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The role of insulin resistance and leptin in the effect of intermittent feeding with a high protein ketogenic diet on body composition in rats

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Highlights

- High-protein ketogenic diets and intermittent nutrition can be protective against obesity and metabolic syndrome.
- Intermittent-high protein ketogenic diet provides low body weight and does not harm body composition.
- High-protein ketogenic diets provides low fasting glucose levels and preserves basal insulin and leptin levels without disturbing the blood lipid profile.

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Abstract

The prevalence of obesity and overweight have been increasing rapidly since childhood in the world and threaten society with various chronic diseases, which are born with them. High-protein ketogenic diets and intermittent nutrition are thought to be protective against obesity and metabolic syndrome. However, the exact effects and results, insulin resistance, and the role of leptin in the functioning mechanism of these diets have not been fully elucidated. In this study, the roles of insulin resistance and leptin hormone in the effect of body composition were investigated with a diet created with a high-protein ketogenic diet and intermittent nutrition combination. Thirty-two young non-obese rats were randomly divided into four equal groups. Both the standard diet and the high-protein ketogenic diet were given as ad libitum and intermittent to the rats for 6 weeks. The body weight and fat mass of the rats were measured at the end of the experiment. The fasting glucose, leptin, insulin, HDL, LDL, and triglycerides were measured with the taken blood samples. The lowest body weight was observed in the intermittent and high-protein ketogenic diet group and followed by the free high-protein ketogenic diet and standard intermittent diet group, respectively. Also, the lowest body fat mass was observed in the intermittent and high-protein ketogenic diet group followed by the standard intermittent diet group. While there was no change in leptin, insulin, HDL, LDL, and triglyceride levels in any group, the lowest blood glucose rate was observed in the intermittent and high-protein ketogenic diet group. The results revealed that an intermittent high-protein ketogenic diet is more effective than others in weight loss without disrupting biochemical health parameters and the applied diets do not prevent growth and development.

Key Words: Ketogenic diet, high protein diet, intermittent feeding, insülin resistance, leptin.

1. Introduction

Obesity is a significant global issue, and there is growing attention within the medical community towards innovative treatments to address its pathophysiological effects. It is recognized as a risk factor for several common and chronic diseases, including glucose intolerance, insulin resistance, diabetes mellitus, hypertension, dyslipidemia, fatty liver, coronary heart disease, heart failure, stroke, respiratory problems, sleep apnea, gallbladder diseases, musculoskeletal problems, osteoarthritis, and various types of cancer. The World

Health Organization has designated obesity as an endemic public health problem. (1). The prevalence of obesity and overweight has been rapidly increasing since childhood, posing a significant threat to society worldwide due to the associated burden of chronic diseases. Despite extensive scientific research conducted for many years on various preventive, protective, and therapeutic dietary approaches and ingredients for obesity, a consensus has yet to be reached regarding a comfortably applied and consistently effective form of healthy diet that fully demonstrates positive effects on weight control and loss. In addition, as the prevalence of obesity has increased, the diet forms recommended for slimming and weight control have also been diversified. Atkins Diet which is the most popular of them, recommends a high-fat (~60), high-protein (~30), and low-carb (~10) diet (2). The Zone Diet which is the second most popular diet following the Atkins Diet, recommends a nutritional form that contains high protein (about 30%), moderate fat (about 30%), and approximately 40% carbohydrates (3). However, there have been conflicting results on whether these diets are effective in weight loss and body weight maintenance, and if so, their effects on body composition (fat or lean tissue mass), metabolic indicators (fasting blood glucose, lipid profile, uric acid, and ketone bodies), and leptin and insulin levels which regulate the long-term energy intake and expenditure have not been fully understood (4,5).

In recent years, it has been suggested that high-protein ketogenic diets show positive improvements in body composition and health indicators compared to classical (high-carbohydrate) diets; they provide a higher rate of satiety, increase thermogenesis, reduce insulin and leptin levels in the blood, and break insulin resistance (6,7). In addition, studies conducted with intermittent feeding in various periods found that significant weight loss was observed and insulin and leptin values decreased compared to free feeding. Therefore, it has been suggested that intermittent feeding may be protective against obesity and metabolic syndrome (8,9). However, studies on intermittent nutrition have generally been conducted with the classical diet, which contains more than 50% carbohydrates and this content has been shown to be insufficient to induce satiety compared to diets with higher protein and fat content (10).

Since the exact effects of the high-protein ketogenic and intermittent nutrition diets and the role of insulin resistance and leptin in the functioning mechanisms of these diets have not been fully elucidated, the advantages and disadvantages of these diets are still being investigated and debated. Although there have been severe advances in the medical treatment of obesity; there is an increased interest in improving and developing nutritional models,

especially with various dietary regimens. There are many known beneficial aspects of intermittent fasting. It is a type of nutrition recommended by health professionals, especially for its benefits on weight management, cardiovascular health, and oxidative stress (11).

The main purpose of this study is to create a healthy diet that can be easily and sustainably applied throughout life. Studies have shown that the primary step of obesity is insulin resistance and the critical point is the hormone leptin so it was also aimed to investigate the role of leptin hormone and insulin resistance (12) on body composition with the created diet for this s study. For this reason, it was aimed to create an untested diet form that would provide healthy weight loss and/or control without disturbing metabolic parameters by combining the intermittent feeding period, which has positive effects in the literature with a high-protein ketogenic diet.

In this study, the developed novel experimental diet model by combining the high-protein ketogenic diet with intermittent feeding holds significant promise to resolve the metabolic syndrome and the pathologies that may develop due to obesity by creating a feasible, repeatable, and comfortable diet.

2. Materials and Methods

2.1. Experimental animals

The study utilized 32 male Sprague Dawley albino rats with an weight range of 170-210 g. The research protocol received approval from the Bezmiâlem Vakıf University Animal Experiments Local Ethics Committee (Date: 28.02.2019, Issue: 2014/172). All experimental procedures were conducted at the Bezmialem Vakıf University Experimental Animals Research and Application Center.

2.2. Experimental Groups

In the study, the rats were divided into 4 groups and 8 subjects were in each group. They were housed in rooms with a temperature of 22-24 °C and a humidity of 50-55%, with 12 hours of light and 12 hours of darkness (light between 07.00 - 19.00).

Group 1 (Control Group): The subjects were fed free (ad libitum) with standard pellet.

Group 2: The subjects were fed with standard pellet intermittently (twice daily at 07.00 in the morning and at 17.00 in the evening).

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Group 3: The subjects were fed as free with special high-fat, high-protein, and low-carbohydrate food.

Group 4: The subjects were fed with high-fat, high-protein, and low-carbohydrate special diets intermittently (twice a day at 07.00 in the morning and at 17.00 in the evening).

2.3. High Protein Ketogenic Diet and Standard Diet Nutrition

The subjects were provided with isocaloric diets. To assess the feeding patterns, the standard fed groups (Groups 1 and 2) were initially given standard pellet on the first day. Groups 3 and 4 receiving special feed were fed high-fat and high-protein food ad libitum and intermittent, respectively. Groups 1 and 2 fed with a standard diet were given 2600 kcal/kg of standard rat food in pellet form (20% of the energy from proteins, 10% from fats, and 70% from carbohydrates). Groups 3 and 4 fed with a high protein ketogenic diet; containing high fat, high protein, and low carbohydrate ratios; A special feed of 5240 kcal/kg was given (60% of the energy was produced from fats, 30% from proteins, and 10% from carbohydrates).

The amount of consumed food was determined by weighing. The subjects consumed about 20 g of pellets and about 15 g of fatty food per day. To provide isocaloric nutrition, the calorie content of the diets was calculated based on the average weight of the subjects based on their daily calorie needs. The daily calorie requirement of a 180-200 g rat corresponds to approximately 70 kcal/kg (13). In addition, all subjects were kept in separate cages to control isocaloric feeding. The subjects were able to access the water freely (ad libitum) during the experiment.

2.4. Collection of Blood and Tissue Samples and Biochemical Measurements

After the experiment procedure was applied for 6 weeks, the subjects were weighed and their heights were measured. Fasting blood glucose was measured with the Accu-Chek Performa Nano glucometer from the blood samples taken from the tail vein of the subjects after 12 hours of fasting. Then, the subjects were anesthetized with a mixture of 10mg/kg xylazine and 40mg/kg ketamine. The anesthetic was given to the subjects intraperitoneally. After checking whether the subjects reached the level of surgical anesthesia with pupillary reflex and withdrawal reflex, 5 ml of blood was taken from their hearts into STR (serum separator tubes).

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The blood samples were centrifuged at 1000xg 6°C for 15 minutes. Serum was separated and stored at +4°C. Each subject from which blood was drawn was sacrificed by cervical dislocation immediately after the procedure. The visceral, mesenteric, and retroperitoneal fat tissues of the experimental subjects were removed and the total weight of the fat tissue removed from each subjects was weighed. Serum insulin, leptin, HDL, LDL, and triglyceride measurements were made with specific rat sandwich ELISA kits in stored blood samples. A commercial kit based on the sandwich ELISA principle (Shanghai Korain Biotech Co. Ltd China, cat.no: E0707Ra) was used to determine serum insulin levels. A commercial kit based on the sandwich ELISA principle (Shangai Korain Biotech Co. Ltd China, cat.no: EO561Ra) was used to determine serum leptin levels. A commercial kit (Shangai Korain Biotech Co. Ltd China, cat.no: E0254Ra) based on the sandwich ELISA principle was used to determine serum HDL levels. A commercial kit (Shangai Korain Biotech Co. Ltd China, cat.no:EA0021Ra) based on the sandwich ELISA principle was used to determine serum LDL levels. A commercial kit (Shangai Korain Biotech Co. Ltd China, cat.no:E0249Ra) based on the sandwich ELISA principle was used to determine serum triglyceride levels.

2.5. Statistical Analysis

SPSS ver.20 statistical analysis program was used for statistical analysis in the evaluation of the findings obtained in this study. The significance of the changes between the experimental groups was analyzed using the post hoc Tukey HSD following the Oneway Anova test, and the significance of the changes compared to the control group (free standard diet) using the Dunnett t-test. As a result of the analysis, values of $p<0.05$ were considered statistically significant.

3. Results

3.1. Effects on Body Weight

At the end of the 6-week experimental period, weight gain was observed in all experimental groups (because they were young adults) as their development and growth processes continued. At the end of the experiment, the body weights of the rats showed a significant change based on the type of diet. The results are shown in Figure 1.

3.2.Effects on Tall Stature

When the heights of all subjects in the groups were compared, it was observed that there was no significant difference between the groups (Figure 2).

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3.3. Effects on Fat Tissue Weights

At the end of the experiment, the total weight measurements of the visceral, mesenteric, and retroperitoneal fat tissues taken from the experimental subjects in all groups were compared. It was observed that the adipose tissue weight of the experimental subjects in the control group (8.21 ± 0.19 g) was significantly higher than the adipose tissue weight of the subjects in Group 2 (7.13 ± 0.23 gr; $p<0.05$). It was observed that the adipose tissue weights of the subjects in Group 3 were significantly higher than the adipose tissue weights of the subjects in Group 4 (5.15 ± 0.31 g; $p<0.001$). The fat tissue weights of the subjects in group 3 (8.16 ± 0.27 g) did not show a significant difference compared to those in the control group. The adipose tissue weights of the subjects in Group 2 were found to be significantly higher when compared to the adipose tissue weights of the subjects in Group 4. It was observed that the fat tissue weights of the subjects in Group 4 were significantly lower than the fat tissue weights of the subjects in all other groups (Figure 3).

3.4. Effects on Fasting Blood Glucose

When fasting blood glucose values were compared in all groups, fasting glucose values of subjects in the control group (115.37 ± 1.25 mg/dl), Group 2 (112.00 ± 1.02 mg/dl), and Group 3 (114.63 ± 1.21 mg/dl) There was no significant difference between them. The fasting blood glucose values of subjects in group 4 (94.75±1.69 mg/dl) were found to be significantly lower than the fasting blood glucose values of subjects in all other groups ($p<0.001$). The results are shown in Figure 4.

3.5. Effects on Insulin and Leptin Values

There was no significant difference between the serum insulin and leptin levels of subjects in all our groups (Figure 5 and Figure 6).

3.6. Effects on HDL, LDL and Triglyceride Values

Upon comparing the serum levels of HDL, LDL, and triglycerides among all subjects groups, no significant differences were observed (Figure 7, Figure 8, and Figure 9).

4. Discusion

In this study, it was aimed to compare the effects of a high-protein ketogenic diet, intermittent-standard diet, and intermittent high-protein ketogenic diet on body composition, body growth (weight and height) in rats, insulin resistance, leptin, and blood lipid profile in

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rats. It was observed that the weight of the rats increased over time. It is important to note that the rats used in the research were young adults and not undergoing the same growth and development process as humans. Despite this, their weight still increased as part of their natural growth and development (14). However, significant differences were observed in the body weights of the rats based on the type of diet they were given. When comparing the body weight gains of the experimental groups, the lowest weight gain was observed in Group 4, which received a high-protein ketogenic diet intermittently. This was followed by Group 3, which received a high-protein ketogenic diet ad libitum, and Group 2, which received a standard diet intermittently. The highest weight gain was observed in Group 1, which served as the control group and received a standard diet continuously. The statistical analysis revealed a significantly lower body weight gain in Group 4 compared to Group 1, indicating that the high-protein ketogenic diet with intermittent feeding led to the lowest weight gain among the groups studied. The order of body weight gain was as follows: Group 4 < Group 3 < Group 2 < Group 1.

In the high-protein ketogenic diet-intermittent group (Group 4), the body weight and adipose tissue mass (visceral, retroperitoneal, and mesenteric adipose tissue) of the subjects were significantly lower compared to all other groups. However, it is noteworthy that despite the lower body weight and adipose tissue mass, the body development in terms of height was normal in Group 4, similar to the subjects in the other groups. At the conclusion of the study, there was no significant difference observed in the heights of the rats among the experimental groups. This finding suggests that the height growth of the rats in all groups, including the high-protein ketogenic diet-intermittent group (Group 4), was not adversely affected. Based on the findings of our study, it can be concluded that the application of a high-protein ketogenic diet during adolescence does not have a negative effect on height growth in rats. This suggests that the high-protein content in the ketogenic diet may have played a role in maintaining normal height development. However, it is important to note that in studies conducted with strict ketogenic diets that have high-fat ratios, such as a 4:1 ratio, on adolescents, there have been reports of side effects such as growth retardation and slowed height growth. These side effects are believed to be associated with the reduced protein ratios in such diets. (15,16). Based on the results of our study, it appears that the high-protein ketogenic diet with increased protein ratios (at least 30% protein content) was able to prevent the side effects, such as growth retardation and slowed height growth. Moreover, it was observed that the rats fed with the high-protein ketogenic-intermittent diet had lower body

weights and adipose tissue weights compared to all other groups. This suggests that the intermittent-high protein ketogenic diet led to lower body weight without adversely affecting body composition. These findings indicate that the increased protein content in the high-protein ketogenic diet and the intermittent feeding schedule may have contributed to the prevention of negative effects on growth and body composition, while still promoting lower body weight. However, it is important to consider that these conclusions are based on research conducted on rats, and further studies would be necessary to confirm the effects and safety of such diets in humans.

In the context of this study, the significant reduction in adipose tissue weights observed in the subjects in the intermittent-high protein ketogenic diet group (Group 4) compared to other groups suggests a potential beneficial effect of this diet on adiposity. This indicates that the diet may have helped mitigate the development of metabolic abnormalities associated with increased adipose tissue mass. It is well-established that an increase in adipose tissue mass, particularly visceral adiposity, is closely linked to various metabolic disorders. Excessive adipose tissue, especially in the visceral region, has been associated with an increased risk of metabolic syndrome, which includes conditions such as obesity, hypertension, dyslipidemia, and insulin resistance (17).

It has been shown that rats consume high-fat foods at a much higher rate than standard feeds, and even when they can reach them ad libitum, they consume high-fat foods even during sleep periods when they do not normally display feeding behavior. For this reason, feeding with high-fat and high-protein feed was determined as 6 meals, as previously done and recommended in the literature, to control the isocaloric nutritional balance (8,18). No significant difference was observed between the adipose tissue weights of the free-fed subjects (Group 3) and the subjects in the control group. The subjects in Group 3 were fed between 07.00 am and 5.30 pm due to the unsuitable technical conditions of the laboratory where we performed our study. However, unlike humans, rats are active at night and feed especially at night therefore rats fed during the day (ie, at the time corresponding to the sleep period of the rats) had a higher lipid synthesis (19) and adipose tissue mass compared to rats fed at night (wake period of rats: ~19.00-7.00) (20). Therefore, it was thought that the subjects in Group 3 could not show low adipose tissue weights because they were fed during the sleep period hours. On the other hand, the body weights of free-fed rats on a high-protein ketogenic

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diet were significantly lower than those in the control group. This result suggests that the ketogenic diet may contribute to the elimination of peripheral edema (21).

It was observed that rats in the intermittent fed standard diet group (Group 2) had lower fat weights compared to the control group and Group 3. This finding aligns with previous studies that have suggested intermittent feeding can lead to more fat loss compared to free (ad libitum) feding (22,23). High-carbohydrate diets alone may not provide sufficient satiety during intermittent fasting, which can make the fasting period more challenging and less comfortable for individuals. Carbohydrates are known to be quickly digested and can lead to fluctuations in blood sugar levels, potentially causing hunger and cravings. In contrast, diets that include adequate protein and healthy fats can contribute to increased satiety and help individuals feel more satisfied during the fasting periods of intermittent fasting (10,24). On the other hand, it is known that feeding with foods containing higher protein and fat, creates longer-term satiety (25). Therefore, it can be said that intermittent feeding with foods containing high carbohydrates cannot be a comfortable and applicable diet. In the intermittent feeding period, which was applied in Group 4, an intermittent feeding procedure was performed with high-protein ketogenic foods in order to provide longer-term satiety. The findings show that this diet program (high protein ketogenic-intermittent) is an effective, easy, and comfortable diet for controlling body weight and adipose tissue mass, and it is a viable diet without hindering growth and development when applied during adolescence.

Examining serum insulin, leptin, fasting glucose, HDL, LDL, and triglyceride levels were examined insights into the mechanisms underlying the different effects of the diet programs on body weights, adipose tissue weights, and changes in fat and carbohydrate metabolism. In our study, no significant difference was observed in the serum insulin and leptin levels among the subjects in the different experimental groups. It is known that both basal and post-feeding concentrations of insulin and leptin correlate and affect each other's oscillations (26). In studies performed in humans and rodents, it has been shown that ketogenic (27,28) or intermittent-fed groups (8,29) have significantly lower insulin and leptin levels than the groups fed standard diets. However, due to inadequate technical conditions in our laboratory during this study, the free-fed high-protein ketogenic diet subjects in Group 3 were fed outside of the active feeding period. In addition, the subjects in Group 2 and Group 4 were fed two meals at 07:00 in the morning and at 17:00 in the evening, which corresponds to the sleep periods of the rats but still falls within proximity to their active phase. When rats

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were fed during their sleep period, it has been observed that the daily rhythm of leptin and insulin release shifts, and their basal levels increase significantly compared to rats fed during their active phase (30,31). Furthermore, it was observed that the daily rhythm of leptin, which undergoes changes based on feeding times, was altered in the rats fed during the day, preventing its rise at night and resulting in postprandial peak levels during the daytime (32). Therefore, in this study, since the subjects in Group 2 and Group 4 were not fed exactly during their active hours, even though they were close to their active periods, and because the subjects in Group 3 were fed exactly during the sleep period, as previously shown in the literature, it was thought that the levels of leptin and insulin increase and the circadian oscillation rhythm of leptin shifts, and for this reason, it could not show the low insulin and leptin levels as expected. According to the literature, it is expected that feeding subjects during their sleep (passive) periods would lead to a significant increase in leptin and insulin levels. However, in our study, such an increase was not observed, and the insulin and leptin levels were similar to the nocturnal fed experimental group (control group). This suggests that the high-protein ketogenic and/or intermittent feeding approach may have the ability to prevent the anticipated elevation in insulin and leptin levels.

Although no significant change was observed between the leptin and insulin levels of groups, significantly lower adipose tissue mass and body weight were detected in intermittent feeding groups (Group 2 and Group 4). Compared to free-fed groups, the low adipose tissue mass and body weights were observed in intermittent fed groups are remarkable. When a detailed literature review is made to explain the mechanism mediating these results, it is seen that leptin has a lipolytic effect during fasting. In a recent study in rats, it was shown that with the decrease of leptin concentrations and increase in corticosterone concentrations starting from the 8th hour of fasting, white adipose tissue lipolysis, hepatic acetyl CoA amount, hepatic pyruvate carboxylase flow were increased and blood glucose was maintained within normal limits (euglycemia) despite decreased glycogenolysis in rats. This study revealed that depletion of glycogen stores from the 8th hour after food intake resulted in decreased plasma glucose and insulin concentrations and glucose uptake by adipocytes and increased corticosterone concentrations, inhibiting glycogenolysis and signaling to fat cells to decrease leptin production, and ultimately plasma glucose and insulin. It is claimed that the concentrations of glucose and insulin, which decrease with the depletion of glycogen stores, send a signal to the adipocytes to decrease leptin concentrations and to increase the activity of the HPA axis, increasing lipolysis and ketogenesis in the white adipose tissue, and energy is

obtained (33). Therefore, based on our findings, it was thought that this mechanism may mediate the decrease in adipose tissue mass and body weight in intermittent fasting groups, and if the leptin and insulin levels were measured of rats during intermittent fasting, it would have detect low levels, which is consistent with this study. On the other hand, the significantly lower fasting glucose levels that observed in the group fed intermittent high protein ketogenic diet (Group 4) are also consistent with the findings of this study. There was no significant difference between serum HDL and LDL and triglyceride values of the subjects in our experimental groups. It has been shown that feeding during the sleep period in rats increases lipid synthesis and triglycerides, and also impairs the lipid profile (34). The fact that the lipid profiles of experimental groups, except the control group, were not elevated, when compared to the nocturnally fed control group, although they were fed during the daytime (sleep period), suggesting that the high protein ketogenic and/or intermittent diet preserves the lipid profile. In addition, HDL, LDL and triglyceride levels of the rats in the high protein ketogenic diet groups (Group 3 and Group 4) did not deteriorate despite the high fat content they consumed. This finding showed that high protein ketogenic and/or intermittent diets are also protective in lipid profile. In addition, it was thougth that the type of fat used in the nutritional content will also be effective in maintaining the lipid profile. Because when a literature review on various fat contents is made, it is claimed that oils containing trans fats such as margarine and palm oil induce obesity in humans and rodents; There are many studies showing that fish oil, butter and tallow do not increase body weight and do not impair the lipid profile (35,36).

As a result, according to the findings, with the advantages demonstrated by this study of intermittent feeding application of a high-protein ketogenic diet, it has been shown that a high-protein and high-fat diet with 2 meals a day is an ideal nutritional model and a ketogenic diet with two meals a day produces lower body and adipose tissue weight compared to the free feeding ketogenic diet. In addition, the fact that no increase was detected in leptin and insulin values in these experimental groups, and the low fasting glucose values and the normal lipid profile especially in the high protein ketogenic group, show that the nutritional models was applied which beneficial for health. On the other hand, since the rats in our experimental groups are young adults and they can be adapted to the adolescence period in humans due to their continued growth and development, it has been demonstrated that the ketogenic nutrition models that performed can be applied as a healthy and comfortable diet in obese adolescents by causing weight and adipose tissue loss, without preventing growth and development.

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Conclusions

It has been suggested that intermittent feeding (fasting) with a high-protein ketogenic diet may provide several benefits, including long-term satiety, therapeutic potential against obesity and metabolic syndrome, reductions in body weight and adiposity, lower fasting glucose levels, and preservation. In this study, rats in all groups exhibited a tendency to gain some weight due to their growth stage. However, it was determined that the weight gain in the intermittent and high-protein ketogenic diet group was significantly lower than in the other groups. There was no difference in height observed among the groups based on the different types of diets applied. Additionally, adipose tissue weight was measured to be the lowest in the intermittent and high-protein ketogenic diet group. Fasting blood glucose values also differed significantly among the groups. The lowest fasting blood glucose value was observed in the group following an intermittent and high-protein ketogenic diet. However, more detailed and comprehensive studies are needed to fully understand the potential therapeutic effects, mechanisms, and long-term implications of intermittent fasting combined with a high-protein ketogenic diet. Human studies, including randomized controlled trials and long-term follow-ups, would provide more robust evidence in this regard.

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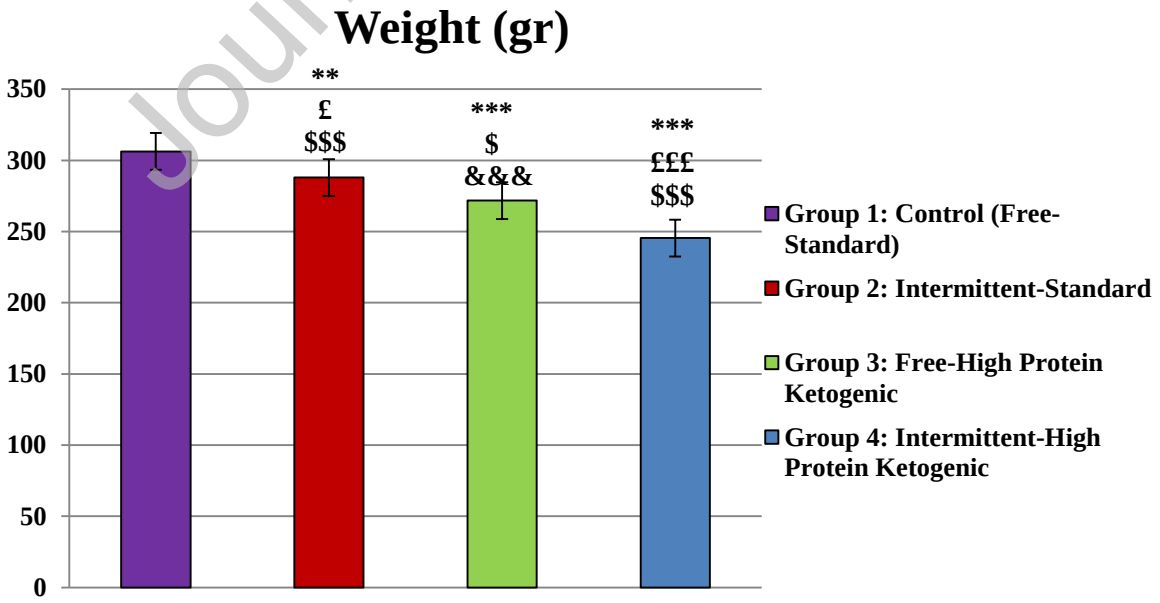
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Figures



*: Indicates the difference with the free standard diet.

$: Indicates the difference with the intermittent standard diet.
£: Shows the difference with the free high protein ketogenic diet.
&: Shows the difference with the intermittent high protein ketogenic diet.

Figure 1. Comparison of body weight values between experimental groups.

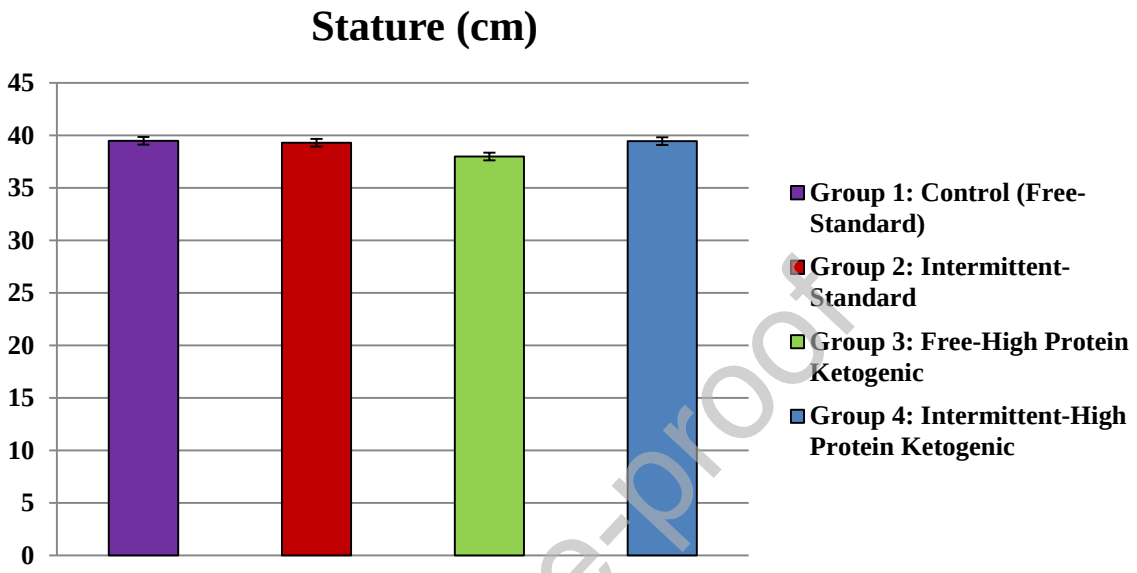
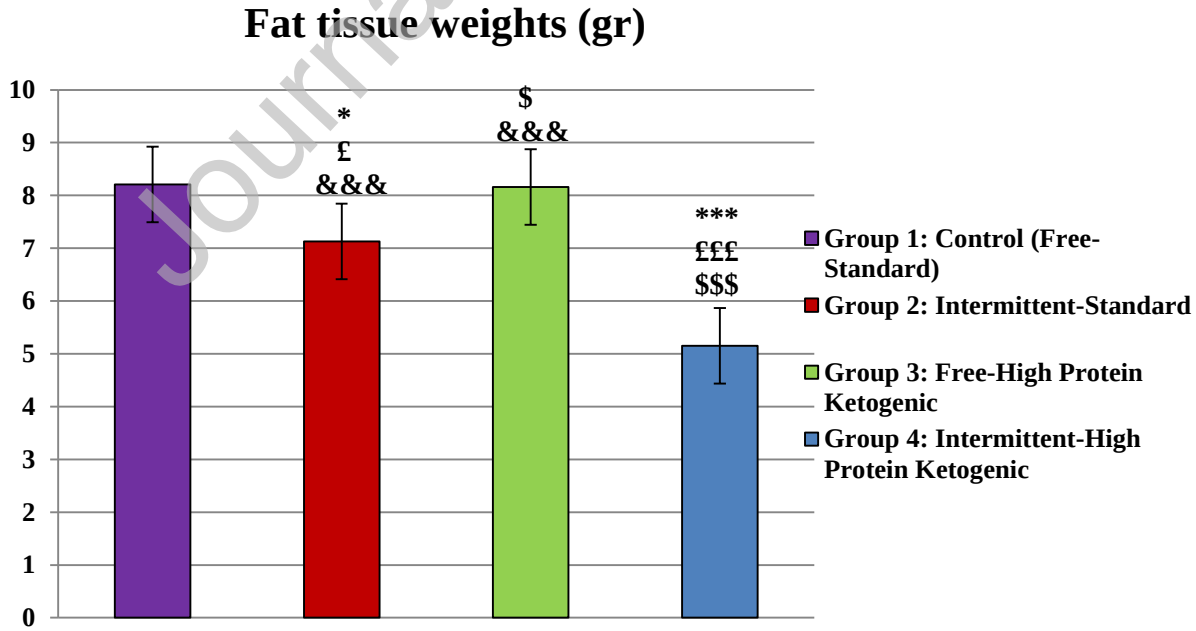


Figure 2. Comparison of tall stature values between experimental groups.

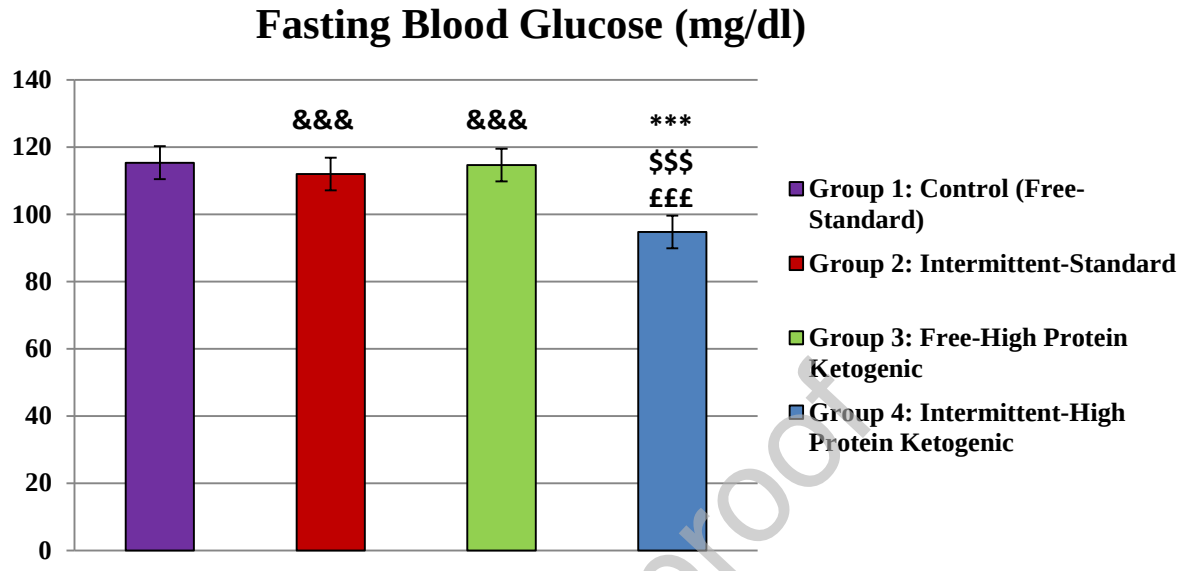


*: Indicates the difference with the free standard diet.
$: Indicates the difference with the intermittent standard diet.

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£: Shows the difference with the free high protein ketogenic diet.
&: Shows the difference with the intermittent high protein ketogenic diet.

Figure 3. Comparison of fat tissue weight values between experimental groups



*: Indicates the difference with the free standard diet.
$: Indicates the difference with the intermittent standard diet.
£: Shows the difference with the free high protein ketogenic diet.
&: Shows the difference with the intermittent high protein ketogenic diet.

Figure 4. Comparison of fasting blood glucose values between experimental groups.

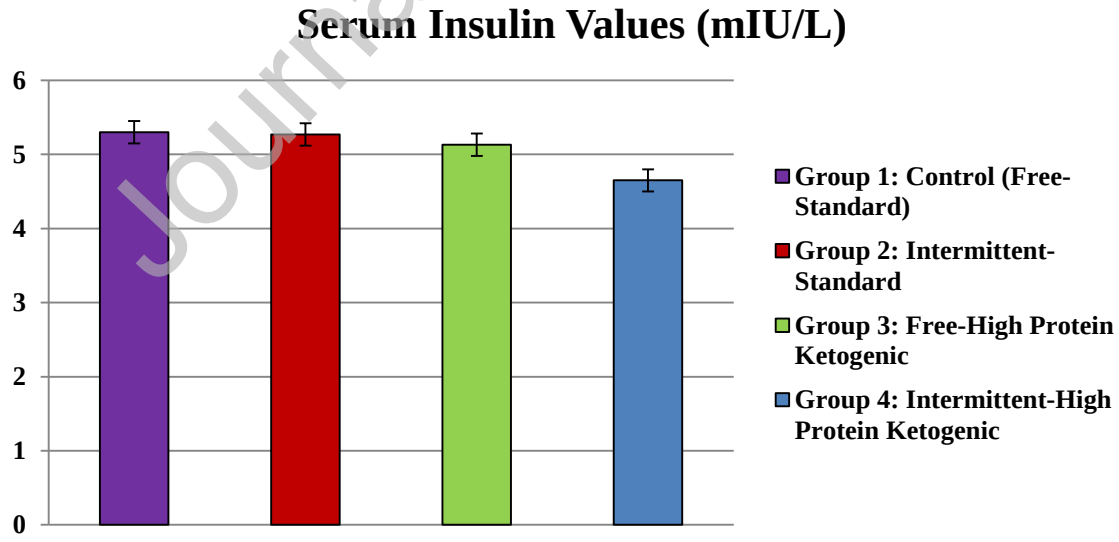


Figure 5. Comparison of serum insulin values between experimental groups.

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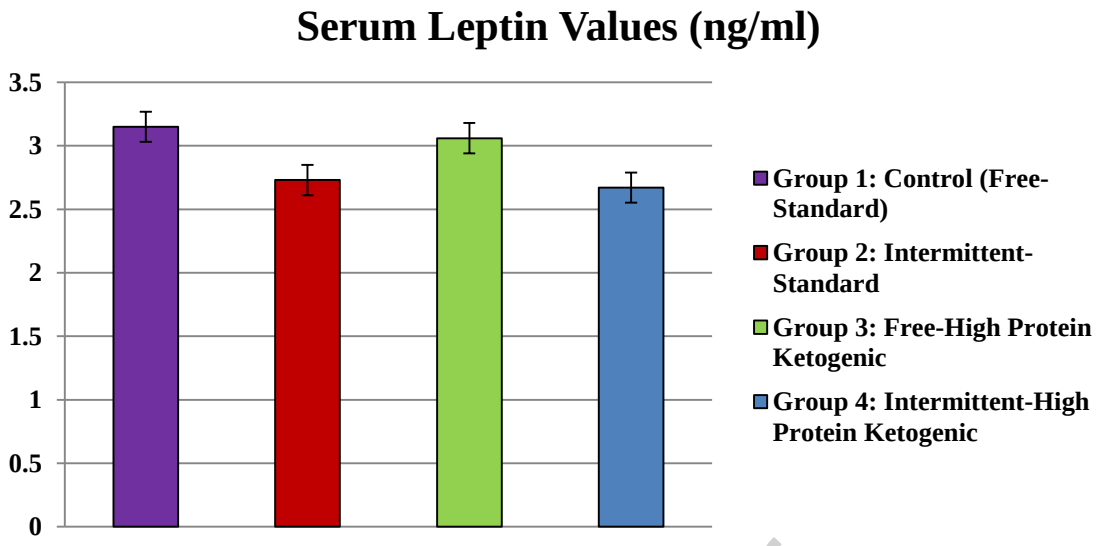


Figure 6. Comparison of serum leptin values between experimental groups.

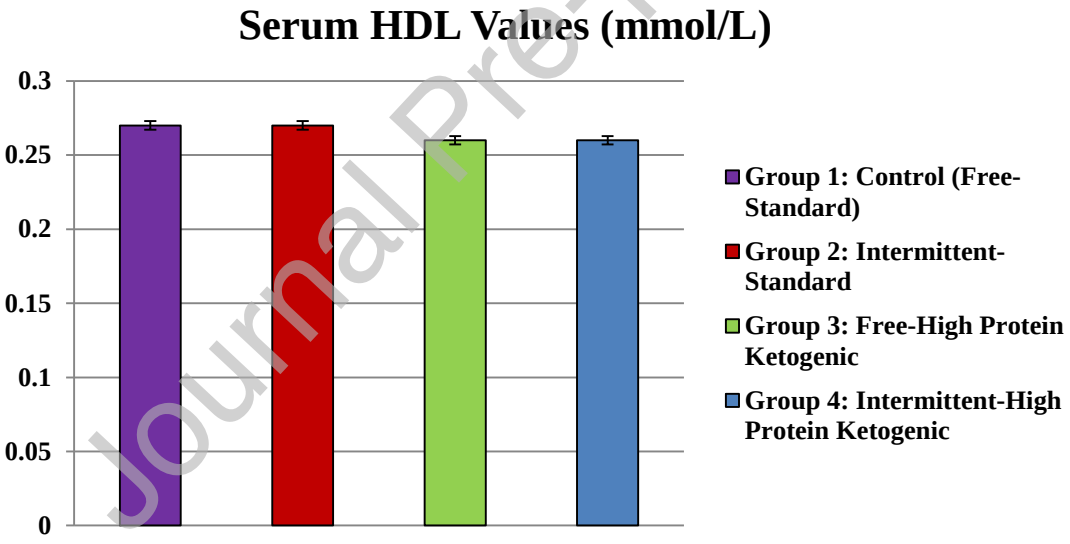


Figure 7. Comparison of serum HDL values between experimental groups.

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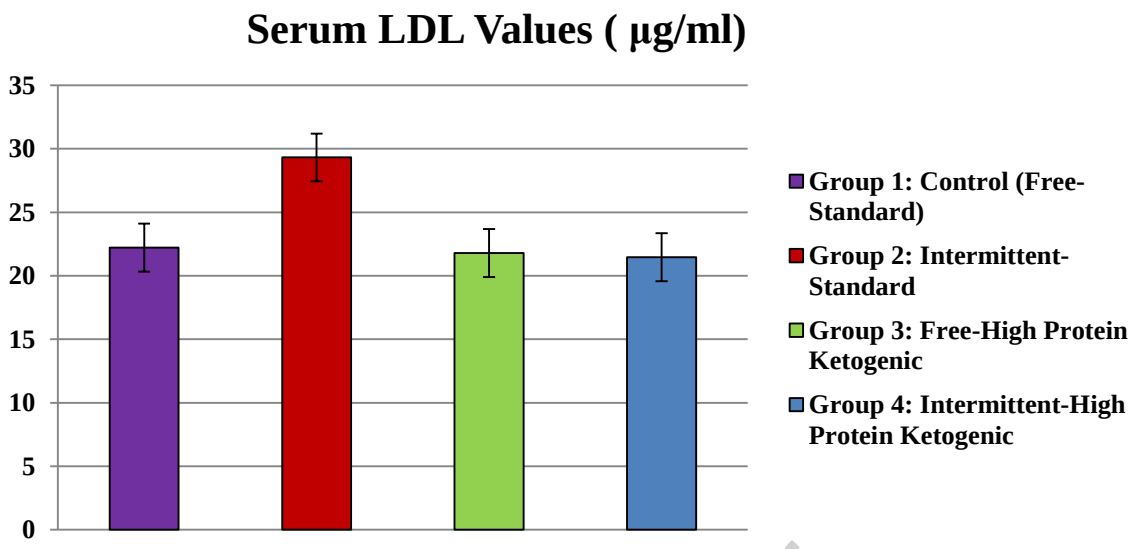


Figure 8. Comparison of serum LDL values between experimental groups.

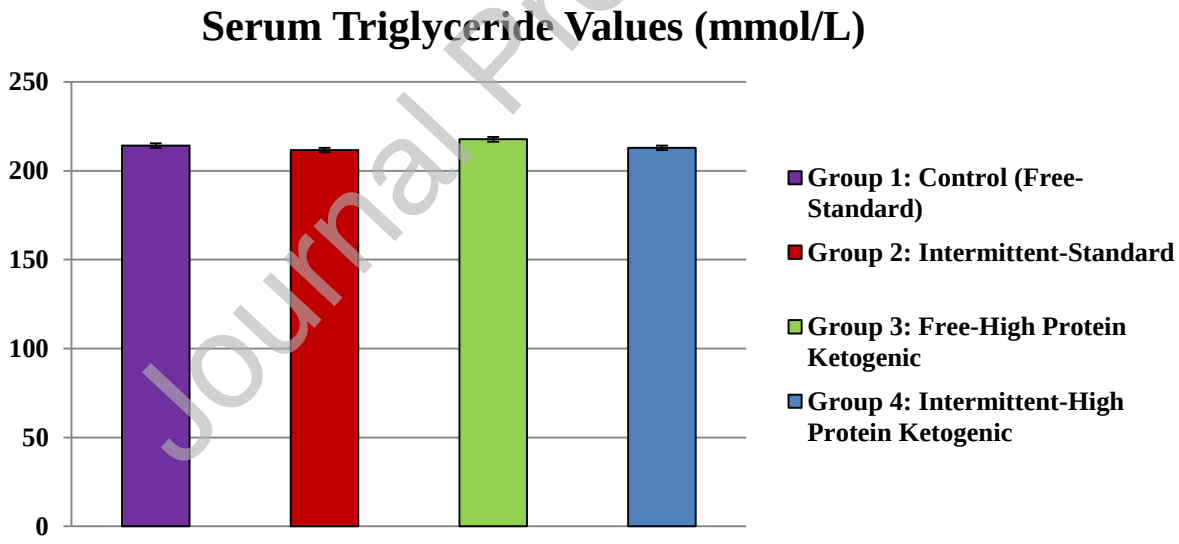


Figure 9. Comparison of serum Triglyceride values between experimental groups.

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Credit Author Statement

Gul Eyikudamacı: Data Curation, Writing-Original draft preperation. **Hasan Ege:** Wrinting-Reviewing and Editing. **Nihal Ensen:** Investigation, visualization. **Nermin Yelmen:** Supervision, methodology

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Declaration of interests

☐The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☒The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Prof. Dr. Nermin Yelmen reports financial support was provided by Istanbul University-Cerrahpasa Cerrahpasa Faculty of Medicine. Nermin Yelmen reports a relationship with Istanbul University-Cerrahpasa Cerrahpasa Faculty of Medicine that includes: consulting or advisory and funding grants.

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