the control (non-obese) group and conclude that individuals with NWO syndrome are susceptible to various inflammatory diseases, including CVD.

This study showed that weight loss through exercising, physical activity, and proper nutrition can be considered front-line therapy. In recent years, the combination of dietary macronutrients and the consumption of certain foods ([36]) and food groups ([37]) have been separately identified to reduce the risk factors for CVDs and NWO

syndrome. Many researchers have broadly investigated the effect of various macronutrients on improving the traditional components of metabolic syndrome and obesity; however, limited information is available on the impacts of these diets on the inflammatory processes in obesity and NWO syndrome. Nevertheless, the inflammatory processes, independent of blood lipid levels, appear to increase the risk of CVDs (38).

The results of this study can be important for several reasons:

(1) Higher concentrations of chemerin and proinflammatory cytokines were observed in women with NWO;

(2) High amounts of chemerin and cytokines were associated with BF%;

(3) Excessive generation of chemerin and cytokines may be involved in the early stages of inflammation and, thus, can be a significant predictor of obesity, CVDs, and metabolic syndrome risks.

Currently, there are some studies underway to analyze the expression of the chemerin gene and proinflammatory cytokines in NWO syndrome, and it is evident that further studies are needed to be conducted to determine the roles of chemerin and cytokines in NWO syndrome.

The findings of the present study could be helpful in designing obesity-related disease prevention programs. Also, it seems that in order to overcome the incorrect classification of obesity only based on the anthropometric measurements, more attention should be paid to both the tissue percentages and the body fat distribution, as inflammatory patterns associated with NWO.

Footnotes

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