The Effect of Excess Iron on the Impairment of Glucose Metabolism in Mice

Selvi Puspa Sari[1], Mas Rizky A.A. Syamsunarno[1,2,3*], Susi Susanah[1], Nur Atik[1], Mohammad Ghozali[1], Ratu Safitri[2], Ramdan Panigoro[1]

[1]Faculty of Medicine, Hasan Sadikin General Hospital - Universitas Padjadjaran, 45363 Bandung, Indonesia

[2]Program Study of Biotechnology, Post Graduate School Universitas Padjadjaran, 45363 Bandung, Indonesia

[3]Central Laboratory - Universitas Padjadjaran, 45363 Bandung, Indonesia

Received 28 Sep 2017.
Revised 13 Nov 2017.
Accepted 27 Dec 2017.
Published Online 31 Dec 2018

*Corresponding author:
Mas Rizky A.A. Syamsunarno
E-mail: rizky@unpad.ac.id

Abstract— Excess iron in the body can trigger pathological conditions through production of reactive oxygen species (ROS) and the deposition in various organs. This condition can also disrupt whole metabolism process, including glucose metabolism. However, the mechanisms between iron and glucose metabolism remain unclear. The study was to investigate the effect of excess iron with glucose metabolism disorder by assessing Glucose Tolerance Test (GTT) and gluconeogenesis rate in mice through Intraperitoneal Glucose Tolerance Test (IPGTT) to determine insulin resistance, Intraperitoneal Pyruvate Tolerance Test (IPTT) to measure hepatic gluconeogenesis, and to assess pancreatic histology for routine histogical examination. In this experimental study, eight-teen male mice were assigned to three equal groups. Mice were divided by the saline injected group (control) and iron dextran injection 0.1 mg/mice (group 2) and 0.3 mg/mice (group 3) dose of injection. Iron dextran was injected daily intraperitoneally. After 14 days of treatment, IPGTT, IPTT and pancreas histology were examined. Repeated analysis of variance (ANOVA) with bonferroni's posthoc multiple comparison test were used for data analysis. IPGTT results showed glucose level was lower 39.85 % in group 3 compared to the control group mice. The results of IPTT showed that glucose level in mice treated with iron dextran were significantly lower in dose dependent manner. Pancreas histology showed islet cells in group 3 decreased in size, which might be due to beta cells depletion. Short term iron injection increases glucose tolerance and supresses hepatic gluconeogenesis in mice.

***Keywords* - Excess Iron, Glucose Metabolism Disorder, Intraperitoneal Glucose Tolerance Test (IPGTT), Intaperitoneal Pyruvate Tolerance Test (IPTT).**

1 INTRODUCTION

Iron is an essential element for synthesis of haemoglobin in the erythrocytes, cofactor for oxidation-reduction reactions, and cell proliferation. The average daily iron intake is 1-2 mg/day and the amount is strictly regulated to fit the body needs. Excess iron in the body can trigger pathological conditions through the production of reactive oxygen species (ROS) that cause organ dysfunction [1,2]. Excess iron accumulate in several organs including the heart, liver, and endocrine organs and can lead to serious problems such as cardiomyopathy, liver failure, and diabetes [1,3].

The first evidence for association of iron with human diabetes mellitus comes from clinical observations in individuals with pathologic iron overload e.g in patients with thalassemia who undergo transfusions on a regular basis to maintain the amount of erythrocytes. The prevalence of diabetes mellitus in thalassemia patients is 6 to 14% [4]. It is suggested that the cause of diabetes mellitus in β thalassemia major patients is due to iron accumulation in the liver which reduce the hepatic clearance of insulin that lead to hyperinsulinemia and insuline resistance. Over time, Insulin resistance will contribute to high-insulin-dependent pancreatic injury. Other than that, diabetes mellitus in β thalassemia major is also caused by pancreatic beta cell damage due to direct oxidant effects of iron accumulation in the pancreas [1,2,5,6]. The deposition of iron in the liver and pancreas can impair glucose tolerance and may progress to overt diabetes mellitus [7,8].

The condition of iron overload can disrupt glucose metabolism and cause hyperinsulinemia through decreased insulin extraction and insulin signaling [9,10]. Insulin has effect on increasing the synthesis of ferritin and redistributing of transferrin receptors (TfRs) in cells so that large amounts of iron can enter cells and tissues, decreasing the regulation of hepsidine expression in hepatocytes and adipocytes, stimulating expression of ferroportin, ferritin heavy chain

(FTH), and ferritin light chain (FTL), reducing the expression of transferrin in adipocytes, and increasing the production of free radicals from hepatocytes [10,11]. Iron can also catalyze the conversion of less-reactive free radicals into highly active free radicals that inhibit antioxidant defenses in cells [11,12]. Beta cells are highly sensitive to free radicals due to the low amount of antioxidant expression such as catalase and superoxide dismutase $(SOD)_2$ [3,11,12].

The mechanisms underlying increased iron deposition in the body during the development of insulin resistance and diabetes mellitus remain unclear. Therefore, animal models are needed to explain the effect of iron overload on glucose metabolism. This study was an attempt to investigate the effect of excess iron content and its relation to glucose metabolism disorder in mice through Intraperitoneal Glucose Tolerance Test (IPGTT) to determine insulin resistance, Intraperitoneal Pyruvate Tolerance Test (IPTT) to measure hepatic gluconeogenesis [13] and to assess pancreatic histology for routine histogical examination.

2 MATERIAL AND METHOD

2.1 Animals

This study was approved by the Ethical Committee on Research of the Universitas Padjadjaran, Bandung, Indonesia No:715/UN6.C.10/PN/2017. All experiments were conducted based on the 3R and 5F principles.

Male white mice (*Mus musculus*), 8-10 weeks old and body weight approximately 25-30 g, were maintained in cages at room temperature and a 12-hour light/dark cycle with adequate air circulation. Mice were purchased from Bio Farma company, Bandung. All mice were provided food and water *ad libitum*.

2.2 Grouping of Experimental Group According to Dosage of Iron Administration

Mice were divided into 3 groups based on the dose of hemadex (iron dextran) injection. The number of mice was six for each group:

a. Group I (control): Saline injection 200 µL/ day
b. Group II: Hemadex injection 0.1 mg/200 µL/day intraperitoneally
c. Group III: Hemadex injection of 0.3 mg/200 µL/day intraperitoneally

Before the treatment, mice were adapted for one week and injection was performed daily for 14 days. The dose was calculated to finding Animal Equivalen Dose (AED) by multiplying human dose (mg/kg) with K_m ratio [14]. Daily transfusion iron loading in human is 0,34 mg/kg and the K_m ratio to convert human dose in mg/kg to AED in mg/kg is multiply human dose by 12.3 [14,15].

2.3 Glucose Tolerance Test

Mice were fasted for 18 hours before test. D-Glucose was intraperitoneally administered at 1 g/kg body weight. Blood sample was collected from the tail vessel and measured by the glucometer (GlucoDr, Korea) at 0, 30, 60, and 120 min [16].

2.4 Pyruvate Tolerance Test

After 18 hours of fasting, sodium pyruvate (1 g/kg body weight) was injected intraperitoneally and blood was collected from tail at indicated time as previously described [17].

2.5 Histology

Mice in each group were sacrificed by cervical dislocation. Pancreas was isolated and tissue sections were fixed in 10% formalin. Tissue sections were stained with hematoxylin and eosin for routine histologic examination and examined under the light microscopy under a ×10 objective in 20 random fields [18].

2.6 Statistical analysis

Statistical analysis was performed with IBM SPSS. Treatment differences were determined using repeated analysis of variance (ANOVA) and Bonferroni's posthoc multiple comparison test were performed to evaluate the differences between groups. A p-value of <0.05 was considered as statistically significant.

3 RESULT AND DISCUSSION

3.1 IPGTT Measurement

The effect of intraperitoneal injection of D-glucose solution at 0, 30, 60, 120 min in the control and treatment groups are shown in Fig. 1. Blood glucose levels were not different among the groups before glucose injection. Thirty minutes after glucose injection, blood glucose levels were strikingly increased in all groups. There were no differences in blood glucose levels between group 1 and group 2. However, the blood glucose

level was much higher in group 1 compared to group 3 at 30 and 60 minutes after glucose injection and there were significant differences in blood glucose level ($p<0.05$).

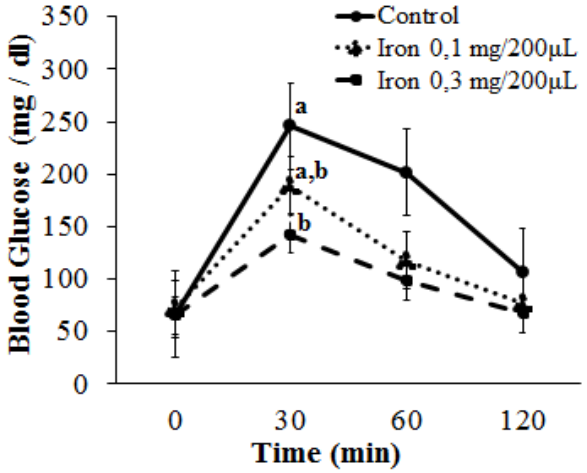

Figure 1: Intaperitoneal Glucose Toerance Test (IPGTT) at 0, 30, 60, 120 min in the control and treatent groups. Different letters indicate stastically significant ($p<0.05$).

The result indicated treatment group show better glucose tolerance than the control, it might be due to short period iron injection. The development of glucose intolerance initiated by hyperinsulinemia due to hepatic disturbance of insulin utilization, this state can persist long time until glucose intolerance become apparent. Prolonged hyprisulinemia causes pancreatic beta cells failure in secreting insulin by increase both cleaved caspase-3 protein and activity, increase apoptosis, and diminish cell number that leads to uncontrolled diabetes [19]. In this study the possibility of diabetes mellitus process has not happened but the process that initiate diabetes mellitus by hyperinsulinemia already seen. This condition may cause the IPGTT result to be lower than the control.

Iron is also known to facilitate insulin secretion from the pancreas. Under normal condition iron in beta cells is important in the process of insulin secretion, either through the fenton reactions that produce ROS or by maintaining the electron transport reaction in the mitochondria to form ATP so that insulin can be secreted through exocytosis [2].

3.2 IPTT Measurement

The effect of intraperitoneal injection of pyruvate solution at 0, 30, 60, 120 min in the control and treatment groups are shown in Fig. 2. Blood glucose level was different between group control and treatment before sodium pyruvate injection. Thirty minutes after sodium pyruvate injection, blood glucose level was strikingly increased in group 1 and group 2 but changes in blood glucose level in group 3 was not to high. However, there was significant different in blood glucose level between group control and treatments ($p<0,05$).

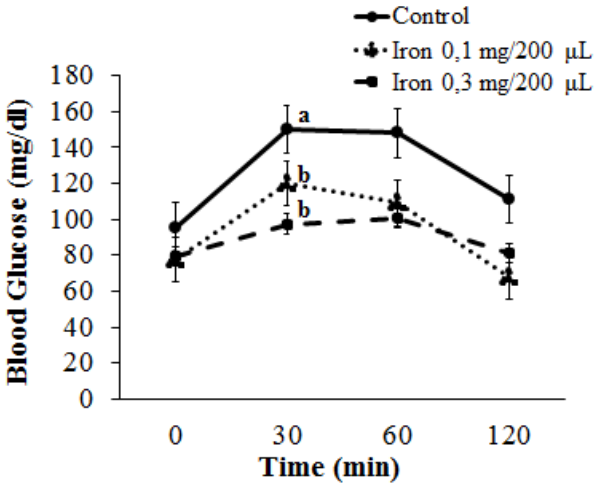

Figure 2: Intraperitoneal injection of pyruvate solution at 0, 30, 60, 120 min in the control and treatment groups. Different letters indicate stastically significant ($p<0.05$).

High iron intake leads to increased iron levels in the liver. This affects the change of circadian rhythm of glucose and gluconeogenesis mediated by the repression of key gluconeogenic enzymes such as glucose-6-phosphatase and Phosphoenolpyruvate carboxykinase (PEPCK) [20].

The results of IPTT measurement in this study were in agreement with study conducted by Simcox et al.[16] mice fed with high levels of iron exhibit increased AMP-activated protein kinase (AMPK) activity and impaired insulin signaling in muscles and liver. Iron activates AMPK by increasing deacetylase and decreasing acetylation of LKB1, in turn stimulating LKB1 and AMPK phosphorylation. In addition, the tissue of iron-fed mice showed a high AMP / ATP ratio, which further contributed to AMPK activation. AMPK is an important regulator of

gluconeogenesis in the liver that can suppress gluconeogenesis. AMPK is known to inhibit hepatic glucose production by suppressed gluconeogenic enzymes. So it can be concluded, a high-iron diet improves glucose tolerance by activating AMPK through a mechanism that includes deacetilation [20,21].

3.3 Pancreas Histology of Mice

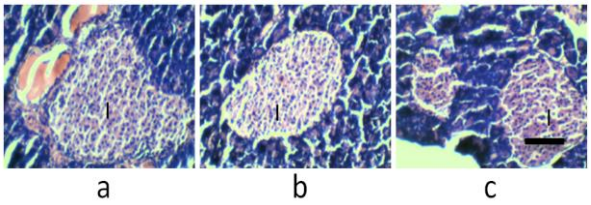

Figure 3: HE staining of pancreas in (a) control group (b) 0,1 iron injection (c) 0,3 iron injection. I= Islet cell. Scale bar=100 µm.

Islet cells in group 3 show decrease in size, it might be due to beta cells depletion by oxidant effect of excess iron. Physiologically, there is significant amounts of hydrogen peroxide are produced in cytoplasm and mitochondria to run cellular metabolism and signal transduction processes. In normal condition, hydrogen peroxide is converted to water by glutathione peroxidase and catalase expressed by beta cell pancreas, but in the presence of highly active iron the hydrogen peroxide will converted to hydroxyl radical which have cytotoxic effect by attacking and denaturing the functional and structural molecule incuding nucleic acid, carbohyrate, protein, and lipids [4,22].

Pancreatic beta cells highly vulnerable to oxidative damage, this condition is probably based on nearly reliance upon mitochondrial metabolism of glucose for glucose induce insulin secretion [23]. Besides that, beta cells have low antioxidant enzymes gene expression. Compared with liver, islets contain only 1% catalase, 2% glutathione peroxidase 1 (GPX 1), and 29% Zn-superoxide dismutase 1 (SOD1) activities. The low expression of antioxidant enzymes makes β-cells susceptible to oxidative stress [24].

Previous studies have shown the effect of excess iron on islet cells is the presence of grouped chromatin, dilation of nuclear membrane, endoplasmic reticulum, and light secretory granules on beta cells. The apoptotic and necrosis features are shown by beta cells, in alpha cells and delta cells occurring accumulation of iron but no apoptosis and necrosis occur [2]. Study by Cooksey also showed general loss of beta cells in iron overload mice, mice with *Hfe*$^{-/-}$ exhibit a 45% decrease in islet surface area compared with control group [22]. Failure of pancreatic beta cells is an early condition in the development of diabetes [2].

4 CONCLUSION

In short periods, iron injections resulted in lower blood glucose levels than the control group. This is likely because the body is in a condition of hyperinsulinemia. The mechanism of glucose intolerance due to iron overload may occur periodically preceded by hyperinsulinemia which then gives negative feedback on pancreatic beta cells so that the pancreatic beta cells are less work and ultimately apoptotic. In addition, beta cells can also experience necrosis due to the accumulation of iron directly on the cell. Depletion of pancreatic beta cells is supported by histology of pancreas that is shrinked in size in group treatment. Iron injections also decrease the rate of hepatic gluconeogenesis due to the suppression of gluconeogenic enzymes.

ACKNOWLEDGMENT

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.The author thank to Academic Leadership Grant Program Internal Research Grant UNIVERSITAS PADJAJARAN year 2016. No financial relationships with organisations that have an interest in the submitted research in the previous three years; no other activities or relationships that could affect the submitted research.

CONFLICTS OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

REFERENCES

[1] Kohgo Y, Ikuta K, Ohtake T, Torimoto Y, Kato J. Body iron metabolism and pathophysiology of iron overload. International journal of hematology. 2008;88(1):7-15.

[2] Backe M Balslev, Ingrid Wahl Moen, Christina Ellervik, Jakob BH, Thomas M-P. Iron Regulation of Pancreatic Beta-Cell Functions and Oxidative stress. Annu. Rev. Nutr. 2016;36:10.1–10.33.

[3] Bas M, Gumruk F, Gonc N, Cetin M, Tuncer M, et al. Biochemical markers of glucose metabolism may be used to estimate the degree and progression of iron overload in the liver and pancreas of patients with beta-thalassemia major. Annals of hematology. 2015;94(7):1099-104.
[4] Simcox JA, McClain DA. Iron and diabetes risk. Cell metabolism. 2013;17(3):329-341.
[5] De Assis RA, Ribeiro AA, Kay FU, Rosemberg LA, Nomura CH, et al. Pancreatic iron stores assessed by magnetic resonance imaging (MRI) in beta thalassemic patients. Eur J Radiol. 2012;81:1465–1470. doi:10.1016/j.ejrad.2011.03.077.
[6] Cavallo-Perin P, Pacini G, Cerutti F, Bessone A, Condo C, et al. Insulin resistance and hyperinsulinemia in homozygous beta-thalassemia. Metabolism.1995;44:281–286.
[7] Suvarna J, Ingle H, Deshmukh CT. Insulin resistance and beta cell function in chronically transfused patients of thalassemia major. Indian Pediatr. 2006;43 (5):393–400.
[8] Khalifa AS, Salem M, Mounir E, El-Tawil MM, El-Sawy M, et al. Abnormal glucose tolerance in Egyptian beta-thalassemic patients: possible association with genotyping. Pediatr Diabetes. 2004;5(3):126–32.
[9] Huang J, Jones D, Luo B, Sanderson M, Soto J, et al. Iron Overload and Diabetes Risk: A Shift From Glucose to Fatty Acid Oxidation and Increased Hepatic Glucose Production in a Mouse Model of Hereditary Hemochromatosis. Diabetes. 2010;60(1):80-7.
[10] Fernández-Real JM, McClain D, Manco M. Mechanisms Linking Glucose Homeostasis and Iron Metabolism Toward the Onset and Progression of Type 2 Diabetes. Diabetes Care. 2015;38(11):2169-76.
[11] Choi JS, Koh IU, Lee HJ, Kim WH, Song J. Effects of excess dietary iron and fat on glucose and lipid metabolism. The Journal of nutritional biochemistry. 2013;24(9):1634-44.
[12] Reardon TF, Allen DG. Iron injections in mice increase skeletal muscle iron content, induce oxidative stress and reduce exercise performance. Experimental physiology. 2009;94(6):720-30.
[13] National Mouse Metabolic Phenotyping Centers. Hepatic gluconeogenesis. [Online]. Available : https://www.mmpc.org/shared/document.aspx?id=144&docType=Protocol.
[14] Article R. A simple practice guide for dose conversion between animals and human. 2016; 27-31.
[15] Taher Ali, Maria D Cappellini, Elliott Vichinsky, Renzo Galanello, Antonio Piga, et al. Efficacy and safety of deferasirox doses of >30 mg/kg per d in patients with transfusion-dependent anaemia and iron iverload. Br J Haematol. 2009 Dec; 147(5): 752–759.
[16] JE Ayala, Samuel VT, Morton GJ, Obici S, Croniger CM, Shulman GI. Standard operating procedures for describing and performing metabolic test of glucose homeostasis in mice. Dis Model Mech. 2010 ;3(9-10):525-534.
[17] Syamsunarno MR, Iso T, Hanaoka H, Yamaguchi A, Obokata M, et al. A critical role of fatty acid binding protein 4 and 5 (FABP4/5) in the systemic response to fasting. PLoS One. 2013 Nov 14;8(11):e79386.
[18] Silva Maisa, Marcelo E. Silva, Heberth de Paula, Claudia Martins Crneiro, Maria Lucia Pedrosa. Iron overload alters glucose homeostasis, causes liver steatosis, an increases serum triacylglycerols in rats. Nutrition Research. 2008;391-398.
[19] Guillen C., A. Bartolome, C. Nevado, M. Benito. Biphasic effect of insulin on beta cell apoptosis dependng on glucose deprivation. FEBS Letters, 522, doi:10.1016/j.febslet.2008.10.020.
[20] Kalhan Satish C., Arnab Ghosh. Dietary Iron, Circadian Clock, and Hepatic Gluconeogenesis. Diabetes. 2015;64:1091–1093.
[21] Huang J, Simcox J, Mitchell TC, et al. Iron regulates glucose homeostasis in liver and muscle via AMP-activated protein kinase in mice. FASEB J 2013;27: 2845–2854.
[22] Masuda Yuichi, Hirohito Ichii, Nosratola D Vaziri. At pharmacologically relevant concentrations intravenous iron preparations cause pancreatic beta cell death. Am J Transl Res. 2014; 6(1): 64-70.
[23] Cooksey Robert C., Hani A. Jouihan, Richard S. Ajioka, Mark W. Hazel, Deborah L. Jones, et al. Oxidative Stress, β-Cell Apoptosis, and Decrease Insulin Secretory Capacity in Mouse Models of Hemochromatosis. Endocrinlogy. 2004;145: 5305-5312.
[24] Lei Xin Gen, Marko Z. Vatamaniuk. Two Tales of Antioxidant Enzymes on β Cells and Diabetes.Antioxid Reodx Signal. 2011 Feb 1; 14(3): 489-503.