Low Carbohydrate – High Protein Diet Does Not Provide Positive Effect on P38 Mitogen-Activated Protein Kinase (p38MAPK) Expression

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Abstract

P38 mitogen-activated protein kinase (p38MAPK) is the activity of the p38MAPK pathway in muscle cells causing mitogen-activated disorders that are responsive to stress stimuli, such as cytokines, and involved in cell differentiation, and apoptosis. Characteristic of p38MAPK expression contributes to metabolic signal on the reduction and excessive production of ovarian androgen found among women with endocrine and metabolic disorders. We suspect that p38MAPK expression changes in ovarian granulosa cells. This research aims to observe changes in p38MAPK expression among PCOS-RI mice fed by a low-protein, high-carbohydrate (RKTP) diet. Testosterone propionate was given to induce the PCOS-IR model, 0.1 ml injection of for 28 days intramuscularly on 18 mice. Each mouse was examined for vaginal swabs and ovarian histology. At the end of treatment, the p38MAPK expression score kept increasing and had a significant negative effect with ANOVA test p value = 0.149 (p >0.05). The results of the treatment group posttest showed p38MAPK expression, and there was a decrease compared to the control group. These occurred because the RKTP diet has not been able to stimulate RE stress through the NFKβ pathway, so the NFKβ pathway increased AGEs receptors on granulosa cells that played a role in oocyte maturation as a response in the follicular development process on PCOS-IR model. Moreover, in this research RKTP diet had not been able to reduce p38MAPK expression in granulosa cells in the ovary.

Keywords: *insulin resistance, p38MAPK expression, polycystic ovary syndrome.*

Introduction

Polycystic ovary syndrome (PCOS) is a hormonal imbalance on the body and most are in the form of endocrine disorders characterized by hyperandrogenic, ovulation dysfunction and infertility[1]. Changes in ovarian morphology are clearly seen in PCOS-insulin resistance, indicating that hyperinsulinemia affects morphology and ovarian function[2]. Further, inflammation related to oxidative stress is due to an increase in insulin that affects proinflammation in serum and ovarian tissue[3]. ROS formation is caused by the increase in activation of oxidative stress pathways induced by glucose increase[4] and activating (nuclear factor-KB) NF-KB and p38 mitogen-activated protein kinase (p38MAPK)[5]. We suspect there is a decrease in p38MAPK expression in ovarian granulosa cells. This research was conducted to determine the effect of a low-protein diet (RKTP) that can reduce p38MAPK expression in ovarian granulosa cells among SOPK-RI model mice.

Many theories state that insulin resistance causes hyper insulin and hypertriglyceridemia due to accumulation of fat in muscles, an increase in adipose mass can cause pathological changes in adipokine hormone that regulates insulin sensitivity[6,7,8,9]. On

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the liver, adiponectin also increases insulin sensitivity, reduces the entry of FA, increases FA oxidation, and reduces hepatic glucose output. In muscles, adiponectin stimulates glucose use and FA oxidation possibly through cellular fuel sensors activation, AMP-activated protein kinases (AMPK), changes in proinflammatory cytokines resulting expression increase. Several potential mechanisms for metabolic effects of TNF-α have been described, including serine kinases activation such as JNK and p38MAPK that increases IRS-1 and IRS-2 serine phosphorylation, resulting in a poor substrate, in which kinase activates insulin receptors and increases IRS degradation[10].

Materials and Methods

Research Design

This research was a true laboratory experimental by posttest only control group design with two control groups and one treatment group. Further, an ethical eligibility test for this research was done by the ethics committee at the Faculty of Veterinary Medicine, Universitas Airlangga with a certificate number of 2.KE.062.04.2019. The research variable was p38MAPK expression, given a high-carbohydrate diet with a high protein composition of 40% carbohydrate, 30% protein and 30% fat in the positive control group and 20 days treatment among POCS insulin resistance mice injected by testosterone propionate for 28 days.

Experiment Unit

Rattus norvegicus was prepared by the Laboratory of Embryology, Faculty of Veterinary Medicine, Universitas Airlangga, and chosen as a model of insulin-resistant PCOS. Acclimated for 2 weeks to adapt and give a standard feed. By using healthy female mice, they were divided into 3 groups, 6 mice were induced with 0.1 ml of testosterone propionate intramuscularly for 28 days and mice normal group were injected with placebo. The treatment group was given RKTP diet food, and the normal group was given standard feed. pP38MAPK (mouse) antibodies were purchased from the Gamma Science of Santa Cruz sc 166182 brands with immunohistochemistry (IHC) examination.

Data Analysis

Data were statistically analyzed using SPSS. Normality analysis used Kolmogorov-Smirnov if the *p* value was >0.05, followed by ANOVA analysis to determine differences between groups and post hoc LSD was tested to discover clearly the differences among each group.

Results and Discussion

The treatment group's mean score was lower than the control group but the p38MAPK expression score was higher in this research. It was possible that RKTP diet by giving a low glycemic index was able to improve insulin sensitivity through glucose uptake GLUT-4, suppressing insulin level secretion. In addition, it suppressed proinflammatory cytokines production so that ROS formation did not occur through oxidative stress signaling pathways. Other pathways such as (Nuclear Factor-KB) NFKβ was different from activation pathway in an insulin resistance, where NFKβ pathway played a role in the secretion of mitogen proteins, namely mitogen-activated protein kinase (MAPK), especially p38 which affected cells on p38 mitogen-activated protein kinase (p38MAPK) to keep activated on granulosa cells through Advanced glycation end products (AGEs) receptors on proinflammatory expression causing increased androgen secretion in the ovary, which then caused impaired ovarian function and abnormal follicular development.

Table 1. Normality Analysis by Kolmogorov-Smirnov.

Variable	**Sample**	**Mean**	**Minimum**	**Maximum**	***p***
p38MAPK expression	18	2.30	0.80	3.20	0.154*

Description: *p* = significance, marked by * normal distribution *p* >0.05

Table 2. Anova Analysis of p38MAPK Expression.

Variable	Group	Mean	D	Confidence Interval by 95%		p
				Lower limit	Upper Limit	
p38MAPK expression	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.

According to Huynh *et al*.[11], in addition to inflammation in SOPK with RI, the increase in cytokine activity and tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and IL-18 inflammation were also found. The increased ROS formation might also be caused by the increase in activation of oxidative stress signaling pathways induced by increased glucose and free fatty acids (FFA)[6,8] which could activate NF-KB and protein kinase C (PKC) activities and p38 MAPK[5]. Moreover, inflammation related to oxidative stress was occurred due to an increase in serum levels of advanced glycation end products (AGEs) and the increased expression of proinflammatory receptor of AGEs (RAGEs) in serum or ovarian tissue[3].

Table 3. Post Hoc Analysis of LSD 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, marked by *mean difference by $p < 0.05$

The table above indicated post hoc analysis of p38MAPK expression comparison in the control and treatment groups without significant difference by *p* value >0.05. The provision of a low carbohydrate diet high in protein had no effect on the p38MAPK expression in the treatment group. Lower p38MAPK expression in the treatment group compared to control group was relevant with the research of Linghui *et al*.[12] stating that insulin resistance state increased proinflammatory cytokines and (endoplasmic reticulum stress) ER stress through activation of c-Jun N-terminal kinase (JNK) and I kappa B kinase (IKKβ) resulting in IRS phosphorylation of serine, inhibiting normal insulin signaling. IKKβ activation also caused phosphorylation

of IκB protein, an inhibitor of kappa β (NFκβ) nuclear factor transcription factors. Protein kinase signaling played an important role in growth and development through mitogen-activated insulin receptor pathways in ovarian granulosa cells[13]. The research indicated changes in glucose and insulin, given the importance of insulin and hyper insulin receptors for compensation in androgen induction.

p38MAPK expression in this research was the lowest in the treatment group, although p38MAPK expression in ovarian granulosa cells kept increasing. During mitogen secretion in granulosa cells functional interference occurred affecting p38MAPK expression, which could be possibly related to ER Stress. Consequently, it caused interference with the insulin receptor signal and its secretion which affected inadequate mitogen signal in granulosa cells. ER stress state increased the misfolded protein amount affecting ER's ability to fold and secrete newly synthesized proteins that were causing ER stress. ER stress-activated unfolded protein response (UPR) pathway and caused apoptosis through the inflammatory pathway of JNK and NFκβ[13].

Conclusion

In summary, low carbohydrate-high protein (RKTP) diet provision added by testosterone propionate injection has a significant negative effect on p38MAPK expression.

Conflict of Interest: The authors declare that they have no conflict of interest.

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