Effect of Cholecalciferol on GLUT4 Expression in Adipocyte of Diabetic Rats

Dewi Ratna Sari,1 Rimbun,1 Tri Hartini Yuliawati,1 Joni Susanto,1 Ari Gunawan,1 Harjanto JM2

1 Department of Anatomy and Histology, Faculty of Medicine, Universitas Airlangga, Surabaya, East Java, Indonesia
2 Department of Physiology, Faculty of Medicine, Universitas Airlangga, Surabaya, East Java, Indonesia

Abstract

This research was conducted to examine the effect of cholecalciferol on fasting blood glucose (FBG), adipocyte diameter and glucose transporter (GLUT) 4 expression in adipocytes of diabetic rats. Nineteen male Wistar strain diabetic rats were divided into 4 groups (K, X1, X2 and X3). Cholecalciferol was administered in the amount of 6.25 µg/kg in X1, 12.5 µg/kg in X2 and 25 µg/kg in X3 per orem, once daily for 14 days. Group K received the placebo. There were no significant differences in FBG (p=0.199) and adipocyte diameter (p=0.218) between groups but there were significant differences in the expression of GLUT4 between control and treatment groups. Thus, cholecalciferol can increase GLUT4 expression in adipocyte without altering FBG and adipocyte diameter of diabetic rats.

Key words: cholecalciferol, GLUT4, adipocyte, diabetes

INTRODUCTION

Recent evidence from animal and human studies suggests that vitamin D, both cholecalciferol and calcitriol, may play a role in modifying the risk of diabetes. Some authors have reported that vitamin D stimulated the expression of insulin receptor, thereby enhancing insulin responsiveness in glucose transport, improving glycaemic control, insulin secretion and insulin resistance.

However, studies on the mechanism and appropriate doses of vitamin D are still unclear and requires further investigation. Further in vivo studies are needed to address the effect and the effective dosages of vitamin D in human adipose tissue as well as its relevance in associated diseases.

GLUT4 has a main role in glucose metabolism and the maintenance of glucose homeostasis in the body; its activation has become a therapeutic target in pharmacological intervention strategies to control diabetes. Adipose tissue is a major site of glucose metabolism and has a critical role in the maintenance of glucose homeostasis.

This study reports the effects of cholecalciferol in the histopathology of adipose tissue in diabetic rats, particularly related to its diameter and expression of GLUT4. This study is expected to reinforce the role of vitamin D as adjunctive therapy in diabetes mellitus.

METHODOLOGY

Twenty eight Wistar strain adult male rats (Rattus norvegicus) matching the inclusion criteria were acclimatized for 7 days. Combination of high fat diet (lard 22.8%) and intraperitoneal injection of 35 mg/kg streptozotocin (STZ) on day 14 were used to induce diabetes. Furthermore, seven days after STZ injection, the FBG from all the rats’ tail vein blood were evaluated. They were defined as diabetic rats when the FBG >135 mg/dl.

All diabetic rats (nineteen) were divided into one control (K) group and three treatment groups (X1, X2, X3). Control group was a group of diabetic rats given propylene glycol in volume of 1 ml/100 gram/body weight (bw). Treatment groups were groups of diabetic rats given cholecalciferol with a dose of 6.25 µg/kg bw (concentration 0.625 µg/ml) in X1 group, 12.5 µg/kg bw (concentration 1.25 µg/ml) in X2 group, and 25 µg/kg bw (concentration 2.5 µg/ml) in X3 group. Cholecalciferol was given in propylene glycol in volume of 1 ml/100 g bw per orem, every day for 14 days, starting on the 21st day. Twenty-four hours after the last treatment, the rats were then fasted for 12 hours and anesthetized with intramuscular injection of ketamine HCl in a dose of 44-60 mg/kg bw, then the FBG was measured from intracardiac blood. The rats were sacrificed by decapitation. Subcutaneous adipose tissue was taken through a 1 x 1 cm incision on the abdominal wall up to

Corresponding Author: Dewi Ratna Sari, dr, MS
Department of Anatomy and Histology, Faculty of Medicine
Universitas Airlangga Prof. Dr. Moentopo 47
Surabaya 60131, East Java, Indonesia
Tel. No.: +62315053804
Fax No.: +62315022075
Email: dr.dewirs@gmail.com

*Part of this paper has been presented in 15th Asian Oceania Congress of Endocrinology (AOCE), October 9, 2014, Cebu City, Philippines.
the subcutaneous layer. The tissue was fixed in neutral buffered formalin solution to be processed into histological preparations by Haematoxylin Eosin (H&E) and immunohistochemistry staining. FBG, adipocyte diameter and GLUT4 expression were analyzed by one-way ANOVA test (α = 0.05) and Least Significant Difference (LSD) for Multiple Comparison Procedure (MCP).

RESULTS AND DISCUSSIONS

There was no significant difference in fasting blood glucose and adipocyte diameter between groups (p>0.05). However, adipocyte diameter was increased in a dose dependent manner after administration of cholecalciferol.

There was significant difference in the expression of GLUT4 between groups (p <0.05). There was an increasing trend of GLUT4 expression along with the increased dose of cholecalciferol (Table 1).

Our findings have shown that cholecalciferol administration could not improve blood glucose homeostasis nor increase adipocyte diameter in diabetic rats. These results were consistent with Calle et al, (2008) who reported that the administration of calcitriol (1,25 dihydroxyvitamin D3) 3.75 µg/kg bw intraperitoneally for 15 days did not correct hyperglycemia, glycosuria and could not increase adipocyte diameter in STZ-induced diabetic rats, but could normalize the number of insulin receptors in adipocytes.14 Another study by Anwar et al, (2013) reported that the subcutaneous administration of 10 µg/100g (0.1 µg/kg bw) cholecalciferol for 6 days can reduce FBG by 26.31% in diabetic rats.2 These findings suggest that the route of administration may affect the effects of vitamin D in improving blood glucose levels and adipocyte diameter in diabetic rats.

Orwoll et al, (1994) reported that vitamin D has no effect on glucose homeostasis in uncontrolled diabetic patients.15 Another study reported that the administration of high dose cholecalciferol in type 2 diabetic patients was not related to improvement in glucose homeostasis but rather to improvement in plasma adiponectin levels.16 Recently, in vitro studies report that vitamin D may increase the surface area of inflammation-induced adipocyte, which is analogous to the diabetic condition.17 Thus, it can be concluded that vitamin D has more influences in inflammatory process mediated by adipokines produced by adipose tissue in diabetes.

The GLUT4 expressions increased dependently along with the increasing dose of cholecalciferol. However, this finding was not statistically significant. In line with the study by Manna and Jain (2012), vitamin D may increase the GLUT4 translocation and glucose utilization in adipocytes through activation of cystathionine-γ-lyase (CSE) and the formation of hydrogen disulfide (H2S).8

| Table 1. Fasting blood glucose, adipocyte diameter and GLUT4 expression in adipocyte |
|-------------------------------------------------|-----------------|-----------------|-----------------|-----------------|
| Fasting blood glucose (mg/dl) | Cholecalciferol 6.25 µg/kg bw, n=5 | Cholecalciferol 12.5 µg/kg bw, n=5 | Cholecalciferol 25 µg/kg bw, n=5 | p value |
| Adipocyte diameter (µm) | 40.68±9.10 | 48.23±16.39 | 50.57±16.29 | 62.40±15.65 |
| GLUT4 expression in adipocyte (Σ cell) | 7.45±1.18 | 10.98±2.14c | 12.12±2.75c | 12.50±3.08c |
| a. Significant difference with Diabetic control group (p= 0.049) |
| b. Significant difference with Diabetic control group (p= 0.013) |
| c. Significant difference with Diabetic control group (p= 0.008) |

Figure 1. Photomicrograph of adipocyte diameter (H&E, x 400).

Figure 2. Photomicrograph of GLUT4 expression in adipocyte, immunohistochemistry, graticulae (H&E, x 400). Arrow head: Adipocyte which did not express GLUT4. Arrow: Adipocyte which expressed GLUT4.
Cholecalciferol can increase the expression of GLUT4 in adipocyte without altering adipocyte diameter and FBG. It might be due to differences in tissue response to insulin. It has been understood that the response of GLUT4 to insulin in adipose tissue is higher than muscle tissue and the rate of fatty acid synthesis in adipocytes is strongly influenced by the plasma insulin concentration. However, in adipocytes of rats on a high fat diet, the fatty acid synthesis is highly unresponsible to insulin in which all lipogenic enzyme activities were decreased. A decreased intracellular capacity to utilize glucose for lipogenesis led to the decreased response of glucose metabolism to insulin in adipocytes of rats on high fat diet. In this study, although administration of cholecalciferol can increase adipocyte glucose uptake, it could not restore the intracellular capacity reduction in utilizing glucose for lipogenesis, which has been proven by non-significant increment in adipocyte diameter. Unfortunately, it has not been supported by the plasma insulin level as well as the rate of lipogenesis and lipolysis.

Adipose tissues only take up a small fraction of total body glucose uptake, but it increases along with the elevation of insulin level.

In addition, administration of cholecalciferol in this study might be unable to inhibit glucose production in the liver and increase glycogen storages both in the liver and muscle tissue. There have been studies reporting that vitamin D administration can improve metabolic disorders in STZ-induced diabetic rats and provide therapeutic or protective effects for the liver, pancreas and kidneys of diabetic mice induced by alloxan. However, no study has reported the effects of vitamin D on metabolic disorders improvement in the liver of diabetic animals induced by a combination of high-fat diet and STZ. Although one study reported that vitamin D can increase GLUT4 translocation in the muscle tissue of STZ-induced diabetic mice, there is no study reporting the same finding in diabetic animal induced by combination method.

Tannenbaum et al (1997) reported that a high fat diet can lower glucose uptake in skeletal muscle and adipose tissue, decrease the number of insulin receptors in the liver, skeletal muscle and adipose tissue, decrease glycolysis and glycogen synthesis in the liver. High fat diet alters the activity of the hypothalamus-pituitary-adrenal in rats, thus increases the production of adrenal glucocorticoid. Increased adrenal glucocorticoid has antagonistic effects on insulin, leading to insulin insensitivity and decreased glucose uptake in insulin target tissues. Therefore, in this study, cholecalciferol could not significantly reduce FBG level, although the expression of GLUT4 in adipocyte was increased.

CONCLUSION

Cholecalciferol administration can increase adipocyte GLUT4 expression without altering fasting blood glucose level and adipocyte diameter in diabetic rats. Increasing the number of experimental animals, dose variations, duration of administration, and caloric restriction, may obtain a better outcome. Other methods, such as GLUT4 quantification in membrane fraction by Western blot may produce a more accurate result.

References

19. Begoña M, Campion J, Dávila N, Calle C. Stimulation by 1,25-


22. George N, Kumar TP, Antony S, Jayanarayanan S, Paulose CS. Effect


24. Sakinah EN. Pharmacodynamics study of cholecalciferol to GLUT4

25. Tannenbaum BM, Brindley DN, Tannenbaum GS, Dallman MF,

Articles and any other material published in the JAFES represent the work of the author(s) and should not be construed to reflect the opinions of the Editors or the Publisher. Authors are required to accomplish, sign and submit scanned copies of the JAFES Declaration: that the article represents original material, that is not being considered for publication or has not been published or accepted for publication elsewhere. Consent forms, as appropriate, have been secured for the publication of information about patients; otherwise, authors declared that all means have been exhausted for securing such consent. The authors have signed disclosures that there are no financial or other relationships that might lead to a conflict of interest. All authors are required to submit Authorship Certifications that the manuscript has been read and approved by all authors, and that the requirements for authorship have been met by each author.