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Review of Decreased IL-6 Levels in SOPK-Insulin Resistant Mice Fed a Low Carb High Protein Diet

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Abstract

Polycystic ovary syndrome with insulin resistance (SOPK-RI) is caused by endocrine disorders with an incidence rate of 5-10% that occurs in women of reproductive age. Characteristics of polycystic ovary syndrome are anovulation, hyperandrogenic, polycystic ovary, and impaired insulin sensitivity involving proinflammatory increases in several inflammatory cytokines including interleukin-6 (IL-6). Increased inflammatory cytokines in women with SOPK-RI found health problems there is fat accumulation in adipocyte tissue such as in an obese person, metabolic syndrome, and type 2 diabetes. The purpose of this study was to determine the effect of a low-carbohydrate high-protein diet on changes in IL-6 levels in SOPK-RI model mice. Laboratory experimental research, namely post-test only control group design. Consisting of three groups, namely, the negative control group (K-) as a normal group that did not get treatment; The positive control group (K+) of the SOPK-insulin resistance model mice were given standard feed, and the treatment group (P) of the SOPK-insulin resistance model mice were given a low-carbohydrate high-protein diet (CTR) with a composition of 40% carbohydrate and 30% protein. The study lasted for 48 days, using serum ELISA examination on IL-6 levels. The average results of IL-6 levels, namely the K- = 0.358 group, K+ = 0.387 and P = 0.442 and obtained different IL-6 levels of significance value p = 0.002 with a low-carbohydrate high-protein diet in SOPK - RI mice showed a significant difference ($p < 0.05$) between the control group and the treatment group. Significantly reduced IL-6 levels in SOPK-RI model mice. A low-carb, high-protein diet may be considered a dietary modality in women with SOPK-insulin resistance. Further research is needed to better determine the other modulators involved in this mechanism in more detail.

INTRODUCTION

Changes in interleukin-6 (IL-6) levels in women with polycystic ovary syndrome (SOPK) are not fully understood, but some sources suggest a relevant link to increased inflammatory cytokines in metabolic disorders and endocrine disorders. According to Rotterdam SOPK is a complex syndrome that is often characterized by hyperandrogens, a comparison of LH levels: FSH, hyperinsulin, anovulation, amenorrhea, acne and hirsutism (hoeger). Obesity is found to be around 50%-60% to be the cause of SOPK and these findings are supported by several evidences. It was shown that obesity increased the risk of SOPK and increased interleukin-6 (IL-6) and other inflammatory factors were seen in women with SOPK (Mazloomi dkk., 2023). Increased insulin resistance and obesity worsen inflammatory conditions (Aryani dkk., 2020).

Changes in diet that lead to the need for high calories and fast food, consumption of food (calories) including carbohydrates every year shows an increase. The addition of calories is known to cause metabolic disorders, lipid disorders, oxidation disorders and impaired use of fatty acids. Some researchers explain glucose uptake disorders, mutations/polymorphisms of GLUT-4 production, insulin receptors, mitochondrial DNA and mitochondrial dysfunction. The influence of the role of increasing proinflammatory cytokines, increasing adipose tissue, increasing free radicals is the pathogenesis of insulin resistance (Khodaeifar dkk., 2018).

Hyperinsulin states in development can cause oxidative stress, increasing the formation of Reactive Oxygen Species (ROS) production (Shan dkk., 2022). The increased formation of ROS can also be due to increased activation of oxidative stress signaling pathways induced by increased glucose and free fatty acid (FFA) (Kordestani dkk., 2018) which can activate (Nuclear Factor –KB) NF-KB and the activity of Protein Kinase C (PKC) and p38 Mitogen Activated Protein Kinase (p38 MAPK) (Shan dkk., 2022).

Some markers as chronic indicators of inflammation such as Tumor Nuclear Factor-α (TNF-α), C-reactive Protein (CRP) and various interleukins (IL-6 and IL-18) (Rudnicka dkk., 2021). Interleukins are multifunctional cytokines that play an inflammatory role in the reproductive system, stimulating the release of progesterone and estradiol and promoting follicular development (Silva dkk., 2020). In addition, several studies have shown that IL-6 as one of the pro-inflammatory cytokines that participate in the development of insulin resistance and type 2 diabetes mellitus thus the possible relationship of metabolic changes in SOPK (Aboeldalyl dkk., 2021).

Low-carbohydrate diets have become a trend in several studies and are used as one of the management in patients with type 2 diabetes mellitus or insulin resistance, which can reduce blood sugar levels, metabolic systems, hormonal and oxidative stress in women with SOPK (Aryani dkk., 2020).

Dietary recommendations on SOPK with Iinsulin Resistance (SOP-RI) with recommended intake of 40% carbohydrates, 30% protein, and 30% fat are in line with the Mediterranean diet pattern on PCOS (Aryani dkk., 2020; Barrea dkk., 2019) were found to reduce glucose levels, increase glucose uptake and improve insulin sensitivity. The addition of protein intake as a balancing composition of food intake needs, where increasing the amount of protein intake can stimulate insulin secretion, beneficial anabolism has the effect of causing satiety and also stimulates glucagon secretion from α cells from the pancreas and stimulates through the process of gluconeogenesis thereby increasing insulin sensitivity (Aryani dkk., 2020).

Until now, the effect of a low-carbohydrate high-protein diet on patients with SOPK-RI on changes in IL-6 levels is needed, so further research is needed to determine the effect of a low-carbohydrate, high-protein diet on changes in IL-6 levels in PCOS-RI model mice. This diet is one way to overcome this syndrome by managing PCOS-RI with a low carbohydrate diet composition of 40% and high protein 30%.

RESEARCH METHODS

This research is a true experimental laboratory research with a research design, namely post test only control group design. The experimental unit in this study was a female white mouse model SOPK-RI (Rattus norvegicus), aged 3 months, weighing 100-200 grams. SOPK-RI modeling was obtained through a 0.1ml testosterone propionate injection protocol intramuscularly once a day for 28 days (Beloosesky dkk., 2004). The determination of the size of the rat sample in each group was calculated based on the formula of Lemeshow (1997). In this study for the treatment group, pellet feed using corn flour reflected the low glycemic index content for carbohydrates with a composition of 40%, egg whites containing protein with a composition of 30% and fish oil containing omega-3 with a composition of 30% fat (Moosheer dkk., 2014) as a composition of a polycystic diet of ovaries with insulin resistance (carbohydrates 40%, protein 30% and fat 30%) (Czyżewska-Majchrzak dkk., 2014).

In this study the sample of each group was added 20% so that the number of samples of each group was added as much as 2 tails for the risk of death during the study, so for each group a minimum of 8 mice were needed. In this test, 3 (three) groups were used, namely (1) negative control group (K-) rats injected with placebo for 28 days and given broiller pellet feed for 20 days, (2) positive control group (K+) SOPK-RI rats (testosterone propionate injection for 28 days), and given broiller pellet feed for 20 days, and (3) treatment group (P) namely SOPK-RI rats given high-protein low-carbohydrate

diet pellet feed with a composition of 40% carbohydrates, 30% protein and 30% fat (RMA, 2012 and Itziar, 2010) for 20 days. Making a SOPK-RI mouse model in (Osuka dkk., 2019) stated that making an SOPK model with TP induction for 21 subcutaneous days can mimic several aspects of SOPK-RI, changes in ovarian histology, as a result of which ovarian volume becomes smaller and inhibition of ovarian function by androgens. Is characteristic of polycystic changes of the ovaries, endocrine and metabolic disorders (Osuka dkk., 2019). Measurement of IL-6 levels was carried out by ELISA examination using Germany Bioassay Kit No. E0177Mo. Data analysis with statistical normality test, homogeneity test, Anova test and Bonferroni post hoc test. The significance level is set at 5%. This research has passed the ethical feasibility test by the ethics committee at Airlangga University, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia with certificate number 2.KE.062.04.2019.

RESULT

This study was to determine the effect of a low-carbohydrate high-protein diet on changes in IL-6 levels in SOPK-RI model mice. The results of the study averaged IL-6 levels of the K- group: 0.358, K + : 0.387, and P : 0.442 with an anova test significance value p = 0.002. While the post hoc test of group K- and group K + p value = 0.488, group K- and group P value p = 0.002, while in group K + and group P value p = 0.038. The study showed that there was a significant difference in the provision of a low-carbohydrate high-protein diet (CTR) with a significance value of p < 0.05 was in group K- and group P and group K + and group P.

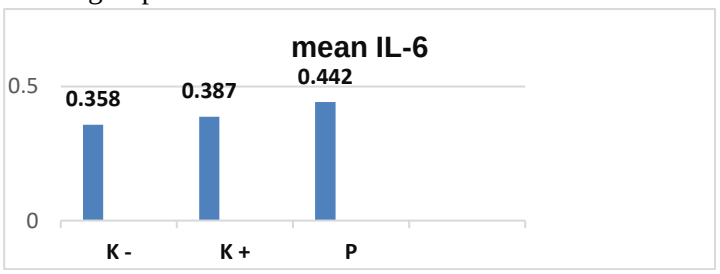

Figure 1. Average IL -6 levels

Source : Hany Puspita Aryani, Dwi Retno Wati and Sugyanta (2023)

In figure 1 the study showed that the average IL -6 levels of K group - 0.358, IL -6 levels of K + 0.387 group and IL -6 levels of P group 0.442. Average group K - < group K + and < group P. This study after giving the CTR diet in group P tended to increase IL-6 levels, but the increase was not too high compared to group K - .

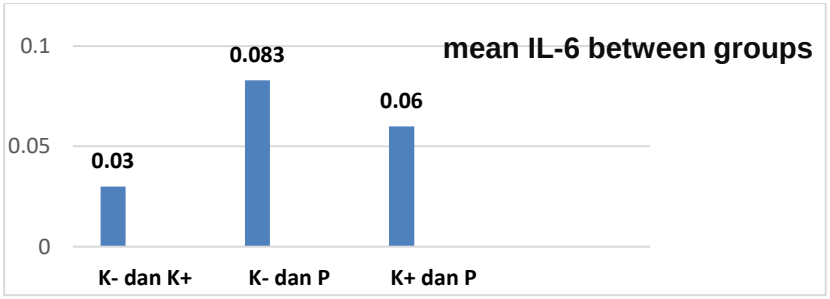

Figure 2. Comparison of mean IL-6 levels between groups

Source : Hany Puspita Aryani, Dwi Retno Wati and Sugyanta (2023)

Figure 2 in this study shows that the average score of IL-6 levels in groups K - and K + = 0.03; groups K - and P = 0.083; and groups K + and P = 0.06. Where IL-6 levels in the K+ and P groups tend to be lower than in the K- and P groups although in the K- and P groups are lower than the K+ and P groups.

Data normality analysis is carried out to determine whether the research data obtained follows or is close to the normal distribution, namely the distribution of data with bell shape. Table 1.1 data were conducted normality test using Kolmogorov smirnov in the control group and treatment group.

Table 1. Analysis of normality Kolmogorov smirnov

Group	SD	Mean	Minimum	Maksimum	p
K-	0,020	0,358	0,321	0,378	0,200*
K+	0,348	0,387	0,345	0,449	0,200*
P	0,420	0,442	0,395	0,516	0,200*

Remarks : p = significance, which is marked * normally distributed p>0.05

In Table 1 above shows the normally distributed data of IL-6 levels in the three groups showing the distribution of normally distributed data of 0.200 (p > 0.05), so statistical analysis was carried out using oneway Anova to determine whether there were differences between groups and then continued Post Hoc Benferroni analysis to find out which average pairs were the most different between the existing pairs of the group.

Table 2 Anova and Post Hoc Analysis

Group	Anova	Post Hoc				
	p	Grup	Interval 95%		Group Mean	p
			Batas bawah	Batas atas		
K-		K+	-0,081	0,024	-0,03	0,488
		P	-0,14	-0,031	-0,083	0,002
K+		K-	-0,024	0,080	0,03	0,488
		P	-0,11	-0,003	-0,06	0,038
P		K-	0,031	0,14	0,083	0,002
		K+	0,003	0,11	0,06	0,038
	0,002					

Remarks : p = significant with p <0.05

In table 2 of the results of the Anova analysis there were significant differences between groups with p values = 0.002 (p < 0.05) and what were the results of Post Hoc analysis comparisons of IL-6 levels between K- and K + groups p values = 0.488 showed no significant differences in giving a low-carbohydrate high-protein diet. In group K + and group P value p = 0.038 showed a significant difference in giving a low-carbohydrate high-protein diet, as well as in group K - and group P the value of p = 0.002 showed a significant difference in giving a low-carbohydrate high-protein diet

DISCUSSION

Polycystic ovary syndrome (SOPK) causes health problems related to the accumulation of adipose tissue, such as insulin resistance, obesity, metabolic syndrome and type 2 diabetes mellitus. Several studies have reported an association between SOPK and chronic inflammation with increased levels of C-reactive protein (CRP), interleukin-6 (IL-6), interleukin-8 (IL-8), tumor necrosis-α factor (TNF-α) and macrophages. Hyperinflammatory states can arise from the mutual effects of hyperinsulinemia, hyperandrogens or obesity that cause oxidative stress and are responsible for fertility, pregnancy complications and infertility.

Carraro et al, (2018) stated a low-carbohydrate or high-protein diet should be considered as a serious treatment option for all insulin resistance patients with obesity and without obesity. That low-carbohirate diet is high in protein has been prescribed since 1979 and is taken seriously by many researchers, although still some consider it skeptical. Hyperphagia leads to impaired positive energy balance and weight gain. People with insulin resistance tend to increase weight easily and have

difficulty losing weight on a diet with normal carbohydrate content, because very high insulin levels due to disruptions in the endocrine system and metabolic pathways that will affect oxidative stress result in an increase in the inflammatory pathway. Increased proinflammatory cytokines such as TNF-α, IL-6 interfere with insulin receptor signaling involved in glucose and lipid metabolism (Carraro dkk., 2018).

The average high IL-6 levels in group P compared to group K - and group K +, the results of the study still tend to increase likely because the time of administration of the RKTP diet is too short so that it has not been able to suppress the inflammatory pathway induced TP for 28 days so that it has not been able to suppress IL-6 levels which may increase with the severity of insulin resistance and androgen status is possible as illustrated by SOPK-RI modeling. However, the results of post Hoc p analysis < 0.005 in groups K- and P p value = 0.002, group K + and P p value = 0.038. As per the research of Rehman and Akash (2016), the state of insulin resistance is directly related to various inflammatory responses (TNF-α, IL-6, and NFKβ) that play an important role in the development of insulin resistance (Rehman & Akash, 2016).

The average results between groups of low-carbohydrate high-protein diets (CTR) in this study obtained IL-6 levels for anova analysis p = 0.002. Decreased IL-6 levels between K- and P groups compared to K + and P, that the provision of the RKTP diet has a positive effect on SOPK and can be considered the provision of the RKTP diet as a modulation of SOPK-RI management interventions caused by lifestyle. In accordance with Rehman and Akash (2016), the state of insulin resistance is directly related to various inflammatory responses (TNF-α, IL-6, and NFKβ) that play an important role in the development of insulin resistance (Rehman & Akash, 2016).

Proinflammatory cytokines play a major role in responding to inflammatory stimulation and tissue damage, IL-6 and TNF-α are cytokines primarily produced by the immune system and various types of adipocyte tissue, hepatocytes and ovarian granulosa cells. TNF-α levels essentially regulated by NFKβ and IL-6 factors cause a stimulus to regulate protein synthesis, activate the hypothalamic-pituitary axis and disturbances in glucose transduction through alterations in serine-theronin kinase activity. Proinflammatory cytokines IL-6 and TNF-α play a role in the pathogenesis of chronic inflammation and insulin resistance in SOPK.

Studies report serum concentrations of IL-6 in women with SOPK with insulin resistance. Interleukin-6 (IL-6) is not characteristic of SOPK, it can be interpreted that IL-6 levels are not a biomarker for the diagnosis of SOPK, but have a potential effect on SOPK with increased inflammation. A low-carb, high-protein diet for 20 days may not have been able to suppress the increase in intramuscular oxidative stress influenced by testosterone propionate injection in this study. Decreased levels of IL-6 in group P compared to group K+ can relieve oxidative stress. Several studies have reported associations between SOPK and chronic inflammation of interleukin-6 (IL-6), tumor necrosis factor (TNF-α), interleukin-8 (IL-8), and macrophages, in addition to women with SOPK also experience increased AGEs and increased RAGE. This chronic inflammation is further aggravated by hyperinsulinemia, hyperandrogenism and inflammatory states. Endothelial cell dysfunction may also be triggered by inflammatory cytokines (Zuo dkk., 2016).

Inflammatory response is one of the main causative factors for the etiology of type 2 diabetes mellitus (DMT2), in summary that proinflammatory mediators, oxidative stress, molecular pathways and metabolic pathways affect cells and tissues which is the pathogenesis of insulin resistance. The best treatment to prevent the pathogenesis of insulin resistance is to improve the increase in proinflammatory response (Rehman & Akash, 2016).

Carraro (2018) states a low-carbohydrate or high-protein diet should be considered as a serious treatment option for all insulin resistance patients with obesity and without obesity. That low-carbohirate diet is high in protein has been prescribed since 1979 and is taken seriously by many researchers, although still some consider it skeptical. Hyperphagia leads to impaired positive energy

balance and weight gain. People with insulin resistance tend to increase weight easily and have difficulty losing weight on a diet with normal carbohydrate content, because very high insulin levels due to disruptions in the endocrine system and metabolic pathways that will affect oxidative stress result in an increase in the inflammatory pathway. Increased proinflammatory cytokines such as TNF-α and IL-6 interfere with insulin receptor signaling involved in glucose and lipid metabolism (Carraro dkk., 2018).

A low-glycemic index diet occurs glucose breakdown where the accumulation of glycogen stores in the liver and muscles is not more dominant, thus improving insulin sensitivity by suppressing insulin secretion so that insulin pathway signals increase and inhibit the proinflammatory secretion of TNF-α in serum and ovarian tissue cells (Szczuko dkk., 2019).

The combination of high-protein, low-carbohydrate (low-glycemic) foods leads to improved insulin sensitivity. In harmony in Wu et al, (2020) SOPK inflammation with insulin resistance was found to have increased activity of proinflammatory cytokines TNF-α, IL-6 and IL-8. This increase increases the formation of ROS production and causes oxidative stress states so that inhibiting PI3K has an impact on insulin sensitivity through GLUT-4 which increases insulin secretion, and increases FFA (Wu dkk., 2020). The involvement of ROS in women of childbearing age with SOPK caused by insulin resistance and obesity triggers oxidative stress to have an impact on reproductive health in ovarian tissue. Oxidative stress states increase ROS production, where ROS has a major role in pathological damage to the reproductive system such as growth, follicular development and oocyte maturation as well as pregnancy. especially in pathological damage in the reproductive system (Shishehgar dkk., 2019).

A diet low in glycemic index balanced with high protein will undergo a proteolysis process or amino breakdown occurs in the fulfillment of energy sources Adenosine triphosphate (ATP) obtained in addition to carbohydrates, in a high-protein diet there is a down regulation of mRNA gene expression and causes a decrease in lipolysis, increased lipogenesis so as to decrease fat stores, and causes obstacles to inflammatory pathways that lead to repair of insulin pathways. In a high-protein diet, the process of gluconeogenesis occurs through the uptake of energy stores other than carbohydrates, high protein increases protein synthesis in muscles, adipocyte tissue and liver resulting in the formation of mammalian target of rapamycin (mTOR) (Mirabelli dkk., 2020) is a gene product or threonine kinase that is involved in controlling in regulating the processes of cell growth, proliferation, metabolism and angiogenesis. mTOR signals can be found in theca cells and granulosa cells to proliferate and differentiate to promote follicular growth and development (J. Liu dkk., 2018). The combination of high-protein, low-carbohydrate (low-glycemic) foods leads to improved insulin sensitivity. Aligned in Wu et al, (2020). SOPK inflammation with insulin resistance was found to have increased activity of proinflammatory cytokines TNF-α, IL-6 and IL-8 (Wu dkk., 2020). This increase increases the formation of ROS production and causes a state of oxidative stress so that inhibiting PI3K has an impact on insulin sensitivity through GLUT-4 which increases insulin secretion, and increases FFA which is illustrated in group P IL-6 levels higher than group K +, but it can be explained that the RKTP diet tends not to be able to inhibit the inflammatory pathway which is the mechanism of SOPK-RI (Aryani dkk., 2020).

The results of this study conducted by group P with K- can be proven that there is an effect of giving the RKTP diet on reducing IL-6 levels so that it has a positive effect on SOPK, although it has not been able to reach normal limits. The involvement of ROS in women of childbearing age with SOPK caused by insulin resistance, obesity triggers oxidative stress to have an impact on reproductive health in ovarian tissue. Oxidative stress increases ROS production, where ROS has a major role in pathological damage to the reproductive system such as growth, follicular development and oocyte maturation during pregnancy. especially in pathological damage in the reproductive system (T. Liu dkk., 2021). ROS and oxidative stress are triggers of insulin resistance and SOPK, SO interferes with

glucose uptake in muscles and adipose tissue and reduces insulin secretion from pancreatic β cells. Increased proinflammatory expression through receptors was found in ovarian tissue in SOPK women (Rostamtabar dkk., 2021).

The results in this study, we suggest in women with insulin resistant polycystic ovary syndrome (SOPK-RI), where IL6 elevation can damage the feedback system of the inflammatory process or vice versa. The data is according to a study by Luotola, 2016 which explains the increase in IL-6 levels in SOPK women. The increase can be caused by lack of ovulas (onvulation) in SOPK-RI women. Regulation of IL-6 adrenal hormone led to significant cytochyma reduction in the serum of the same SOPK-RI woman in experimental Bosmann, 2013. Disrupted cytokines play a major role in the immunopathogenesis of SOPK-RI (Mazloomi dkk., 2023).

CONCLUSION

This study confirms that SOPK-RI is an inflammatory syndrome with metabolic abnormalities that may be associated with dysfunction in neuroimmunity. A low-carb, high-protein diet can reduce IL-6 levels in SOPK-insulin resistance models compared to SOPK-insulin resistance model mice given standard feed.

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