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The Effect of Low-Calorie High Protein Diet on Insulin, TNF- α and P38MAPK Levels in Insulin-Resistant PCOS Mice Models

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ABSTRACT

Introduction: Polycystic Ovary Syndrome (PCOS) is found in 33% -38% of women who are overweight. The characteristic correlation of PCOS with insulin resistance, hyperandrogenemia and chronic inflammation such as tumor necrosis factor-α (TNF-α) involves activity of the mitogen protein signaling pathway (MAPK). Calorie restriction or a low carbohydrate diet for women with PCOS is beneficial for their hormonal and metabolic profile.
Objective: The purpose of this study is to determine the effect of low carbohydrate high protein diet (LCHP) in reducing insulin levels, TNF-α levels and p38MAPK expression.
Methods: This study used PCOS mice models which were divided into three groups: K- (untreated mice given standard feed), K+ (PCOS-IR mice model given standard feed) and P (PCOS mice model given the LCHP diet) for 20 days. The PCOS mice model was obtained from the injection of testosterone propionate for 28 days. Insulin and TNF-α levels of the blood serum were measured using ELISA and p38MAPK examination was done using immunohistochemicals on ovarian tissue. **Results:** Results showed that there was a significant difference of insulin and TNF-α levels between the group given the LCHP diet and the other groups (p <0.05). In contrast, the expression of p38MAPK showed a negative effect on the LCHP diet (p >0.05).
Conclusion: The findings in this study could potentially be due to the activity of the MAPK pathway caused by oxidative stress in cells that effects p38MAPK activity, in which it is still unclear whether oxidative stress causes the occurance of kinase activation or not. However, it has no effect on insulin signaling pathways and the proinflammatory cytokine TNF-α.

Keywords: Low calorie high protein diet (LCHP), insulin resistance, PCOS, insulin levels, TNF- α and p38MAPK expression.

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INTRODUCTION

Polycystic ovary syndrome (PCOS) is a metabolic and endocrine disorder characterized by amenorrhea, oligomenorrhea, anovulation, hirsutism, nigrican acantosis and infertility found in 5-10% of women of childbearing age all across the world[1]. It is believed that there is an interaction between insulin resistance, hyperandrogenemia, chronic inflammation and oxidative stress in PCOS[2].

Polycystic ovary syndrome (PCOS) is a gynecological disorder that causes reproductive issues in women of childbearing age[3]. The pathophysiology of PCOS patients is known to show an increase in androgen compensation from hyperinsulinemia due to insulin resistance, which causes changes in steroid biosynthesis that affects steroidogenic enzymes[4]. There is an increase in luteinizing hormone (LH), hyperandrogenemia, chronic anovulation such as oligomenorrhea and infertility[5]. Many researchers found that the risk of PCOS in women of childbearing age may increase due to genetics, body weight, obesity, insulin resistance, cardiovascular disease, hypertension, dyslipidemia, poor eating habits and a lifestyle with lack of exercise[5,6]. Although the mechanism underlying the pathogenesis of PCOS is still difficult to understand, women of childbearing age with PCOS may have changes in sex steroid hormones that affect ovarian secretion or changes in the adrenal that stimulate the metabolism of estrogen and androgens in the ovaries. In addition, sex steroids also affect fat metabolism of estrogen, and androgen is a pathogenesis of PCOS[7].

Insulin resistance is caused by the condition where the amount of calorie intake exceeds the body's need, along with lack of exercise. Limiting the number of calories or a low-carbohydrate diet is believed to be able to increase insulin sensitivity and glucose uptake, thereby reducing insulin levels, increasing sex hormone binding globulin (SHBG) in the liver, suppressing ovarian androgen production which increases aromatase in granulosa cells so that folliculogenesis occurs[8,9].

The substrate receptor insulin signaling pathway (IRS) plays a role in steroidogenesis. With increased oxidative stress, various protein kinases are activated to induce IRS serine/threonine phosphorylation and inhibit IRS tyrosine phosphorylation, reducing the capacity of the IRS to bind with insulin receptors and activate phosphatidyl inositol-3 kinase (PI3K). Because of this, insulin signal to the effector via the IRS to activate PI3K is disrupted. The insulin signaling pathway can also be activated by oxidative stress through the Jun N-terminal kinase (JNK) pathway/mitogen protein kinase activation (MAPK)[10], as well as the IKKβ inflammatory pathway. Nuclear factor Kβ (NFKβ) causes insulin resistance through disruption of insulin receptors[11]. However, the involvement of low carbohydrate high protein (LCHP) diet with insulin levels, TNF-α levels and p38MAPK expression on the pathogenesis of PCOS cases with insulin resistance has yet to be explored with more detail. Because of this reason, further research and investigation is needed.

MATERIALS AND METHODS

Research Design

The design used in this research was true experimental, using posttest only control group design. This study used female rats (*Rattus norvegicus*) with body weight of 100-200 grams, each group consisting of 6 mice, thus requiring a total of 18 mice. Testosterone propionate was injected intramuscularly with dose of 0.1 ml for 28 days to create insulin resistant PCOS mice models[12]. 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. Three groups were used in this research, namely, the negative control group (K-) as the normal group not given treatment, the positive control group (K+) insulin-resistant PCOS mice models given standard feed and the treatment group (P) insulin-resistant PCOS mice models (PCOS-IR) given a low carbohydrate high protein diet (LCHP). The LCHP diet refers to RMA (2011), consisting of 40% of carbohydrates, 30% of protein and 30% of fat for 20 days[13]. After the 48th day, surgery was performed to collect serum through the heart and collect ovarian tissue of the mice. Serum was analyzed using ELISA to obtain insulin (Thermo Scientific USA insulin kit No. 743021419) and TNF-α (Kit TNF-α Bioassay Germany No. E0177Mo) levels. Analysis was conducted in the Embryology Laboratory of the Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia.

Measurement of insulin and TNF- alpha levels using ELISA

Serum was obtained from the heart of *Rattus norvegicus* on the 48th day post treatment. The examination of insulin and TNF-alpha levels was carried out using ELISA (Thermo Scientific, USA No. 743021419) and Horseadish geroksidase enzyme to label antibody. Materials must be attached to the solid phase (microtiter well) prior to detection using ELISA. The material (Ag/Ab) that attaches to the microtitter well should be coated with polysterine/polyvinylchloride[14].

Examination of Immunohistochemical (IHC) p38MAPK expression

Examination of p38MAPK expression was done using an immunohistochemical test (IHC) on ovarian tissue, which was then observed using a microscope with 400× magnification. The observation of p38MAPK expression was done by measuring the percentage of immunoreactive cells with color intensity score on immunoreactive cells through observation of 5 (five) different fields of view.

Data Analysis

Anova test analysis was performed to determine the difference between groups and followed up with the Post Hoc Least Significance Different (LSD) test as a reference in determining whether the average of the two treatments was statistically different or not. Data normality analysis was performed to determine whether the research data obtained in this research followed or approached normal distribution, namely the bell-shaped data distribution. Normally distributed data (p >0.05) was analyzed with ANOVA analysis to determine whether there was a difference between groups, then continued with Post Hoc analysis to determine the mean pair with the greatest difference among the other pairs of the group.

RESULTS

This study aims to determine the effect of low carbohydrate high protein diet on reducing insulin levels. All variables of insulin levels, TNF-α levels and p38MAPK expression were tested using normality analysis. Normally distributed data (p >0.05) was analyzed further using Anova test to understand the difference between groups and Post Hoc Least Significant Difference (LSD) test to specifically clarify the difference between each group. This study used a significance level of 0.05 (5%), so that statistical test results with p-values of p <0.05 is said to be significant, indicating a significant effect, whereas p >0.05 is said to be insignificant, indicating no effect.

Table 1. Kolmogorov-smirnov normality test.

Variable	Sample	*Mean*	Minimum	Maximum	*p*
Insulin Level	18	14.87	10.59	23.62	0.200*
TNF-α Level	18	45.65	36.85	54.13	0.064*
p38MAPK Expression	18	2.30	0.80	3.20	0.154*

Note: p = significance, * indicates normal distribution p >0.05.

Table 1 above shows that the data is normally distributed (p >0.05). Data was then analyzed further with Anova analysis.

Table 2. TNF-α levels, insulin levels and p38MAPK expression in this research.

Group	TNF-α (ng/ml)	Insulin (μU/ml)	p38MAPK expression (Score/cell count in 5 fields of view)
K-	50.43 ± 1.26	18.21 ± 1.10	2.65 ± 0 .16
K+	44.48 ± 2.25	12.66 ± 0 .81	2.28 ± 0 .19
P	42.06 ± 1.89*	13.75 ± 0 .31*	1.98 ± 0 .30
(K-) + (K+)	5.94 ± 2.61*	5.54 ± 1.15*	0.36 ± 0.32
(P) + (K-)	--8.36 ± 2.61*	-4.45 ± 1.15*	-0.66 ± 0.32
(K+) + (P)	2.42 ± 2.61	-1.09 ± 1.15	0.30 ± 0.32

Note: K- (negative control group or normal group), K+ (positive control group, PCOS-IR mice models given standard feed) and P (treatment group, PCOS-IR mice model given LCHP diet). Data is presented as mean ± SE. * indicates a significant difference between the positive control group (K+) and treatment group (P) (p <0.05).

Analysis of Insulin Levels

Serum insulin levels were obtained on the 48th day post treatment and can be seen in Table 3. Results show that data is normal distributed with p = 0.200 and there is a significant difference of insulin levels between groups, analyzed with ANOVA with significance of p = 0.001.

Table 3. Anova analysis of insulin levels.

Variable	Group	*Mean*	SD	Confidence level 95%		*p*
				Lower limit	Upper limit	
Insulin level	K-	18.21	2.71	15.36	21.06	0,001
	K+	12.66	1.99	10.57	14.75	
	P	13.75	0.76	12.95	14.56	

Note: p = significance, SD = standard deviation.

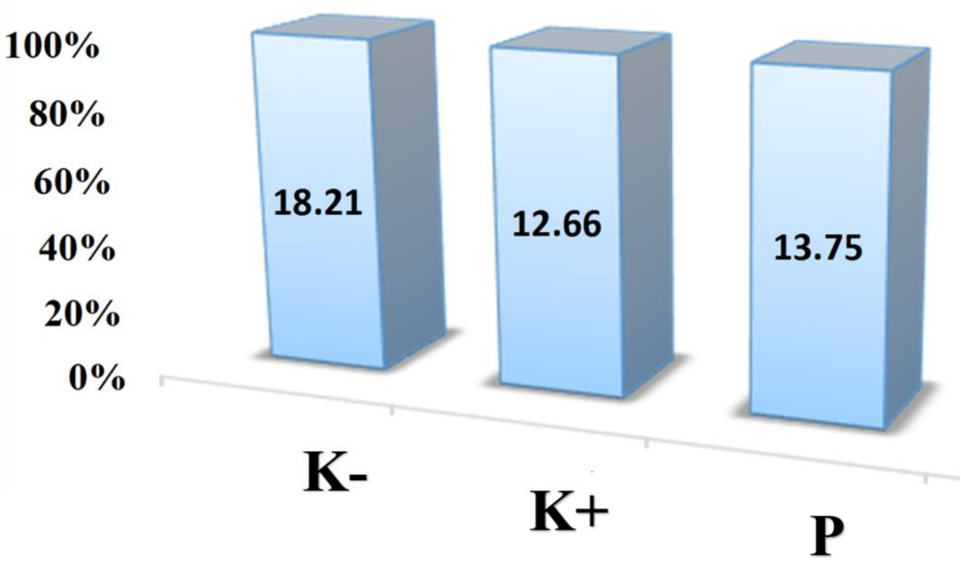


Figure 1. Mean of insulin levels of PCOS mice models. K- = 18.21; K+ = 12.66; P = 13.75. p = 0.001.

Figure 1 above shows a bar chart of the mean insulin levels in the serum of the insulin-resistant PCOS mice models with values of K- = 18.21; K+ = 12.66; P = 13.75 and p-value of p = 0.001. The positive control group (K+) shows the lowest value, however there is not a great difference between this group and the treatment group (P). Even so, results show a significant difference between groups.

Analysis of TNF-alpha Levels

TNF-α levels were obtained post treatment and presented in Table 4. Normality test was then done to all of the groups' data and showed a significance value of 0.064, which implies that the data is normally distributed. Further analysis was done using Anova analysis. Results revealed that there is a significant difference between groups with a significance of p = 0.017.

Table 4. Anova analysis of TNF-α levels.

Variable	Group	Mean	SD	Confidence level 95%		*p*
				Lower limit	Upper limit	
TNF-α levels	K-	50.43	3.09	47.18	53.67	0.017
	K+	44.48	5.53	38.67	50.28	
	P	42.06	4.63	37.19	46.92	

Note: p = significance, SD = standard deviation.

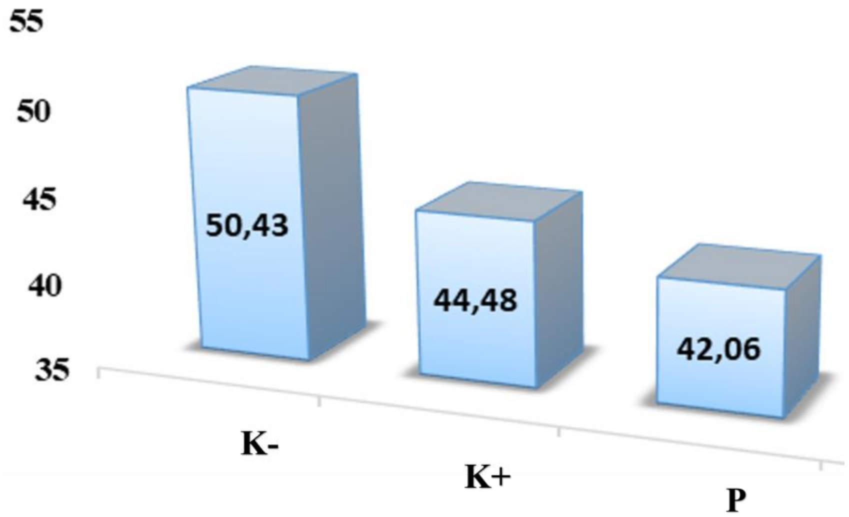


Figure 2. Mean of TNF- α levels in PCOS-IR mice model serum. K- = 50.43; K+ = 44.48; P = 42.06. p = 0.0017.

In Figure 2, TNF- α levels of insulin-resistant PCOS mice models on the 8th week of treatment showed values of K- = 50.43; K+ = 44.48; P = 42.06 with p-value of p = 0.0017. The control group showed the highest mean compared to the treatment group and showed a significant difference between the groups.

In PCOS, an increase of proinflammatory cytokine TNF-α was seen due to the increase of insulin secretion. Increase of IGF-1 correlates with PCOS patients with insulin resistance. Hormonal imbalance can cause disruption between the pituitary gland - hypothalamus and ovaries (H-P-O) which is one of the causes of PCOS. IGF-1 increases the steroid cycle and IGF-1 depends on the TNF-α factor[15]. TNF-α is a cachectin which is mostly produced by macrophages. Biological activities depend on TNF-α concentration, at low concentrations it induces autocrine and paracrine regulation of the immune response, whereas at high concentrations TNF-α effects the endocrine system[16].

Ovulation, fertilization and embryo implantation is influenced by TNF-α, which is an important factor in the pathogenesis of PCOS. Higher androgen levels increase the release of TNF-α from mononuclear cells (MNC)[17]. In this study, we attempt to determine the effect of TNF-α, insulin and free tetstosterone on biochemical parameters after administering LCHP diet with the aim of reducing adipose tissue and lowering the concentrations of TNF-α, insulin and free testosterone in laboratory mice.

P38MAPK Expression in PCOS Mice Models.

The expression of p38MAPK from the ovarian tissue of insulin resistant PCOS mice models can be seen in Table 5. Results were obtained on the 48th day post treatment. Data was normally distributed with p = 0.200. Insulin levels were analyzed with ANOVA and results showed a significance value of p = 0.001, indicating a significant difference between groups.

Table 5. Anova analysis of p38MAPK expression.

Variable	Group	Mean	SD	Confidence level 95%		*p*
				Lower limit	Upper limit	
p38MAPK	K-	2,65	0,39	2,23	3,06	0,149
	K+	2,28	0,47	1,78	2,78	
	P	1,98	0,73	1,21	2,75	

Note: p = significance, SD = standard deviation.

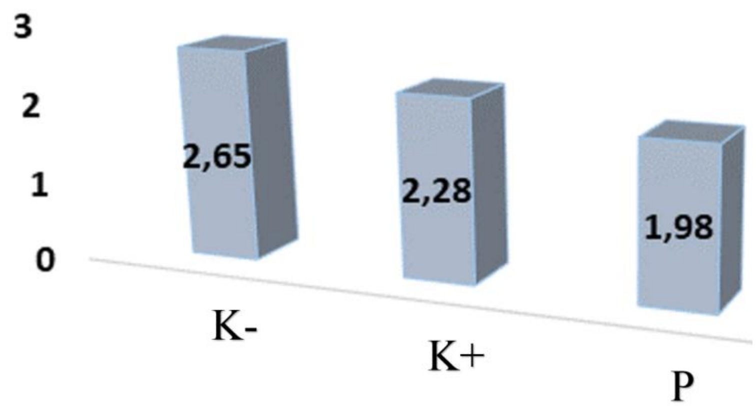
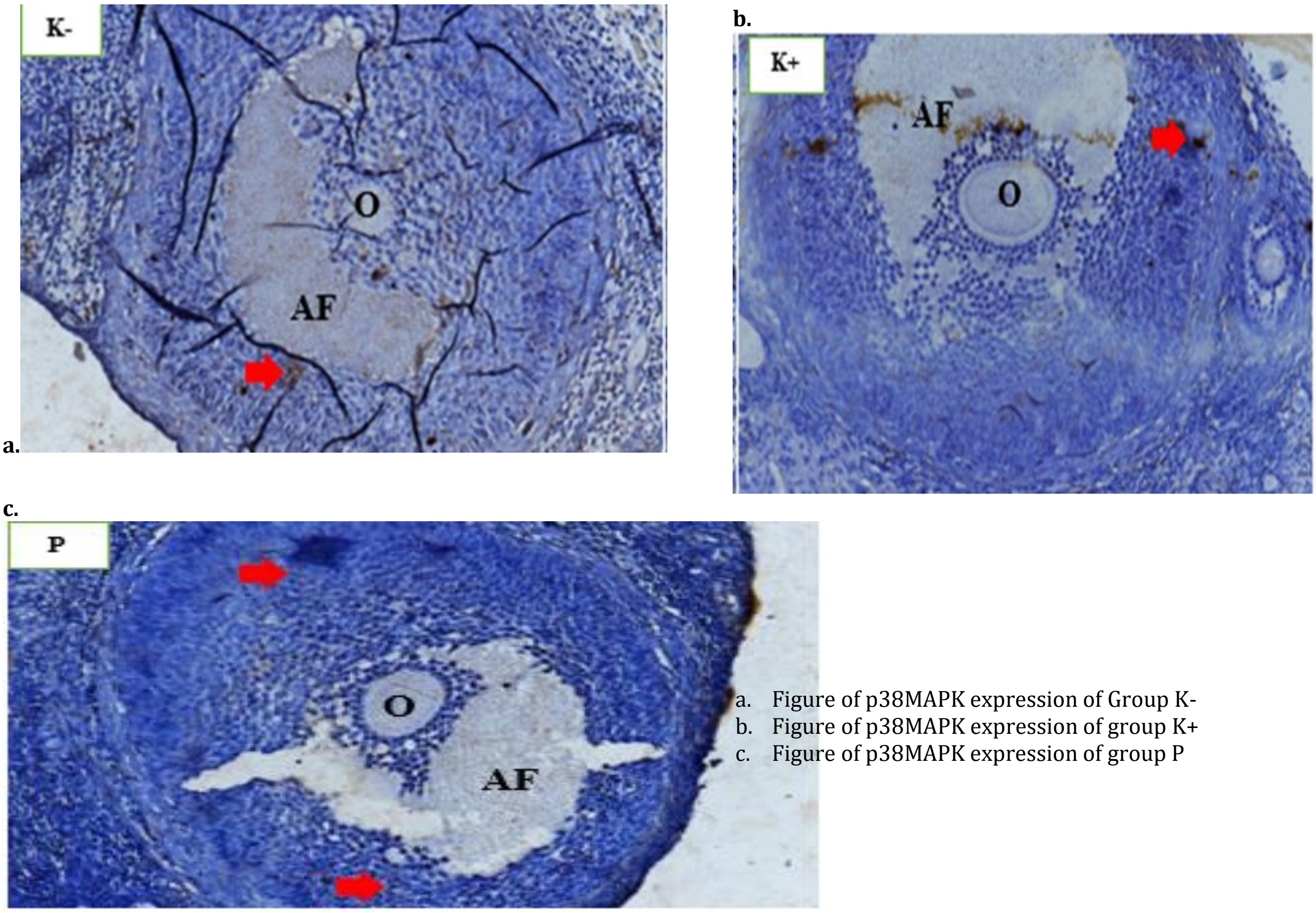


Figure 3. Mean of p38MAPK expression from ovarian tissue of PCOS-IR mice models. K- = 2.65; K+ = 2.28; P= 1.98. p = 0.149.



a. Figure of p38MAPK expression of Group K-
b. Figure of p38MAPK expression of group K+
c. Figure of p38MAPK expression of group P

Figure 4. Comparison of p38MAPK expression (arrows) between groups. On the slide above, it can be seen that the p38MAPK expression in the negative control group is stronger (percentage of positive cells and color intensity) compared to the other groups. Immunohistochemical staining (IHC), 1000× magnification (Figure a, b, and c).

Figure 3 reveals that the mean of p38MAPK expression from the ovarian tissue of PCOS-IR mice models have values of K- = 2.65; K+ = 2.28; P = 1.98 with significance value of p = 0.149. Results show that there is no significant difference between groups even though the treatment group showed higher p38MAPK expression compared to the control group. In Figure 4, p38MAPK expression shows that the mean value of p38MAPK expression score in theca cells and granulose cells in the ovarian tissue of insulin-resistant PCOS mice models for each group are K =2.65; K+ = 2.28, and P = 1.98.

There was an improvement in p38MAPK expression in theca cells and ovarian granulosa cells between K- (normal mice) and K+ (PCOS-IR mice models) groups, K+ (PCOS-IR mice models fed standard feed) and P (PCOS-IR mice models given LCHP diet) groups, as well as between K- (normal mice) and P (PCOS-IR mice models) groups. A decrease in the mean value of p38MAPK expression was seen in the insulin resistant PCOS model group that was given the LCHP diet and the group that was given standard feed. However, there was no significant difference between the ANOVA and post hoc test results between the groups.

The average score of the treatment group was lower compared to the control group. The high p38MAPK expression score in this study could be due to the LCHP diet with low glycemic index which has the ability to improve insulin sensitivity through GLUT-4 glucose uptake, which suppresses the secretion of insulin, and also suppresses the production of proinflammatory cytokines. Thus, the formation of ROS does not occur through the activity of the oxidative stress signaling pathway. Other pathways such as NFKβ are different compared to activation pathways in insulin resistance conditions, where the NFKβ pathway plays a role in the secretion of mitogen proteins, such as MAPK, especially p38 which affects cells. p38 MAPK remains activated in granulosa cells via AGEs receptors for proinflammatory expression causing an increase in androgen secretion in the ovaries, leading to impaired ovarian function and abnormal follicular development. Along with inflammation, increased activity of cytokines and inflammation of TNF-α, Interleukin-6 (IL-6), and IL-18 is also found in PCOS with insulin resistance[18]. The increased formation of ROS can also be due to increased activation of the oxidative stress signaling pathway induced by an increase in glucose and free fatty acids (FFA) which can activate NF-κB, along with PKC and p38 MAPK activity[19,20]. Inflammation associated with oxidative stress is due to an increase in serum levels of

AGEs and an increase in the expression of proinflammatory RAGEs in serum and in ovarian tissue[21]. A futher reaserch regarding the regulation of cytochrome P450 subfamily and testosterone as well as the regulation of progesterone observed in granulosa cells of mice with insulin resistance[22]. Protein kinase is inhibited to activate mitogen p44/42 after a state of insulin resistance in mice granulosa cells, thus decreasing the phosphorylation of protein kinase p44 / 42, which has an effect on the downregulation of cytochrome p450 subfamily 17 and decrease of progesterone production. This results in endocrine disorders, especially in the reproductive system, causing disturbance in the steroidogenesis signaling pathway in ovarian cells.

Post-Hoc Analysis

There was a significant difference (p <0.05) in the insulin levels, TNF-α levels and p38MAPK expression between the three groups. Further analysis was performed using post hoc least significant difference (LSD) to test the difference in insulin levels between groups. Results are shown in Table 6.

Table 6. LSD analysis of insulin levels.

Variable	Group	Sample	*Mean*	*p*
Insulin levels	K-	2	5.54*	0.000
		3	4.45*	0.002
	K+	1	-5.54*	0.000
		3	-1.09	0.356
	P	1	-4.45*	0.002
		2	1.09	0.356

Note: p = significance, *mean difference with p <0.05.

Results in Table 6 reveal that there is a significant difference between insulin levels of the control group and treatment group. The largest difference was seen between the negative control group sample 2 and positive control group 1 with significance of 0.000, and between the negative control group sample 3 and treatment group sample 1 with significance of 0.002. The administration of low carbohydrate high protein diet reduced insulin levels in the treatment group.

Table 7. Post hoc least significant difference (LSD) analysis of TNF-α levels.

Variable	Group	Sample	Mean	*p*
TNF-α levels	K-	2	5.94*	0.038
		3	8.36*	0.006
	K+	1	-5.94*	0.038
		3	2.42	0.370
	P	1	-8.36*	0.006
		2	-2.42	0.370

Note: p = significance, *mean difference with p <0.05.

Table 7 shows that there is a significant difference of TNF- α levels between the control group and treatment group. Largest difference was seen between the negative control group sample 3 and treatment group sample 1 with significance of 0.006, and between the negative control group sample 2 and the positive control group sample 1 with significance of 0.038. The administration of low carbohydrate high protein diet had an effect on reducing TNF-α levels in the treatment group.

Table 8. Post hoc LSD analysis of p38MAPK expression.

Variable	Group	Sample	*Mean*	*p*
p38MAPK expression	K-	2	0.36	0.271
		3	0.66	0.055
	K+	1	-0.36	0.271
		3	0.30	0.365
	P	1	-0.66	0.055
		2	-0.30	0.365

Note: p=significance, *mean difference with p <0.05.

Table 8 above shows the post hoc analysis results of p38MAPK expression. There was no significant difference between the control group and treatment group with significance value of p >0.05. The administration of low carbohydrate high protein diet did not affect the expression of p38MAPK in the treatment group.

DISCUSSION

Polycystic ovary with insulin resistance will aggravate PCOS pathology with the development of hyperandrogemia, increase of proinflammatory cytokine which activates polyol, hexoxine, and PKC pathways[23]. Low carbohydrate high protein diet on insulin-resistant PCOS mice models enhances insulin sensitivity through GLUT-4-facilitated glucose uptake, which then leads to suppression of insulin level and also fat accumulation present in muscle and adipocytes tissues, thus reducing oxidative stress secretion which will suppress ROS production and block TNF-α secretion, the proinflammatory cytokine, through NFKβ pathway[24].

Low-carb diet results in low TNF-α level. Consumption of low glycemic index diet in PCOS patients reduces fat mass and suppresses excessive adipocyte tissue accumulation, which will inhibit proinflammatory cytokines and growth factors, including IL-6 and TNF-α, in adipocyte tissue which increase glucose transport and suppress lipolysis[25]. We previously also found that suppression of adipocyte tissue accumulation will boost glycogen, lipid, and protein degradation and affect the conversion of the ovarian hormone androgen by aromatase into estrogen[8]. So that the decrease in insulin and TNF-α levels in PCOS patients can lead to ovulation improvement by way of stereoidogenesis process of androtenedione-testosterone aromatase into estrogen in granulosa cells, which certainly induces oocyte maturation and folliculogenesis[26]. ANOVA analysis of p38MAPK expression with *p* >0.149 indicates that the low carbohydrate high protein diet has a negative impact, in which the expression score in the treatment group was lower but still high. In hyperglycemia mechanism via polyol, AGEs, hexoxine, and PKC pathways, these pathways supposedly influence the secretory activity of insulin level and androgen production, this certainly correlates with TNF-α increase due to increased insulin/p38MAPK production. However, this has no effect on insulin and TNF-α levels, it is likely that the activated pathways are the NFKβ and IKKβ pathways, namely the PKC pathway[23]. These pathways still activate mitogen proteins. Increase in ROS level due to a high glucose metabolic rate triggers glycation, this leads to endoplasmic reticulum (ER) stress which increases the amount of misfolded protein to fold using the ER's ability to export newly synthesized proteins. ER stress activates unfolded protein response (UPR) pathway and causes apoptosis via the inflammatory pathway JNK and NFKβ[24]. Therefore, p38MAPK expression still induces secretion in granulosa cells which will interfere with the stereoidogenesis process in theca cells, which inhibits aromatase by the p450 enzyme into estrogens that undergo oocyte maturation and follicular atresia, but not folliculogenesis.

With the increasing interest in research on the effects of low carbohydrate diet on fertility in PCOS women, we systematically conducted a research to determine the extent to which the LCHP diet may affect fertility. A high number of overweight women are at risk for insulin resistant PCOS, which disrupts the steroid metabolism and menstrual cycle. It was reported that almost 75% of women of childbearing age suffer from PCOS[27]. In line with this, unhealthy lifestyle and a high-calorie diet are some of the causes of PCOS[28]. High calorie intake causes disruption of lipid and metabolic profiles, as well as increased fat and insulin accumulation, which eventually lead to obesity[29]. Raise in insulin level due to high secretion of androgen is the clinical symptom of PCOS. This affects GnRh to decrease the FSH ratio and increase LH, which then interfere with the steroidogenesis process, as well as the follicle growth and development. It is known that there is a significant improvement in the menstrual cycle and/or rate of ovulation after a low carb diet[28]. In general, PCOS women have an increased pulsatile GnRh activity, high LH levels, theca cell hyperactivity, and altered granulosa cell activity which reduces the production of estradiol (E2) and progesterone (P4)[4].

Ovaries and adrenal glands are in charge of the conversion of androstenedione and testosterone production, whereas the adrenal glands are responsible for dehydroepiandrosterone sulfate (DHEAS). Increased level of androgens such as dehydroepiandrosterone (DHEA), androstenedione, and testosterone was also found. The increase in enzymes in theca cells affects the conversion of testosterone to estrogen in granulosa cells by the p450 aromatase enzyme. The steroid hormone synthesis pathway begins with the conversion of cholesterol to pregnenolone by p450scc, pregnenolone to 17 hydroxypregnenolone and progesterone to 17 hydroxyproegesterone (17OH-P) by CYP17A1 (17 α-hydroxylase and 17, 20 lyase). Then 17 hydroxypregnenolne is converted into DHEA through 17.20 lyase activity of the same enzyme. DHEA is converted into androstenedisone by the 3β HSD enzyme into testosterone in theca cells. While in the granulosa cells, testosterone by the p450 enzyme becomes estrogen. The role of both FSH and LH receptors affects the growth and development of follicles and ovulation in PCOS women[4].

It was discovered that approximately 80% of women with insulin resistant PCOS also develop compensatory hyperinsulinemia. Both underweight and obese PCOS women are also known to have high insulin levels. Insulin also inhibits the synthesis of liver SHBG, which is a circulating protein that binds the testosterone and increases testosterone production. Dietary arrangement such as the low-carb diet has been studied and shown to lower insulin levels and improve ovulation. LCHP diet reduces glucose level, thereby reducing insulin release which results in a decrease in free testosterone[30,31]. Low carbohydrate diet is defined as a meal structure that helps to manage or prevent diseases by limiting carbohydrate consumption and increasing protein and/or lipid intake[6]. It has been shown that a low carbohydrate diet is effective for weight loss and facilitates infertility treatment in PCOS patients.

Increase in inflammatory cytokines is caused by a shortage of oxygen in ovaries, which will lead to reperfusion (detortion), causing injury to endothelial cells in blood cells and disturbances in microcirculation, which are responsible for damage in ovarian tissues. Ischemia-reperfusion damage stimulates reactive oxygen species (ROS) such as hydroxyl radical (•OH), hydrogen peroxide (H_2O_2), and superoxide radical (O_2-). Involvement of ROS in women of childbearing age with insulin-resisant PCOS was also found. Obesity triggers oxidative stress which affects the reproductive health, particularly in the ovarian tissues. Oxidative stress increases ROS production, wherein ROS has a major role in pathological damage to the reproductive system such as growth, follicular development and oocyte maturation after pregnancy, especially pathological damage in the reproductive system[32].

Oxidative stress plays an important role in the risk of developing PCOS, both underweight and obese PCOS women often have excessive body fat, central adiposity, and insulin resistance. High adipocytes factor in hypoadiponectinemia as well as adipose tissue dysfunction such as excess cytokines and oxidative stress. Abdominal adiposity increases androgen production and directly channels ovarian and adrenal responses to pro-inflammatory cytokine mediators such as (TNF-α, IL-6) or indirectly with the development of insulin resistance and hyperinsulinemia which will lead to increased androgen production. ROS and oxidative stress are triggers for insulin resistance and PCOS, oxidative stress interferes with glucose uptake in muscle and adipose tissue and reduces insulin secretion from pancreatic β cells. Increased expression of proinflammatory receptors was found in ovarian tissue in women with PCOS[33]. Through the steroidogenesis pathway, increase in steroids expression and metabolic cholesterol in ovarian tissue occur. Synthesis of estrogenic hormone in ovarian granulosa cells is compensated by the increase in cholesterol and fatty acids. In granulosa cells, the conversion of testosterone to estrogen undergoes changes caused by inhibition of the p450arom enzyme. The ovaries become smaller and do not develop and grow (polycystic). Hyperketotic stroma, which is a characteristic feature of PCOS, is also found[34].

Insulin resistance increases proinflammatory cytokines and endoplasmic reticulum (ER) stress through the activation of c-Jun N-terminal kinase (JNK) and I kappa B kinase (IKKβ), resulting in IRS phosphorylation in serine, which inhibits normal insulin signal. Activation of IKKβ also causes phosphorylation of the protein IκB, which is an inhibitor of NFκβ transcription factor[22]. Protein kinase signaling plays an important role in growth and development through mitogen-activated insulin receptor pathways in ovarian granulosa cells[24]. We obtained a positive relationship between LCHP diet and decreased level of TNF-α and insulin, but not on p38MAPK expression. Our findings indicate that the decreased level of TNF-α and insulin after LCHP diet could be due to a mechanism through a sequential pathway starting with inhibition of TNF-α and insulin secretion. The main effect of the low-carb high-protein diet is to inhibit the TNF-α inflammatory pathway on the insulin-resistant PCOS mechanism. Inhibition of the TNF-α inflammatory pathway on the insulin-resistant PCOS mechanism should be put into consideration as a strategy for developing insulin-resistant PCOS therapeutic interventions in the future. If the LCHP diet is given in a longer period, p38MAPK expression tends to improve.

CONCLUSION

One of the main roles of p38MAPK in PCOS is the stimulation of steroidogenesis and aromatization of androstenedione to estrogen. Increased p38MAPK expression after the LCHP diet affects folliculogenesis. It is assumed that oxidative stress is not able to block the activity of MAPK pathway. However, the pathogenesis of insulin-resistant PCOS has several pathways such as the polyol activity, hexoxine, and AGEs pathways, in which the insulin signaling pathway is able to suppress the proinflammatory activity of TNF-α and insulin level in general. The contribution of LCHP diet to p38MAPK expression stimulated by insulin and TNF-α is unclear.

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