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Effect of different diet methods on body composition and insulin, leptin, and ghrelin levels in overweight women

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Abstract

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Keywords

fat tissue, frequent meals, intermittent feeding, obesity, overnight fasting

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Article

Effect of different diet methods on body composition and insulin, leptin, and ghrelin levels in overweight women

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1. Introduction

Overfeeding leads to excessive fatness and obesity, and an increase in the body mass index (BMI) used as an indicator of obesity is associated with cardiovascular diseases, diabetes, and many types of cancer. Observational evidence indicates that weight loss with energy restriction reduces a risks of a disease [1]. It is claimed that the development of obesity, a universal problem, can be reduced by changes in lifestyle and dietary habits. While the amount and frequency of the meal are considered as the primary nutritional approach to energy restriction [2], intermittent energy restriction or intermittent fasting methods is an alternative approach that has recently attracted more attention.

The general opinion is that there is an inverse relationship between the number of consumed meals and the body weight, thus a smaller but frequent feeding can help prevent obesity [3]. It is assumed that this inverse relationship is related to eating frequency, which increases appetite control and digestive tract peptides. However, previous studies that compared the frequent (three meals and three snacks) and the intermittent (only three meals) diet methods revealed that participants have similar improvements in the body mass, both fat and lean body mass [4]. Beside food type and amount, frequent feeding has been shown to play a decisive role in fasting serum lipid levels and insulin secretion change [5].

In contrast, hypotheses argued that decreasing the frequency of eating with intermittent fasting favorably regulates gene expression that allows cells to cope with severe stress [6]. Intermittent fasting with energy restriction has been reported to have positive effects on growth factors with signal transmission, the release of neurotransmitters and gene expression, and it may help increase insulin sensitivity, reduce blood pressure and oxidative stress, and improve fat metabolism [2]. Intermittent fasting practice may occur during the day every other day, as well as it can be provided as 12–14 hour fasting at night [7].

Appetite and energy intake are physiologically regulated by the neuroendocrine system, which involves complex interactions between central and peripheral mediated pathways. Appetite regulating hormones contain episodic bowel signals that are sensitive to short-term stimulation in nutritional behavior, control hunger and saturation relative to eating (for example, acylated ghrelin), and tonic hormonal signals regulate energy balance and long-term changes in body fat (for example, insulin and leptin). An increased insulin level with increased body weight causes adipose cell hypertrophy, and, despite the increase in insulin requirement, the decrease in the number of receptors leads to insulin resistance [8]. Leptin and ghrelin work together as antagonists in the organism, and there is a negative correlation between the serum leptin level and the ghrelin level [9].

Due to a limited number of scholarly works, it is not precisely known if a frequent-meal or intermittent overnight fasting diet, both with similar calorie restrictions (-20%), is more effective in fighting obesity. The purpose of this study is to compare the effects of similar calorie-restricted intermittent overnight fasting diet with the frequent-meal diet to understand their influence on the body composition and the insulin, leptin and ghrelin levels in overweight women.

2. Materials and Methods

2.1. Participants

The research has an experimental, semi-controlled study design that includes quantitative and repeated measurements. The inclusion criteria of the participants were as follows: female, aged between 35–55, BMI >25 kg/m², not having any health problems that would prevent the application of the diet methods, having healthy ranges of hormone levels, especially the ones that are known to affect the metabolic rate [thyroid stimulating hormone (TSH), free thyroxine (T4) and free triiodothyronine (T3)], being sedentary or lightly active for three months before the start date of the study (i.e., <3 h/week), not using alcohol, having a stable weight for three months before the start date of the study (i.e., <5 kg weight loss or gain), having a regular menstrual cycle, and having follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol and progesterone within the reference ranges.

The exclusion criteria for the study were failure to adapt to the protocol included in tests and measurements, and the presence of special conditions (e.g., drug use, breaking the diet) that would prevent participation in the study and challenges from complying with the diet (e.g., severe injury/disease). Initially, 49 people who meet the sought criteria were willing to participate in the study after hearing about it from an advertisement. 46 of them accepted to participate in the study after completing the preliminary questionnaire. Consequently, the study was completed with 30 people due to exclusion criteria. The groups were determined based on the participants' ability to maintain the diet.

This study was approved by a local medical research ethics committee (no: 70198063-050.06.04) and was undertaken in compliance with the Helsinki Declaration.

2.2. Study Design

Before and after the 4-week diet period, participants' height, body weight, body composition and basal metabolic rate were measured. Additionally, their daily energy expenditure and the substrate oxidation rate were calculated. Markers associated with the criteria and aims of the study were evaluated from the venous blood samples. The content of the diet was private.

2.3. Anthropometric and body composition measurements

Height and body weight were measured with minimal clothing (Seca 767, Hamburg, Germany) using standard methods. Body composition was measured on a body analysis device (Tanita MC 780MA, USA) which operates with the bioelectric impedance method. Body fat amount (kg and %), right-left arm and leg fat amount (%), lean body mass (kg and %), and body fluid amount (kg and %) was measured. Each measurement was repeated twice consecutively; the intra-class correlation coefficient was calculated as 98%. For these measurements, participants were asked to come to the laboratory in the morning right after night fasting and to avoid physical activity and caffeine consumption the day before.

2.3.1. Blood Analysis

Venous blood samples were drawn in the morning (between 09.00 and 10.00 a.m.) by a nurse, while the participant was sitting. One sample was collected with a 4 mL purple cap with EDTA and the other one with a 9 mL red cap flat tube. Following a 20-min rest period, at room temperature, tubes were centrifuged at 2000 g for 15-min (Nüve NF 200, Ankara, Turkey), and the serum was separated. Serum sample analyses were conducted on the same day.

To get information about the individuals' general health and metabolic status, before starting the diet program serum hemogram (WBC, RBC, PLT), ferritin, iron, hemoglobin, blood lipids (triglyceride, total cholesterol, HDL-K), serum TSH, T4 and T3, vitamin B12, serum 25 (OH) vitamin D were evaluated. LDL-K levels were calculated according to the Friedewald formula ($\text{LDL-K} = \text{total cholesterol} - \text{triglyceride} / 5 - \text{HDL-K}$).

The markers in the research hypothesis were investigated twice. Fasting blood glucose, serum leptin, ghrelin (using the commercial kit with enzyme-linked immunosorbent assay (ELISA) method) and plasma insulin (microparticle enzyme immunoassay method (AxSYM Abbott Diagnostics Division)) levels were analyzed at the beginning and end of the diet program. Insulin resistance ($\text{HOMA-IR} = \text{fasting glucose (mmol / l)} \times \text{fasting insulin (mU / l)} / 22.5$) was calculated.

2.3.2. Resting Metabolic Rate Measurement, Daily Energy Requirement, and Metabolic Measurement Calculations

The resting metabolic rate was measured with the help of a gas analyzer (Innocor Inno500, Odense, Denmark) after the participants rested for 15-min in the supine position. VO_2 and VCO_2 respiratory values were recorded from the inhaled air by employing the breath-by-breath method, and RER was calculated accordingly. The rate of carbohydrate (CHO) and fat oxidation were determined with formulas along with the average of the last 5-min data [10]. In addition to the measured basal metabolic rate (kcal) value of the participants, daily energy requirements were calculated individually using the physical activity factor.

2.3.3. Diet applications

In diets created with 20% restriction of daily energy need, 50–60% of the energy is formed from CHO, 10–15% from proteins, 25–30% from fats, and the content was arranged to take into account the participants' wishes. The overnight fasting diet group (OFD) did at least 12 hours of night fasting (dinner at 7 p.m. and not eating anything until breakfast) and had three meals and two snacks a day, while the frequent meal diet group (FMD) had three meals and three snacks a day, and were allowed to have dinner at the desired time and then have a snack.

A social network was established with the participants over the phone, and continuous communication was carried out for a month. The necessary motivation was provided, and their compliance with diets was checked precisely.

2.3.4. Determination of physical activity levels

International Physical Activity Inventory (IPAQ), which was adapted to Turkish, was applied to the participants twice, at the beginning and at the end of the diet program, and they were warned not to change their physical activity habits during the study [11].

2.4. Statistical analysis

Statistical analyses were performed using IBM Statistical Package for Social Science (SPSS) for Windows, Version 25.0. (IBM Corp., Armonk, NY, USA). Descriptive statistics were reported as the mean \pm SD. After the normality test (Shapiro-Wilk), the pre- and post-test comparison of the groups was done with the Paired Sample t-Test or the Wilcoxon Signed Ranks Test. Inter-group differences of the data were analyzed by the Independent Samples T-Test or the Mann Whitney U Test. Data not showing normal distribution are marked in tables with the ¥ sign. The Spearman Rho Correlation Coefficient test was used for correlation analysis between parameters. The effect size of the difference in the data with normal distribution was evaluated using the classification of Cohen d (< 0.2 trivial, $0.2 \leq d < 0.5$ small, $0.5 \leq d < 0.8$ moderate, $d \geq 0.8$ large effect size). $p \leq 0.05$ was accepted as the level of statistical significance..

3. Results

When the groups were compared in terms of body composition parameters before (1) and after (2) the diet period, statistical improvement was found in all parameters except lean mass (kg) and body water (kg) in both groups, and fat amount (%), right and left arm fat (%) and left leg fat (%) results only in FMD (Table 1). When the two groups were compared within themselves, there was no significant difference between the groups ($p > 0.05$).

Table 1. Pre- (1) and post- (2) test results regarding the body composition.

Body composition		OFD (n=15)	% diff p value	FMD (n=15)	% diff p value	% diff p value*	d value
Body mass (kg)	1	84.0 \pm 13.6	-%2.3	90.7 \pm 14.1 ¥	-%2.5	0.198	
	2	82.1 \pm 13.3	0.000	88.5 \pm 13.8	0.003	0.205	-0.472
BMI (kg/m ²)	1	32.6 \pm 5.43	-%2.2	34.9 \pm 5.17	-%2.7	0.256	-0.433
	2	31.9 \pm 5.29	0.000	34.0 \pm 5.13	0.000	0.266	-0.403
Fat mass (kg)	1	33.8 \pm 8.46	-%4.0	36.9 \pm 11.1 ¥	-%7.0	0.476	
	2	32.5 \pm 7.97	0.000	34.5 \pm 10.3 ¥	0.005	0.696	
Fat ratio (%)	1	38.9 \pm 5.23 ¥	-%1.0	39.9 \pm 5.54	-%3.0	0.982	
	2	38.5 \pm 3.11	0.001	38.9 \pm 5.23	0.108	0.821	-0.092
Right arm fat ratio (%)	1	45.5 \pm 5.94	-%1.8	47.5 \pm 6.84	-%1.9	0.415	-0.312
	2	44.7 \pm 5.93	0.001	46.6 \pm 6.15	0.369	0.418	-0.314
Left arm fat ratio (%)	1	46.5 \pm 5.90	-%2.4	45.5 \pm 7.43	%2.4	0.705	0.149
	2	45.4 \pm 5.81	0.000	46.6 \pm 6.57	0.659	0.630	-0.193
Right leg fat ratio (%)	1	44.7 \pm 4.46	-%1.1	45.6 \pm 4.19	-%1.6	0.572	-0.207
	2	44.2 \pm 3.75	0.000	44.9 \pm 4.22	0.049	0.664	-0.175
Left leg fat ratio (%)	1	44.7 \pm 4.46	-%0.9	45.4 \pm 4.24	-%1.8	0.658	-0.160
	2	44.3 \pm 3.77	0.000	44.6 \pm 4.39	0.072	0.823	-0.073
Fat free mass(kg)	1	48.2 \pm 5.29	%0.4	50.8 \pm 4.02	-%1.4	0.156	-0.553
	2	48.4 \pm 5.40	0.766	50.1 \pm 3.66	0.587	0.307	-0.368
Fat free mass (%)	1	57.4 \pm 3.27	%1.7	56.9 \pm 5.11	%1.7	0.734	0.116
	2	58.4 \pm 2.95	0.000	57.9 \pm 4.99	0.032	0.725	0.121
Body fluid (kg)	1	36.0 \pm 4.36	%1.1	38.2 \pm 3.03	-%1.3	0.124	-0.585
	2	36.4 \pm 4.06	0.528	37.7 \pm 2.63	0.414	0.319	-0.380
Body fluid (%)Body mass (kg)	1	42.8 \pm 2.86	%2.7	42.8 \pm 3.84	%1.1	0.987	0.000
	2	44.0 \pm 2.27	0.019	43.7 \pm 3.67	0.030	0.830	0.098

BMI, body mass index; OFD, overnight fasting diet group; FMD, frequent meal diet group; ¥, shows not normally distributed data; p value, shows 1.-2. measurement differences within each group; *p value, shows the measurement difference between groups.

The measured O₂ consumption and CO₂ production (lt), calculated CHO and fat oxidation rate (g/min), and energy amount (kcal) as variables of the basal metabolic rate are presented in Table 2. While there was no significant difference in the pre- and post-test results in OFD, a significant increase in the resting fat oxidation rate (g/min) and the resting heart rate (pulse/min) were observed in FMD ($p < 0.05$). There was no statistical difference between the groups for all data ($p > 0.05$).

Table 2. Pre- (1) and post- (2) test results regarding the resting metabolic rate.

Resting metabolism		OFD (n=15)	% diff p value	FMD (n=15)	% diff p value	% diff p value*	d value
CHO oxidation rate (g/min)	1	0.02 ± 0.03 ¥	0.925	0.04 ± 0.03	0.211	0.165	
	2	0.03 ± 0.03 ¥		0.02 ± 0.02 ¥		0.917	
Fat oxidation rate (g/min)	1	0.14 ± 0.03	0.871	0.12 ± 0.02	0.046	0.107	0.784
	2	0.13 ± 0.02		0.13 ± 0.03		0.636	0
VCO ₂ (lt)	1	0.19 ± 0.02	0.577	0.18 ± 0.02	0.413	0.165	0.500
	2	0.20 ± 0.04		0.19 ± 0.02		0.365	0.316
VO ₂ (lt)	1	0.28 ± 0.04	0.897	0.25 ± 0.03	0.077	0.086	0.848
	2	0.28 ± 0.04		0.27 ± 0.04		0.506	0.250
RER (VCO ₂ /VO ₂)	1	0.73 ± 0.03 ¥	0.733	0.74 ± 0.03 ¥	0.256	0.106	
	2	0.73 ± 0.02 ¥		0.73 ± 0.22 ¥		0.917	
RMR (kcal/day)	1	1892 ± 286	0.893	1738 ± 175	0.096	0.087	0.649
	2	1881 ± 302		1812 ± 244		0.501	0.251
HR (pulse/min)	1	72.0 ± 7.15	0.117	71.5 ± 7.30	0.045	0.850	0.069
	2	69.8 ± 8.21		67.6 ± 9.52		0.502	0.247

CHO, carbohydrate; VCO₂, the amount of carbon dioxide produced; VO₂, the amount of oxygen consumed; RER, respiratory exchange ratio; RMR, resting metabolic rate; HR, heart rate; OFD, overnight fasting diet group; FMD, frequent meal diet group; ¥, shows not normally distributed data; p value, shows 1.-2. measurement differences within each group; *p value, shows the measurement difference between groups.

Evaluation of some parameters affecting fat metabolism is shown in Table 3. It was found that glucose and insulin levels in OFD and insulin levels and insulin resistance index in FMD significantly decreased with diet ($p < 0.05$). While there was a significant decrease in the leptin level and a significant increase in the ghrelin level in FMD, only a significant decrease in the leptin level was detected in OFD ($p < 0.05$).

Table 3. Pre- (1) and post- (2) test results regarding fat metabolism.

Fat metabolism		Reference range	OFD (n=15)	% diff p value	FMD (n=15)	% diff p value	% diff p value*	d value
Glucose (mg/dL)	1	74-106	90.9 ± 6.81	-%6.6	95.7 ± 17.3	-%4.4	0.325	-0.365
	2		85.3 ± 6.92	0.002	91.7 ± 13.9	0.114	0.122	-0.582
Insulin (µIU/mL)	1	1.9-23	12.2 ± 5.94 ¥	-%72	12.9 ± 5.44	-%47	0.443	
	2		7.10 ± 2.80	0.002	8.78 ± 4.47	0.002	0.227	
HOMA- IR	1	0.5-1.4	2.24 ± 1.59	-%30	2.97 ± 1.47	-%47	0.204	-0.477
	2		1.72 ± 0.55	0.224	2.02 ± 1.12 ¥	0.005	0.820	
Leptin (ng/mL)	1		13.9 ± 4.92	-%49	14.8 ± 7.67	-%37	0.715	0.574
	2		9.33 ± 4.77	0.000	10.8 ± 4.82	0.002	0.424	0.425
Ghrelin (ng/mL)	1		8.75 ± 3.35 ¥	%1.5	8.49 ± 3.64 ¥	%5.8	0.632	
	2		8.88 ± 2.80 ¥	0.754	9.01 ± 3.18 ¥	0.044	0.868	

HOMA-IR: homeostatic model assessment- insulin resistance, OFD, overnight fasting diet group; FMD, frequent meal diet group; ¥, shows not normally distributed data; p value, shows 1.-2. measurement differences within each group; *p value, shows the measurement difference between groups.

Significant positive correlations were found between body weight (1) and leptin levels (1, $p = 0.005$) (2, $p = 0.040$), between body weight (2) and leptin levels (1, $p = 0.001$) (2, $p = 0.005$), insulin (2, $p = 0.034$), HOMA-IR (2, $p = 0.037$), between fat mass (1) and insulin (1, $p = 0.039$), leptin level (1, $p = 0.008$), HOMA-IR (1, $p = 0.012$), between fat mass (2) and

insulin (1, $p = 0.045$), insulin (2, $p = 0.049$), leptin (1, $p < 0.001$), leptin (2, $p < 0.001$), HOMA-IR (1, $p = 0.015$), HOMA-IR (2, $p = 0.043$), between leptin (1) and HOMA-IR (2, $p = 0.024$), between leptin (2) and HOMA-IR (2, $p = 0.006$), between insulin (2) and leptin (1, $p = 0.011$), leptin (2, $p = 0.003$), ghrelin (1, $p = 0.049$), HOMA-IR (2, $p < 0.001$), between ghrelin (1) and HOMA-IR (2, $p = 0.037$) in both diet groups.

4. Discussion

Similar improvements in body composition were observed in both groups after the 4-week diet administration. A significant increase in the fat oxidation rate and a significant decrease in the heart rate solely in FMD is observed. There was a significant decrease in insulin, leptin levels in both groups. A significant decrease in the glucose level in OFD and a significant improvement in HOMA-IR and ghrelin levels in FMD were noted. Furthermore, significant positive correlations were found between body weight, fat mass and leptin levels, between insulin and leptin levels, leptin and ghrelin levels, and HOMA-IR in both diet groups.

Although there has been an ongoing discussion over the subject matter for many years, the relationship between feeding frequency and body mass/composition cannot be clearly determined. Beside those who say that there is a significant negative relationship between body mass and feeding frequency [12, 13], there are also results arguing that there is no such relationship [5, 14, 15]. Supporting our study methodology and the results, a study that examines the effects of snack consumption after dinner found no significant difference in body composition between the two diets [16].

The intermittent fasting model has often been tested on experimental animals. In one study, there was no difference in body weight or energy intake in rats that ate at 12-hour intervals; however, positive effects on glucose and insulin regulation were observed regardless of calorie intake [7]. In one of the few studies on humans, the participants' body weight decreased by an average of 9% in the 20-week intermittent diet model which was administered by taking very low calories once a week [17]. In this current study, when comparing OFD and FMD in terms of body composition, similar significant decreases were observed in both group' BMI and body fat amount (kg) after only four weeks. This result reveals that the main change in body composition occurs by not the cutting the nightly food consumption, but limiting the daily energy intake (-20%). In our study, the increase in lean body mass (%) obtained in both diet groups suggests that frequent feeding effect to regulate the release of blood sugar and insulin. The achieved increase in body fluid (%) may be owing to the fact that the participants started to drink water regularly during the month of dieting.

Obesity is characterized by high fasting plasma insulin and exaggerated insulin response to oral glucose uptake. A positive correlation is observed between increased central obesity and insulin resistance measurements [18]. Previous research investigating the very low-calorie diet effect in obese women for three months showed that leptin, insulin, BMI, and HOMA-IR levels significantly decreased compared to baseline values [19]. In our study, while it was observed that in both diet groups fasting insulin, leptin levels, and BMI decreased, there was also a significant decrease in HOMA-IR only in FMD. From this point of view, it can be suggested that eating frequency in the form of three meals and three snacks a day will have a more positive effect in the treatment of insulin resistance and can be investigated in more detail.

The mean fasting blood glucose and insulin levels measured in FMD and OFD at the beginning of our study decreased with 4-week diet and the values were within the expected reference ranges in healthy individuals. Moreover, a reduction in insulin resistance was observed after the diets, although it was within the reference values in both diet groups, and this reduction in FMD is statistically significant to suggest that calorie restriction positively affects fasting glucose. However, in terms of its positive effect on insulin resistance, it is proposed that frequent meals with energy restrictions are more effective than an intermittent diet with overnight fasting.

When two groups fed regular and irregular meals were compared within themselves and between groups, no significant difference was found in the glucose and insulin response [20], and irregular meal frequency has been reported to impair energy metabolism. Meal frequency is not effective in individuals' total energy intake throughout the day [21]. There are other studies showing that differences in meal frequency do not significantly affect glucose and insulin responses [13, 22]. In a study investigating the relationship between the resting metabolic rate and feeding frequency, no significant relationship was found, but the frequency of meal was shown to increase satiety [6].

Leptin acts as a sensor that actively regulates energy balance changes by creating signals of hunger and satiety. It can be said that it decreases in nutrition with negative energy and in plasma with weight loss, increases in positive energy nutrition and regulates the amount of body fat and the person's required weight [23]. It is also a hormone proportional to the BMI and the waist circumference [24]. Most obese individuals have a high level of leptin concentrations in their serum and plasma but show leptin resistance due to reduced leptin transport to the central nervous system or leptin receptors. A higher leptin level is required to overcome leptin resistance. For this, more leptin is released from the adipose tissue, which leads to an increase in the fat tissue that produces it [25]. In a study performed in two lean and obese groups of adults who fasted for 53–96 hours, it was observed that their body weight decreased by less than 4%, while leptin concentrations decreased by 54–72%. Studies with short-term fasting indicate that the leptin concentration reflects triacylglycerol synthesis or glucose uptake rather than the existing adipocyte stores since the fat mass gained in short-term fasting still exists. It is associated with factors that reflect reduced glucose availability and increased lipolysis, such as a decrease in leptin during fasting, an independent decrease in glucose, and an increase in beta-hydroxybutyrate [24].

Previous studies reported that no significant correlation was found between increased meal frequency and the leptin level [5]. On the other hand, a positive correlation was found between leptin and BMI [26] in a low-calorie diet, where most of the CHO was taken at dinner, balanced leptin and ghrelin levels throughout the day, and it supported weight loss better than other diet methods [27]. According to a study supporting the effects of meal frequency on energy balance regulation, individuals who consumed three meals and four meals (three meals + one snack) had increased body fat mass, leptin concentration, and respiratory capacity with meal skipping [28]. In adult individuals who lost weight with a calorie-restricted diet program, leptin levels significantly decreased, and the ghrelin level decreased compared to the level before the diet program. On the other hand, no change in the leptin level was found, while the serum ghrelin level significantly decreased in the group who could not lose weight [29]. Our study results support the results of the study mentioned above both with positive correlations determined between the leptin level, body weight and body fat amount, and a decrease in the serum leptin level in parallel with decreasing the body weight as a result of the diet programs.

Participating directly in short-term regulation of energy balance, ghrelin, after being produced in the stomach, reaches its receptors in the anterior pituitary and hypothalamic region, where it stimulates the release of the growth hormone and regulates energy homeostasis, nutrient uptake and fatty tissue-increasing information to the brain [30]. The ghrelin level decreases with obesity and calorie intake and increases in hunger. It has been reported to have an inverse proportion to the BMI and the waist circumference [24]. In our study, serum ghrelin levels increased in both groups, and this increase was statistically significant in FMD (%6.3, $p = 0.044$). Besides, significant positive correlations between insulin (2) and ghrelin (1) and between HOMA-IR (2) and ghrelin (1) in OFD as well as between glucose (2) and ghrelin (1) and ghrelin (2) in FMD are thought to reflect the effects of ghrelin on energy balance despite similar body weight losses. Also, it was found that the level of ghrelin in only FMD showed a significant increase in parallel with the significant increase in fat oxidation, which requires further investigation of the possible relationship between the ghrelin level and the body fat oxidation rate.

Although the short-term findings obtained in our study are positive, testing the long-term effects of these dietary strategies in a large-scale group, evaluating the physical activity factor, due to the positive effects on weight control and insulin levels, and controlling the stress and sleep levels, due to the effects on diet periods, may warrant further investigation.

5. Conclusions

After a 4-week same calorie-restricted frequent meal diet with six meals and an overnight fasting diet with a 12 hour-overnight fasting, similar improvements in body composition and insulin, leptin, and ghrelin levels have been achieved.

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