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Effectiveness of Low Glycemic Index High Protein Diet on Ages and Interleuikin-6 Levels in PCOS Insulin Resistance Model Mice

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ABSTRACT

Background: A dietary approach that is low in carbohydrates and high in protein may enhance the management of polycystic ovary syndrome (PCOS) by assessing serum concentrations of Interleukin 6 and AGEs. This study aimed to evaluate the effects of a low carbohydrate, high protein diet in PCOS insulin resistance model mice on the level of AGEs and Interleukin 6.
Subjects and Method: This study used a post test only control group design, true experimental design. The number of samples in this study was 18 mice divided into 3 groups. The number of samples in each group was added 20% of the initial number of samples as a reserve for mice that died. The sample comparised six female rattus norvegicus mice aged 2-3 months, weighing 150-200 gr. The independent variable was the low glycemic index high protein (LGHIP) diet. The dependent variables were AGEs and Interleukin-6. Examination of blood serum of mice ELISA measurement of AGEs and IL-6 levels. Anova and Post Hoc tests were performed to compare AGEs and IL-6 levels between groups.
Results: LGIHP diet decreased IL-6 levels of PCOS-IR model mice (p= 0.002). There was a significant difference between the K- and P groups in IL-6 levels (p= 0.002). Changes in AGEs levels between groups showed a significant difference (p= 0.015). Post Hoc test for AGEs levels showed significant differences between the K- and P groups (p= 0.023), the K+ group with the P group (p= 0.047).
Conclusion: The LGIHP diet may serve as a potential dietary strategy for managing PCOS-IR. Regulating diet especially carbohydrates and glucose can improve the response to glucose secretion and inflammatory pathways, thereby affecting the significant risk of PCOS severity.

Keywords: low glycemic index high protein diet, AGEs, IL-6, polycystic ovary syndrome.

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BACKGROUND

Polycystic ovary syndrome, commonly known as PCOS is a prevalent endocrine disorder found in women during their reproductive years, affecting approximately 4% to 20% of this population. The key indicators of PCOS include irregular, excessive hair growth, insulin resistance, increased body weight, and metabolic syndrome. The Rotterdam criteria are used for diagnosing PCOS, requiring the presence of at least two out of three indicators: irregular or absent

ovulation, elevated androgen levels, polycystic ovaries identified through an ultrasound, which must show 12 or more follicles ranging from 2 to 9 mm in size or an ovarian volume exceeding 10 mL in at least one ovary.

Previous studies have indicated that women with PCOS often experience inflammation that heightens insulin resistance. Factors such as diet, lifestyle choices, and certain environmental exposures can adversely affect PCOS, directly impacting both fertility and overall health (Saleh et al., 2023). The cause of PCOS remains unclear, yet it has been linked to various theories involving both genetic and environmental influences. These elements can disrupt normal bodily function through various metabolic processes, leading to issues like insulin resistance, irregularities in fat tissues, unusual production of steroid hormones and impairment of the hypothalamic pituitary ovary system (Deswal et al., 2020). PCOS frequently comes with a greater likelihood of having other health issues like metabolic conditions, which include obesity and insulin resistance. Moreover, it is well understood that the condition of PCOS tends to become more severe in the presence of obesity.

Women with PCOS exhibit elevated levels of Tumor Necrosis Factor (TNF) and C-Reactive Protein (CRP), along with increased amounts of monocytes, lymphocytes, and inflammatory cells in ovarian tissue. This ongoing state of inflammation is intensified by obesity and elevated insulin levels. Research has highlighted the mutual impact of high insulin levels and obesity, high androgen levels, and inflammatory conditions (Rudnicka et al., 2021). Cytokines are substance found in the body that assist in cell communication and are created by various cell types, including adipocytes. They also play a role in several biological activities that help attract macrophages. A number of studies have shown that women with PCOS have higher levels of Interleukin-6 (IL-6), Monocyte Chemoattractant Protein-1 (MCP-1), and Macrophage Inflammatory Protein-1α (MIP-1α). Further investigations have revealed that genes associated with interleukins are crucial in the inflammatory response and are involved in functions such as cell growth, differentiation and apoptosis (Pan et al., 2021).

The uncertainty about whether inflammation is a direct effect of PCOS or results from insulin resistance and obesity emphasizes the need to understand the exact procesess behind the increased inflammatory markers in women with PCOS. Several inflammatory markers, such as TNF, CRP, and different interleukins, particularly elevated IL-6 may stem from metabolic problems. IL-6 prompts the liver to produce CRP, which acts as an indicator of inflammation in women with PCOS. Aromatase mainly regulates sex hormone levels during periods of inflammation. Studies indicate that women with PCOS have lower aromatase activity, resulting in higher serum androgen levels (Rudnicka et al., 2021).

Proinflammatory cytokines enhance the activity of aromatase, and inflammatory states transform androgens into estrogens. Additionally, testosterone can suppress aromatase function. The diverse impact of estrogen on inflammation is linked to immune responses. Thus, cytokines and additional elements associated with inflammation can trigger aromatase and alter the estrogen to androgen ratio, including IL-6 and TNF have been shown to stimulate aromatase in different kinds of cells. They are involved in the process of fertility advancement and the attachment of lized eggs in the uterus and their effects can sometimes cause issues that result in miscarriage and difficulty conceiving (Kolanska et al., 2021).

Several scientists have noted earlier that reactive alterations occur in substances known as Advanced Glycation End products (AGEs). AGEs are initiated through non-enzymatic reastions that occur between reducing sugars and amino acids, proteins, fats, or nucleic acids. Women diagnosed with PCOS have shown an increased amount of AGEs as well as a greater presence of receptor for advanced glycation end products (RAGE) (Rudnicka et al.,2021). The interaction between AGE and RAGE triggers multiple inflammatory signaling pathways inside cells, along with pathways related to oxidative stress. This results in tissue harm through a positive feedback mechanism that promotes the regulation of RAGE receptor expression. Studies using immunohistochemistry have indicated that RAGE and AGE modified proteins are expressed differently in the ovaries of women who have PCOS compared to those who do not. In women with PCOS, granulosa cells exhibited a greater expression of RAGE in comparison to theca interna cells. These results imply that this scenario poses a risk since the multi proinflammatory molecule and its receptor, RAGE are harmful and play a significant role in reproductive conditions that lead to ovarian dysfunction (Mouanness et al., 2022).

Advanced Glycation End Products (AGEs) are reactive compounds produced through non enzymatic glycation reactions involving reducing sugars and proteins, lipids, or nucleic acids. These AGEs can build up from external food sources, particularly from foods that are cooked and processed at high temperatures, and are linked to reproductive and metabolic issues commonly seen in PCOS (Mouanness et al., 2022). Women with PCOS who consumed a diet high in AGEs for two months exhibited significantly elevated levels of serum AGEs, androstenedione, and free androgens compared to their counterparts on a low AGE diet. The introduction of AGEs has been shown to impact granulosa cell function, leading to enhanced proliferation of these cells within the ovaries, as well as incease production of pprogesterone which act to inhibits aromaterase and estrogen production. This effect results from a decrease in the regulatory activity of LH/cAMP receptors, resulting in irregular menstrual cycles, an increased number of follicles or cysts, thinning of the granulosa layer, and reduced serum levels of progesterone (Azhary et al., 2020).

Sources indicate that a low carbohydrate diet consists of 40% carbohydrates, 20% protein, and 40% fat. This diet was created in the early 2000s by incorporating various sources of vegetables proteins, nuts, and phytosterols. Improvements in lipid profiles and increase in High Density Lipoprotein (HDL) cholesterol have been observed. Furthermore, additional research verifies that a low carb diet leads to reductions in waist size, fasting glucose, serum insulin levels, and body weight among women diagnosed with PCOS. Thomsom and his team demonstrated that a low carb diet containing 30% protein, 40% carbohydrates, and 30% fat led to a decrease in fat mass and free fat by approximately 2-3 kg over a 20 week period in 94 overweight and obese women with PCOS. Moreover, this diet offers positive effects for various conditions, including type 2 diabetes mellitus, cancer, neurological issues, obesity, and PCOS (Hsu et al., 2022).

As demonstrated, research has validated the beneficial impacts of diets low in carbohydrates and ketogenic diets. Reducing carbohydrate intake in women with PCOS can influence insulin release, leading to a significant decrease in hyperandrogenism. Nonetheless, nutritional approaches that lack distinct underlying therapeutic reason

may vary. To our knowledge, there hasn't been a significant investigation into inflammatory alterations. It's also unclear if this type of diet can be more effective in addressing PCOS. Thus, this study aimed to asses if a high protein, low carbohydrate diet could yield greater positive effects on PCOS by evaluating the levels of serum IL-6 and AGEs.

SUBJECTS AND METHOD

1. Study Design

This study used a true experimental research design with post test only control group design in the laboratory of the Faculty of Veterinary Medicine, Airlangga University in August 2024.

2. Population and Sample

In this study, the subjects were female white mice of the PCOS-IR strain (Rattus norvegicus), approximately 3 months old and weighing between 100 and 200 grams. The number of samples in this study was 18 mice divided into 3 groups. The number of samples in each group was increased by 20% of the initial number of samples as a reserve for mice that died. White mice were chosen for their stable genetics shorter reproductive life span, short estrogen cycles, and ease of handling.

Before the study began, the mice went through a week long acclimatization period to confirm they were healthy, exhibited typical behaviors, and had normal vaginal swab results. Certain criteria led to the exclusion of mice from the study. These included anatomical abnormalities (such as injured or missing ears, shortened or absent tail, legs with deformities thathinder standing, sores on the body, or unclear eyesight) and any signs of pregnancy during the adjustment phase. The mice were divided into three main groups: a normal control group (K-), PCOS-IR mouse model that was fed standard broiler food (K+), and a PCOS-IR mouse model provided with a low glycemic index, high protein diet (LGIHP) (P).

The number of mice in each group was calculated using the Lameshow formula. There was a notable increase in the insulin resistance index in mice receiving testosterone propionate for 14, 21, and 28 days compared to the control group. Vaginal swabs were collected before and after treatment to monitor any changes in the reproductive cycle resulting from the treatment. On the 48th day, the mice were euthanized using ether anesthesia and dislocation of the neck, after which ovaries and blood samples were collected.

3. Study Variables

The the independent variables was low glycemic index high protein (LGIHP) diet, while the dependent vairabel in this study were AGEs and Interleukin-6.

4. Operational Definition of Variables

Low glycemic index high protein (LGIHP) diet emphasizes consumption of foods that have a low glycemic index and are rich in protein sources. The group receiving treatment was given palled feed that consisted of corn flour, which has a low glycemic index for carbohydrates and is made up of 40%. Additionally it contained egg white with a protein content of 30%, along with fish oil that provides omega-3, accounting for 30% fat. This formulation is specifically intended for a diet aimed at managing PCOS-IR.

Advanced Glycation End products (AGEs) are highly reactive compounds created through non enzymatic procesess that involve the bonding of reducing sugars with proteins, lipids, or nucleic acids.

Interleukin-6 is a type of cytokine, which serves as a signaling molecule inflammatory responses in the body.

5. Study Instrument

The Enzyme Linked Immunosorbent Assay (ELISA) was used to detect the amounts of IL-6 AND AGE IN blood obtained from mice's blood vessels. A labeled immunoassay known as ELISA is regarded as the gold standard for immunoassay. At the conclusion of the trial, blood was drawn for AGEs and IL-6, the units obtained are ng/mg. Ratio is the data scale. ELISA analysis was used to measure the level of IL-6 using the Germany Bioassay Kit No. E0177Mo. The AbbVie Cambridge UK Kit No. abx054078 was used for the ELISA test to determine the AGEs level.

6. Data Analysis

This study will documnet research finding using a data gathering from created specifically for this investigation to examine the levels of IL-6 and AGEs in the PCOS-IR mice model. Analysis of variance using ANOVA. Because there were significant differences in variables between groups, the Bonferroni test was performed. Statistical test using SPSS version 22.

7. Research Ethics

The letter approving ethical clearance for research was received from the Research Ethics Committee at the Faculty of Veterinary Medicine, Airlangga University, Indonesia with number 3.KEH.092.06.-2024.

RESULTS

Based on the output of the One-Way ANOVA statistical test above, it was found that the Sig. value was 0.015 <0.05, so it can be concluded that the average OD450 AGEs value of the three treatment groups had a significant difference.

Table 1. One-Way ANOVA Test for AGEs and IL-6

Variables	Variant	Mean difference	p
Test for AGEs	Between Groups	0.036	0.015
	Within Groups	0.006	
Test for IL-6	Between Groups	0.011	0.002
	Within Groups	0.001	

Notes: One Way Anova Test. A Significant difference if p value <0.05. There are 3 groups consisting of K- : a normal control group, K+: a PCOS-IR mice model that was fed standard broiler food, P: a PCOS-IR mouse model provided with a low glycemic index, high protein (LGIHP) diet.

According to the findings from the output of the One-Way ANOVA statistical test above, it was found that the Sig. The p-value was 0.002, so it can be concluded that the average serum IL-6 concentration of the 3 treatment groups had significant differences. Based on the results of the data analysis above (using the Bonferroni test) it was found that there was a significant difference in the OD450 AGEs value between the P1 group and the K- group (p= 0.023) and the K+ group (p= 0.047). The results of the data analysis also showed that there was no significant difference in the OD450 AGEs value between the K- group and the K+ group.

Findings of Bonferroni test, it was found that there was a significant difference in the concentration of IL-6 Serum in the P1 group with the K- group (p= 0.002) and the K+ Group (p= 0.037). The results of the data analysis also showed that there was no significant difference in the IL-6 Serum Concentration in the K- group with the K+ Group (p= 0.437).

Table 2. Post Hoc Test for AGEs and IL-6

Variable	Group 1	Group 2	Mean Difference	p
Test for AGEs	Groups K-	Groups K+	-0.14	1.000
		Groups P1	-0.27	0.023
	Groups K+	Groups K-	-0.11	1.000
		Groups P1	-0.25	0.047
	Groups P1	Groups K-	0.02	0.023
		Groups K+	0.01	0.047
Test for IL-6	Groups K-	Groups K+	-0.01	0.437
		Groups P1	-0.02	0.002
	Groups K+	Groups K-	-0.01	0.437
		Groups P1	-0.01	0.037
	Groups P1	Groups K-	0.01	0.002
		Groups K+	0.01	0.037

Notes: One Way Anova Test. A Significant difference if p value <0.05. There are 3 groups consisting of K- : a normal control group, K+: a PCOS-IR mice model that was fed standard broiler food, P: a PCOS-IR mouse model provided with a low glycemic index, high protein (LGIHP) diet .

DISCUSSION

The findings from the ANOVA analysis on AGEs levels showed a reduction following the introduction of a low carbohydrate, high protein diet in mice modeled for PCOS with insulin resistance. With a p= 0.015, indicates a significant difference in the provision of the diet. Adjustments in AGEs concentrations following the administration of a low carb, high-protein diet represent one of the uses of lifestyle changes, like diet and exercise, which are suggested as primary treatment for women with PCOS.

Treatment today takes into account both changes in lifestyle and medication, and it should be customized each individual (Armanini et al., 2022). Hypocaloric diets are said to be effective in regulating insulin secretion, the endocrine system, lipid levels and reducing weight and improving the menstrual cycle (Najafabadi et al., 2023).

Cooking food at high temperatures, eating fast food, and consuming high calorie meals can lead to a rise in the amount of Advanced Glycation End Products (AGEs) in the bloodstream. This increase plays a role in the development of ovulation issues. The disruption of the hypothalamic pituitary ovarian axis further contributes to the causes of Polycystic Ovary Syndrome (PCOS) due to elevated AGEs levels. The interaction between AGEs and their receptor RAGE activates numerous inflammatory and oxidative stress pathways that result in tissue harm.

Such damage also affects the positive feedback that normally enhances the regulation of RAGE receptors. In women with PCOS, granulosa cells exhibit a higher expression of RAGE compared to theca cells, which is significant in the context of reproductive disorders that can lead to ovarian dysfunction (Mouanness et al., 2022). AGEs are very reactive compounds that can enter the body through food or be produced internally as a result of metabolic activities. The accumulation of AGEs tends to rise with age, high blood sugar, insulin resistance, and a diet rich in glycotoxins (Tatone et al., 2021). The reduction of AGE levels observed in this research aligns with multiple studies indicating that a diet low in AGEs contributes to decreased levels of these compounds, as well as enhancements in metabolism, hormones, and markers of oxidative stress in women with PCOS.

Post hoc examination of AGE levels in this research sought to assess the variations between the groups, which was underscored by implementing a low carb, high protein

dietary approach. The findings revealed a significant distinction between the K- and P groups, with p value 0. 023, as well as between the K+ and P groups, which had a p value 0.047. Nevertheless there was no noteworthy difference found in the K- and K+ groups with p-value exceeding 0.005, specificially at 1.000. The primary advantage of dietary intervention is its impact on insulin sensitivity. The consumption of carbohydrates influences blood sugar levels after meals. Moreover, a low carbohydrate diet has anti inflammatory properties and is and id related to hormonal and metabolic profiles. It is crucial to track the specific diet that is likely to promote weight reduction, androgen levels, and fertility in women with PCOS. The type and amount of carbohydrates ingested are essential aspects of the diet for those with PCOS.

Incorporating complex carbohydrates and foods high in unsaturated fats has been shown to mitigate chronic inflammation, enhance metabolic function, reduce insulin resistance, and address hormonal imbalances in PCOS (Calcaterra et al., 2023). The Association of Clinical Endocrinologists and the American College of Endocrinology suggest that women with PCOS should aim for a weight loss of 5-10% or more to enhance ovulation, normalize menstrual cycles, lessen hirsutism, boost insulin, sensitivity, and lower androgen levels (Moore et al., 2021). Consequently, adopting a calorie-restricted diet is seen as a crucial dietary approach for individual with PCOS. Studies on different dietary modifications like low-fat diets, low carb diets, high protein diets, and ketogenic diets have shown a notable effect on central obesuty among these individuals by affecting insulin levels and fat metabolism (Mei et al., 2022).

Reproduction ASRM the initial approach to treating PCOS involves modifying one's lifestyle, which includes managing diet and increasing physical activity. Changes in diet have proven effective in alleviating symptoms associated with PCOS, such as irregular periods, hormonal imbalances, and issues with ovulation. It's crucial to manage macronutrient intake, particularly carbohydrates, as a diet consisting of 10% carbs can stimulate AMPK and SIRT-1, leading to positive effects on blood sugar levels, inflammation, and reproductive health (Najafabadi et al., 2023). A low carbohydrate diet has been linked results similar to a high protein dietary models in earlier research focused on PCOS, revealing differences in lipid profiles (Mei et al., 2022).

There was a significant difference in the results of the post hoc test on IL-6 levels between groups in this study with a p value <0.005, namely the K- group with the P group (p = 0.002) and the K + group with the P group (p = 0.037), while the K- group with the K + group p value > 0.005, namely 0.437, stated that there was no significant difference. t is believed that a diet low in carbohydrates and high in protein may enhance the inflammatory pathway. The study demonstrated that providing a surplus of fat leads to a decrease in carbohydrate oxidation, while fat oxidation remains largely unaffected. When there is an overconsumption of carbohydrates, it leads to an increase in fat storage via lipogenesis, resulting in lipid build up in different tissues, which can lead to cellular damage, cell death, and the release of inflammatory substances from monocytes in fat tissue. Following this, increased intake of glucose and saturated fats can worsen metabolic and reproductive issues that heavily influence the onset of PCOS (Calcaterra et al., 2023).

Diet significantly influences the management of ongoing inflammation. Inflammation triggered by diet is linked to insulin resistance and elevated androgen levels,

which are key factors in PCOS. Research indicates that certain food elements, such as glucose, can ltrigger chronic inflammation via oxidative stress. The Diet Inflammatory Index (DII), created by Shivappa et al, offers a numerical measure to categorize individuals based on their dietary patterns' potential to cause inflammation. This index looks into how various food elements correlate with inflammatory markers such as IL-1, IL-4, IL-6, IL-10, TNF, and CRP. On the other hand, a diet that promotes inflammation includes high amounts of saturated fats, grains, fruit juices, red meat, processed meats, sugary drinks, sugar, and honey. Recent research has shown that elevated values on the dietary inflammatory index are linked to an increased likelihood of developing PCOS.

Lifestyle interventions strategies, particularly focusing on weight loss and physical activity, are essential components in the management of women with PCOS. Changes in diet composition aimed at managing, treating, and even preventing PCOS have gained significant focus recently. Consequently, identifying an appropriate diet is crucial, particularly for women affected by PCOS (Azarbayjani et al., 2024). The results align with recent studies indicating a decrease in inflammatory markers such as IL-6 and AGEs among women with PCOS. Adjusting dietary intake, particularly concerning carbohydrates and glucose, can enhance the body's response to glucose release and inflammatory processes, thereby influencing the minor risk associated with the severity of PCOS. Tailoring a diet to fit the individual circumstances of each person could be a subject for future studies.

AUTHOR CONTRIBUTION

Hany Puspita Aryani: conceptualization, writing original draft, and validation; Sugiyanta: methodology, writing original draft, and writing review and editing; Eliza Zihni Zatihulwani: investigation, resources, supervision.

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CONFLICT OF INTEREST

There was no conflict of interest in this study.

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