Response of Pancreatic AKT1 Gene Expression, Insulin and Glycemic Indices to the Aerobic Training Period in Type 2 Diabetes Wistar Rats

Vahid Imanipour[1], Nader Shakeri*[2], Khosro Ebrahim[2], Shahram Soheyli[3]

1. PhD, Department of Physical Education and Sport Sciences, Faculty of Humanities and Social Sciences, Science and Research Branch, Islamic Azad University, Tehran, Iran.
2. PhD, Department of Physical Education and Sport Sciences, Faculty of Humanities and Social Sciences, Science and Research Branch, Islamic Azad University, Tehran, Iran.
3. PhD, Department of Physical Education and Sport Sciences, Shahr-e-Qods Branch, Islamic Azad University, Tehran, Iran.

***Correspondence:**
Nader Shakeri, PhD, Department of Physical Education and Sport Sciences, Faculty of Humanities and Social Sciences, Science and Research Branch, Islamic Azad University, Tehran, Iran.
Tel: (98) 910 450 0995
Email: nsprofsport@gmail.com

Received: 3 January 2018

Accepted: 20 March 2018

Published in April 2018

Abstract

Objective: Laboratory studies on diabetic rats have shown that diabetic rats have a lower beta cell mass than healthy rats. On the other hand, over the years, with tremendous advances in genetic science, many studies have been done on the expression of genes and its effective factors, such as physical activity. One of these genes is AKT / PKB. Due to the association between the expression of this gene and type 2 diabetes, the effect of physical activity on the expression of this gene seems to be necessary.

Materials and Methods: 30 male Wistar 10 week olds were randomly divided into three groups: healthy control, diabetic control and diabetic training. The diabetic control group was diabetic and injected with nicotinic amide and streptozotocin. The diabetic group was trained for 12 weeks Aerobic on the treadmill.

Results: Based on independent t-test, aerobic training showed a significant increase in the relative expression of AKT / PKB in the pancreatic tissue of the aerobic diabetic group compared to the diabetic control group.

Conclusion: The AKT / PKB signaling pathway not only plays an important role in insulin resistance, but also plays an important role in the ability of beta cells to adapt to increased insulin levels. Therefore, it can be concluded that environmental factors such as aerobic activity. The expression of the AKT / PKB gene expression can be effective in improving the treatment of type 2 diabetes.

Keywords: AKT / PKB gene expression, Type 2 diabetes, Aerobic exercise

Introduction

Type 2 diabetes (T2DM) is the most common metabolic disorders. In addition to hyperglycemia and insulin resistance, T2DM causes many diseases, especially cardiovascular disease. Beside genetic and hereditary factors, high-calorie diets, obesity, overweight, an inactive and sedentary life style are the most important causes of T2DM.Obesity is associated with insulin resistance as the primary cause of T2DM. (1). Also, imbalance of some hormonal factors, such as inflammatory or anti-inflammatory cytokines, influences on the blood glucose and insulin level. Today's studies strongly emphasize the recognition of genetic factors affecting diabetes and other chronic diseases. In the last decade, several genetic factors that are important in the

prevalence or severity of type 1 and 2diabetes have been identified. Recently, some genetic factors associated with increased risk of T2DMwere identified by genetic link studies (2). So that the disorder or polymorphisms in that area may predispose to the prevalence or increased severity of diabetes due to impaired secretion of insulin or damage to some insulin stimulants or the synthesis of insulin. One of these genes is the AKT1 / PKB, which were considered by the researchers as the effect of increasing and decreasing the expression of this gene on diabetes in recent years. Various studies suggest that the signaling pathway controlled by AKT1 / PKB not only play an important role in insulin resistance, but also contribute to increasing the compatibility of beta cells to release more insulin.(3). AKT1 / PKB activity is regulated by dependent and non-dependent mechanisms of PI3K and requires multiple steps involving in their translocation to the plasma membrane and subsequent phosphorylation. The activity of AKT1 / PKB lead to phosphorylation of several substrates, which control several signaling cascades, such as glucose transfer by insulin, protein synthesis and glycogen, cell growth, cell differentiation and survival (4). In transgenic mice, whose overexpress a constitutively active variant of AKT1 / PKBin beta cells, there has been an increase in proliferation, neogenesis, cell size, insulin secretion and synthesis which indicate the effective role of this protein in the physiology of beta cells in the pancreas. In other words, increasing the expression of AKT1 / PKB in beta cells leads to increased proliferation, cell mass, the synthesis and secretion of more insulin (5). Therefore, the present study aimed to evaluate the effect of an aerobic training period on the expression of AKT1 / PKB, insulin, glucose and beta cell function in male Wistar rats with T2DM.

Materials and Methods

The studied sample consisted of all male Wistar rats in the animal house of the Pasteur Institute, among them 30 male Wistar 10 weeks old in a weighing range of 20 ± 220 gr were selected to participate in the study. Wistar rats were randomly divided into 3 groups including 2 diabetic and 1 healthy control groups. The rats were kept at the animal house of Azad University of Parand in a 5 to 10 meter room under controlled light conditions (12 hours of light and 12 hours of darkness), temperature (22± 3 ° C), and humidity in the range of 30 to 60. At first, the rats were acquainted with the environment in the animal house for 2 weeks and how to run on the treadmill. Then, after a fasting night (12 hours), nicotineamide and streptozotocin were injected to induce T2DM. Initially, a solution of nicotinamide at a dose of 110 mg per kg of rat mice was injected to peritoneal; after 15 minutes, the freshly prepared STZ solution in the citrate buffer with PH = 4.5 was also administered at a dose of 60 mg per kilogram were injected. The healthy control group received only the same volume citrate buffer . After one week, fasting blood glucose was measured and glucose level above 150 mg / dL was considered as T2DM (6). A training program for 12 weeks of aerobic training and 5 sessions per week with gradual increase in speed (18-26 m / min) and time (10 to 55 minutes) in the form of running on treadmill with the aim of determining its effect on fasting glucose, serum insulin, beta cell function and relative expression of AKT1 / PKB in pancreatic tissue were compared to the control group that did not participate in the training program. About 48 hours after the last training session (10 to 12 hours fasting), all rats were anesthetized by injecting 10% ketamine (50 mg/kg) and xylosin 2% (10 mg/kg). After assuring anesthesia, the blood samples were taken directly from the animal's heart. Blood samples were centrifuged for 20 minutes for serum separation and maintained at 80 ° C for glucose and insulin measurement. Rat pancreatic tissue was sampled and washed in physiological serum in RNAlater^{TM} liquid micro-tubes and transferred to the laboratory for genetic tests. Also, the function of beta

cells was calculated using fasting insulin and glucose level in the software HOMA2-Calculator. All statistical analyzes were performed using SPSS / Win software version 16. The Kolmogorov-Smirnov test was used to ensure that the distribution of data is normal. Data analysis was performed using independent T-test. Changes less than 5% were reported significant.

Results

Based on the independent T-test, aerobic training caused a significant decrease in fasting glucose and a significant increase in serum insulin and beta-cell function in the aerobic diabetic group compared to the diabetic control group. (Table 1)

Also, aerobic exercise led to a significant increase in the relative expression of AKT1 in the pancreatic tissue of the aerobic diabetic group compared to the diabetic control group.

The relative expression of AKT1 was 1 in Diabetic control group and 1.60 ± 0.47 in Aerobic diabetic group (*P*-value: 0.006)

Discussion

The main pathophysiological reasons of T2DM are insulin resistance and beta cell dysfunction. (7). In healthy people, secretion of insulin from the pancreas is linked through a negative feedback loop to the peripheral body insulin sensitivity that allows beta cells to compensate for changes in whole body sensitivity to insulin through proper secretion of insulin (7). However, in the pathogenesis of diabetes, degeneration or progressive impairment of beta cell function leads to a failure in sufficient insulin secretion to overcome insulin resistance (8). On the other hand, over the years, researchers have shown that diabetes is the result of complex interactions between genetic and environmental factors on fat and glucose metabolism such as impairment of liver, muscle, insulin secretion, fat metabolism, total body lipolysis, and metabolic impairment in other organs of the body. Although the main cause of type 2 diabetes is insufficiency of insulin secretion from pancreatic beta cells to compensate for insulin resistance (9). Therefore, many studies are underway to determine the effect of external stimuli for the treatment of type 2 diabetes. Meanwhile, the importance of exercise and physical activity in diabetes management is so much that some studies have suggested that exercise even in the absence of weight loss or body mass decreased glycosylated hemoglobin and blood glucose levels in diabetic patients (10). In the present study, induction of type 2 diabetes in Wistar rats led to a significant decrease in beta cell function compared to healthy group. Also, since genetic interventions are one of the most important factors affecting the dysfunction of beta cells, and the consequence of lowering the secretion of insulin from these cells, it is questioned whether external stimuli such as diet modification or participation in different training programs are able to correct the genetic factors that affect the function of these cells. Although studies in this area are very limited and it is difficult to provide a conclusive conclusion, however, various studies have shown that the signaling pathways controlled by AKT / PKB not only play an important role in insulin resistance, but also in the ability of beta cell to adapt to an increase in insulin demand. AKT / PKB activity is regulated by dependent and independent PI3K mechanisms and requires multiple steps involved in their translocation to the plasma membrane and subsequent phosphorylation.

In transgenic mice, which have overexpress a

Table 1. Fasting glucose, insulin and beta cell function in aerobic and controlled diabetic groups

Variable	Diabetic control Mean ± SD	Aerobic diabetic Mean ± SD	P-value
Glucose (mg/dL)	294 ± 11	240 ± 14	0.001
Insulin µIU/ml)	4.06 ± 0.21	5.11 ± 0.25	0.001
Function of beta cell (HOMA-BF)	6.32 ± 0.31	10.42 ± 0.81	0.001

SD: standard deviation

constitutively active variant of AKT / PKB in beta cell, an increase in proliferation, neogenesis, cell size and synthesis secretion, provide additional evidence of the involvement of this protein in the physiology of pancreatic islets (8). In other words, increasing the expression of AKT / PKB in beta cells leads to increased proliferation and cell mass, and the synthesis and secretion of more insulin (11). In contrast, a mutation in the AKT / PKB gene, which leads to a 80% reduction in kinase activity, is associated with glucose intolerance and insufficiency of insulin secretion (12). Reduction of glucose or glycemic control by AKT is not limited to pancreatic cells and is probably because of prolonged muscle contractions due to AKT signaling pathways in protein production processes.AKT is probably activated by the non-PI3K mechanism and increases the intracellular concentration of calcium ions. These effects result from an increase in CAMP concentration during muscle contractions (13,14). In addition, there are reports of increased activity and phosphorylation of AKT / PKB due to muscle hypertrophy by activating mTOR and stopping glycogen synthase kinase 3B (GSK-3B), which ultimately make important changes in the level of insulin sensitivity and the concentration of this hormone in the body (15). Presse et al., Found that the regulation of AKT / GSK-3B and AKT / mTOR in human skeletal muscle is the basis of muscle hypertrophy. In a similar study, Chui and Bumkim (13) found that inhibiting the AKT1 gene destroys the growth pattern in mice. Increased insulin sensitivity is due to increased PK3 / PKB phosphorylation by PI3K (16,17). In muscle fibers, contractile stimulation increases molecular variations by activating two pathways of mTOR and GSK-3B, leading to muscle hypertrophy (15). The AMPK activity increases in response to increased glucose tolerance due to participation in exercise training (18). Anderson et al. showed that in skeletal muscle, increased transcription of mRNA in GLUT4 and several other proteins involved in insulin signals and glucose metabolism such as AKT, PI3K, AMPK, and mTOR occur in aerobic exercise and resistance exercises (19). The current research and other studies in this field provide evidence that various exercise training influence on the intracellular regulation of signaling pathways that lead to glucose uptake. In this regard, the interaction of insulin resistance, AKT and PI3K lead to protein synthesis and GLUT4 displacement towards the cell membrane. Therefore, participation in regular exercise training is effective in control and treatment of metabolic diseases such as diabetes and insulin resistance. In general, the disorder or decline in the function of beta cells is associated with a decrease in the synthesis and secretion of insulin. On the other hand, Available evidence has strongly emphasized that, in addition to increasing insulin resistance, the decrease in beta cell function is also one of the most important causes of the prevalence or increased severity of type 2 diabetes. Although increased insulin resistance occurs in response to lack of dietary control and sedation and obesity, but impairment in beta cell function or decreased synthesis and insulin secretion appears to be largely due to genetic factors. Clinical and laboratory studies, especially in the last decade, have supported the potential impact of transcription factors and their variants on the prevalence of type 2 diabetes. Meanwhile, AKT1 gene expression has been described as an important genetic factor of type 2 diabetes. Increasing the expression of AKT in beta cells of the pancreas reduces the desire for type 2 diabetes which is associated with increased activity of beta cells and insulin synthesis. In the present study, induction of type 2 diabetes by injection of nicotinamide and STZ was associated with a decrease in the expression of AKT1 in the pancreatic tissue and a significant reduction in serum beta and insulin serum levels, with a significant increase in glucose in diabetic rats, than in healthy subjects. However, 3 months of aerobic exercise training resulted in a significant reduction in glucose and increased beta cell function along with higher serum

insulin levels than in the control group that did not participate in the training.

Conclusions

Therefore, according to the results of this research and previous studies, the possibility of the effect of changes in the relative expression of this gene on beta function and insulin secretion in response to aerobic exercise for 12 weeks was reported. Although it should be noted that in addition to aerobic exercise, other types of exercise training, environmental factors and direct and indirect pathways associated with other genes can also contribute to the level of insulin secretion.

References

1. Catherine M. Nutrigenetics and Metabolic Disease: Current Status and Implications for Personalised Nutrition. Nutrients. 2013;5(1):32-57.
2. Russell MA, Morgan NG. The impact of anti-inflammatory cytokines on the pancreatic β-cell. Islets. 2014;6(3):950547.
3. Manning BD, Toker A. AKT/PKB Signaling: Navigating the Network. CELL. 2017;169(3):381-405.
4. Elghazi L, Balcazar N, Bernal-Mizrachi E. Emerging role of protein kinase B/Akt signaling in pancreatic beta-cell mass and function. Int J Biochem Cell Biol. 2006;38:157-63.
5. Bernal-Mizrachi E, Wen W, Stahlhut S, Welling CM, Permutt MA. Islet beta cell expression of constitutively active Akt1/ PKB alpha induces striking hypertrophy, hyperplasia, and hyperinsulinemia. J Clin Invest. 2001;108(11):1631-8.
6. Ueki K, Okada T, Hu J. Total insulin and IGF-I resistance in pancreatic beta cells causes overt diabetes. Nat Genet 2006;38:583-8.
7. Anders R, Ola H. Mechanisms whereby genetic variation in the TCF7L2 gene causes diabetes: novel targets for anti-diabetic therapy? New grants from Hjelt foundation. 2013.
8. Bergman RN, Finegood DT, Kahn SE. The evolution ofβ -cell dysfunction and insulin resistance in type 2 diabetes. Eur J Clin Invest 2002;32(3):35-45.
9. Amra C. The T-Allele of TCF7L2 rs7903146 Associates with a Reduced Compensation of Insulin Secretion for Insulin Resistance Induced by 9 Days of Bed Rest. DIABETES. 2010;59:836-43.
10. Boule´ NG, Haddad E, Kenny GP, Wells GA, Sigal RJ. Effects of exercise on glyce- mic control and body mass in type 2 diabetes mellitus: a meta-analysis of con- trolled clinical trials. JAMA 2001;286:1218-27.
11. Bernal-Mizrachi E, Wen W, Stahlhut S, Welling CM, Permutt MA. Islet beta cell expression of constitutively active Akt1/ PKB alpha induces striking hypertrophy, hyperplasia, and hyperinsulinemia. J Clin Invest. 2001;108(11):1631-8.
12. Bernal-Mizrachi E, Fatrai S, Johnson JD, Ohsugi M, Otani K, Han Z, et al. Defective insulin secretion and increasedsusceptibility to experimental diabetes are induced by reduced Akt activity in pancreatic islet beta cells. J Clin Invest. 2004;114(7):928-36.
13. Melo SFS, Amadeu MA, Magalhães CF, Fernandes T, Carmo EC, Barretti DLM, et al. Exercício de forçaativa a via AKT/mTorpelo receptor de angiotensina II tipo I no músculocardíaco de ratos. Rev Bras EducFísEsporte São Paulo. 2011;3(25):377-85.
14. Najeeb AS, Zou MH. AMPK: A cellular metabolic and redox sensor. A mini-review. Front Biosci (Landmark Ed). 2014;(1):19:447-74.
15. Pauli JR, Cintra DE, Souza CT, Ropelle ER. Novosmecanismospelosquais o exercíciofísicomelhora a resistência à insulina no músculoesquelético. Arq Bras EndocrinolMetab. 2009;53(4):399-408.
16. Sakamoto K, Arnolds DEW, Ekberg I, Thorell A, Goodyear LJ. Exercise regulates Aktand glycogen synthase kinase-3 activities in human skeletal muscle. Biochemical and Biophysical Research Communications. 2004;325:419-25.
17. Sharma N, Arias BE, Bhat AD, Sequea DA, Ho S, Croff KK, et al. Mechanisms for increased insulin-stimulated Akt phosphorylation and glucose uptake in fast- and slow twitch skeletal muscles of calorie-restricted rats. Am J PhysiolEndocrinolMetab.2011;300: 966-78.
18. Shaw M, Cohen P, Alessi DR. The activation of protein kinase B by H2O2 or heat shock is mediated by phosphoinositide 3-kinase and not by mitogen-activated protein kinase-activated protein kinase-2. Biochem J. 1998;(15):336:241-6.
19. Zanchi NE, Lancha AH Jr. Mechanical stimuli of skeletal muscle: Implications on mTOR/p70s6k and protein synthesis. Eur J Appl Physiol. 2008;102(3):253-63.