Research in Health Science
ISSN 2470-6205 (Print) ISSN 2470-6213 (Online)
Vol. 8, No. 4, 2023
www.scholink.org/ojs/index.php/rhs

Original Paper

Effect of Aerobic Exercise Intervention on Renal Insulin Signaling Pathway in db/db Mice

Qingyi Liu[1]

[1] University of Edinburgh, Edinburgh, United Kingdom

Received: August 9, 2023 Accepted: September 2, 2023 Online Published: September 6, 2023
doi:10.22158/rhs.v8n4p1 URL: http://dx.doi.org/10.22158/rhs.v8n4p1

Abstract

Objective: To study the effects on insulin signaling pathway in db/db mice during aerobic exercise and discuss the related mechanisms. Methods: Ten BKS mice were selected as the quiet control group (WT group), and 20 BKS-db/db mice were randomly divided into two groups as the diabetic quiet control group (C group) and the diabetic simple exercise group (E group), with 10 mice in each group. The mice in E group were acclimatized to the low-intensity criterion (2~7m/min, 10min/day, 5 days/week) for one week, and then subjected to 8 weeks of aerobic endurance exercise training on a running platform (0°incline, 7~12m/min, 30~40min/day, 5 days/week), while the mice in groups WT and C were kept quietly. At the end of the experiment, the fasting blood glucose content, glucose tolerance, insulin tolerance of each group of mice were detected respectively, and the expression levels of AKT protein and insulin receptor protein were detected by Western Blot technique. Results: (1) Compared with the diabetic quiet control group (Group C), the glucose tolerance and insulin tolerance in the quiet control group (Group WT) showed significant changes (P <0.05); in the diabetic simple exercise group (Group E), there was no significant change in glucose tolerance and insulin tolerance (P >0.05). (2) Compared with the diabetic quiet control group (Group C), the expression level of insulin receptor protein was down-regulated (P<0.05) and AKT protein expression was up-regulated (P<0.05) in the diabetic simple exercise group (Group E); compared with the quiet control group (Group WT), the expression level of insulin receptor protein was increased (P<0.05) and AKT protein was decreased (P<0.05) in the diabetic quiet control group (Group C), expression level decreased (P<0.05). Conclusion: Exercise alone could not improve insulin resistance in db/db mice; eight weeks of aerobic exercise improved and corrected renal insulin signaling pathway and alleviated renal insulin resistance in db/db mice.

Keywords

Aerobic exercise, db/db mice, Insulin signaling pathway

1. Research Purpose

Diabetes mellitus (DM) is a clinical syndrome resulting from the interaction of multiple factors and other synergistic or combined impairments. Many of the complications that affect the health and quality of life of patients such as retinopathy, DPN, diabetic nephropathy, diabetic foot, etc. are due to diabetes, and these complications can be life-threatening in severe cases. Diabetic Kidney Disease (DKD) is one of the major complications of DM, which is a microangiopathy caused by high glucose microenvironment. In terms of histological characteristics, it is mainly characterized by extracellular matrix accumulation, excessive thickening of the basement membrane and abnormal cell proliferation caused by tethered dilatation disease, as well as glomerulosclerosis and interstitial fibrosis of the kidneys, and the main manifestation is the gradual decrease of renal function, and the kidneys may ultimately fail and gradually develop into end-stage renal disease.

Patients with DKD may develop insulin resistance (IR) in other tissues of the body, such as skeletal muscle or kidney, as part of a series of cardiovascular metabolic abnormalities, which are due to genetic factors and environmental dysfunctions, as well as a variety of other detrimental influences, causing a decrease in the rate and utilization of glucose uptake; at the same time, a series of negative feedback regulatory mechanisms based on phosphorylation in the cellular insulin signaling pathway are disrupted, resulting in a variety of physiological conditions. At the same time, a series of negative feedback regulation mechanisms based on phosphorylation in the cellular insulin signaling pathway in the body start to be disturbed, leading to a variety of physiological characteristics, such as insulin sensitivity and responsiveness will be reduced, which will lead to metabolic disorders within the body tissues (glucose, lipid-related), and ultimately may lead to a variety of metabolic disorders, such as DM, CM, CVD, and other diseases (Niu et al., 2009; Zick, 2004; Zick, 2005). IR is a major pathogenic risk factor for DKD and one of the mechanisms to control its progression.The common pathogenic factors of IR in patients with DKD syndrome include systemic factors and other local factors, and the mechanism also involves glomerular cells, tubular epithelial cells, and other intrarenal tissue cells. tissue cells. In the kidney, GLUT is the main transporter on glomerular thylakoid cells, and increasing the activity of AKT can increase the activity of GLUT and increase the uptake of glucose by a variety of target cells; if the expression of GLUT is abnormal, it will lead to the dysfunction of glucose uptake and absorption by the target cells, and thus IR will occur.

A large number of studies have demonstrated that it is possible to induce IR production in the organism by enhancing the activity of serine/threonine phosphorylation on insulin receptor substrate 1 (IRS1), which allows for the blockage of insulin signaling (Cullen, Brice & Kahn, 2006). Protein kinase B (PKB), also known as AKT, is a serine/threonine kinase involved in a large number of cellular processes, including glycolysis, glycogen synthesis, adipogenesis, gluconeogenesis inhibition, and cell survival; one of the many important functions of AKT is to regulate the metabolic action of insulin in order to stimulate glucose transport (Whyte, Gill, & Cathcart, 2010), and it is a key protein on the PI3K signaling pathway. AKT can be activated by a variety of growth signals and there have been many

studies on the biochemical mechanisms of AKT activation. Once activated, AKT proteins become involved in regulating many biological functions regarding cell survival, proliferation, migration, metabolism, and synthesis of downstream proteins in the vasculature. At the same time, AKT is a central node in many signaling pathways. Insulin receptor protein (INSR) is one of the ligand-activated receptors and tyrosine kinase proteins of transmembrane signaling proteins, and INSR is an important signaling regulator and activator of cellular value-added and differentiation, growth metabolism, and nutrient metabolism. INSR possesses a number of distinctive physiological and biochemical properties, and its main physiological role is metabolic regulation, which is distinct from other receptor tyrosine kinases involved in the regulation of cell growth and/or differentiation. Therefore, insulin receptor protein's are the most common cause of abnormal insulin signaling pathways in IR (Wang, 2017).

The main relevant signaling sources and signal-regulated protein pathways of IR include insulin receptor substrate (IRS) proteins, serine-threonine kinase (AKT) pathway, adenosine monophosphate-activated protein kinase (AMPK) pathway, and mitogen-activated protein kinase (MAPK) pathway. Studies and reports have found that aerobic exercise can significantly correct the structural-functional imbalance of the PI3K/AKT pathway molecules in db/db mice, thus helping to regulate the utilization and reuse of sugar in the cells of db/db mice, which confirms that maintaining the stability of the PI3K/AKT pathway is an important goal and a key target for clinical interventions in diabetes mellitus (Liu et al., 2020). However, aerobic exercise has a significant impact on the AKT pathway in the kidney. intervention on AKT and insulin receptors in the kidney needs to be further investigated. In summary, this experiment was conducted by testing glucose tolerance, insulin tolerance, renal AKT protein level and renal insulin receptor protein level change, summarizing the change rule and exploring the mechanism of action.

2. Research Methods

2.1 Experimental Animals and Grouping

The experimental subjects were purchased from Jiangsu Jicui Pharmachem Technology Co., Ltd, Animal License No.: SCXK (Su) 2018-0008, and all the mice were safely and separately caged in the Animal Experiment Center of Jiangsu Academy of Agricultural Sciences, and fed with normal feed (purchased from Qinglongshan Animal Breeding Center, Jiangning District, Nanjing City) during the modeling period, and all the mice were free to eat and drink during the feeding period, allowing the mice to live a normal life. During the feeding period, all mice were free to eat and drink water. To allow the mice to live normally, the test center chose a light ratio of 12h:12h in the rearing environment, and regulated the temperature to maintain at 22±2℃.

2.2 Methods of Intervention

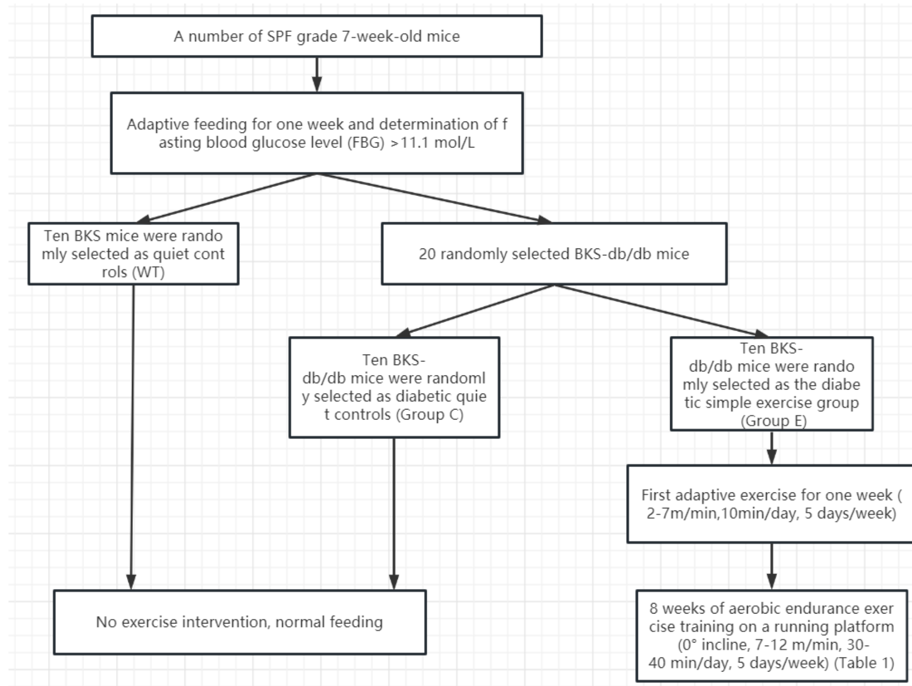

Figure 1. Grouping of Experimental Animals and Intervention Methods

All mice were first acclimatized and fed for 1 week, 10 BKS mice were used as the quiet control group (WT group), and diabetic-onset mice were screened based on fasting blood glucose level (>250 mg/dL) among multiple BKS-db/db mice. The eligible diabetic mice were then randomly divided into two groups: diabetic quiet control group (Group C) and diabetic simple exercise group (Group E), with 10 mice in each group. Except for the mice in the WT and C groups, which were not subjected to exercise, the feeding conditions, etc., were kept the same, except for the different conditions of exercise intervention (Figure 1).

Table 1. Exercise Intervention Table for Mice

Number of Weeks	1	2	3	4	5	6	7	8
Speed (m/min)	7	9	10	10	11	12	12	12
Time (min)	30	30	30	40	40	40	40	40

2.3 OGTT (Glucose Tolerance), ITT (Insulin Tolerance) Testing

After the mice were fasted without water for 4h, the mass of the mice would be weighed, and the OGTT test would be performed by gavage using a glucose solution standardized at 1g/kg body weight, and the ITT test would be performed by intraperitoneal injection using an insulin solution standardized at 0.75 U/kg body weight. In all mice, blood was collected from the tail vein at time points of 0, 15, 30, 60, and 120 min, and blood glucose values were determined.

2.4 Taking Materials

Mice were anesthetized intraperitoneally using 5% sodium pentobarbital (30 mg/kg) intravenously. The abdominal cavity of all mice was opened, and 5 ml of blood was taken from the inferior vena cava of all mice in the three groups, which was placed for a period of time and then centrifuged for 10 min using a machine at 3000 r/min to obtain its plasma, which was then stored in a refrigerator at -80 degrees Celsius. After blood sampling, 6 mice were randomly selected from each of the three groups. After anesthesia, the mice were anesthetized, and the kidneys were found on both sides of the spine and in the extraperitoneal lumbar region in the shape of a fava bean, with a smooth and slightly flattened surface, and then fresh kidneys were taken out bilaterally, and the fascial tissues around the kidneys of the mice were removed carefully, and then repeatedly washed in sterile saline in a petri dish. After that, the kidneys were wrapped in tinfoil and placed in sterile cryopreservation tubes and frozen in liquid nitrogen, and then transferred to an ultra-low temperature refrigerator at -80℃ for freezing.

2.5 Western Blot Assay

2.5.1 Extraction of Kidney Tissue Proteins

Take out the corresponding kidney tissue samples from -80℃ refrigerator, rinse with pre-cooled sterile saline and remove the excess sterile saline on the surface of the kidney tissue samples with clean filter paper, after each group of samples was selected for quantification, the samples were fully ground by adding RIPA lysate and protease inhibitor, and the supernatants were taken as cytoplasmic proteins by centrifugation of the grinding liquid after 5min (14,000RPM). Protein quantification was performed.

The protein concentration of each sample in each group was determined using the BCA method, and the samples were added to the loading buffer according to 4:1 and put into a metal bath (97℃) for 10 min protein denaturation and then stored in a refrigerator at -30℃.

2.5.2 Gel Configuration

Wash the glass plate with purified water, mount it on a stationary rack, test the device for leaks, prepare a 10% SDS-PAGE gel and fill it up, add the gel to three-quarters of the glass plate and seal it with isopropyl alcohol to ensure that the gel surface can be leveled. After waiting for the separation gel to solidify (about 30 min), pour out the isopropyl alcohol in the slot and absorb it with filter paper, after that, prepare 10% concentrated gel and fill it up, insert the comb quickly while making sure there are no air bubbles, and wait for the concentrated gel to solidify and then carry out electrophoresis.

2.5.3 Sampling and Electrophoresis

Put the prepared gel into the electrophoresis tank, pour the electrophoresis solution in the middle of two glass plates, draw the comb in the electrophoresis solution, take the samples sequentially and put the pre-stained protein Marker on both ends of the samples (5ul at the beginning and 2ul at the end), connect the electrophoresis instrument and then start electrophoresis at a constant voltage of 90V, and then continue to electrophoresis at a constant voltage of 110V after the samples have passed through the concentration of the gel until the samples stop electrophoresis after they have run to the bottom.

2.5.4 Transfer and Closure

Select the appropriate size of PVDF membrane, put it into methanol to soak and then put it into equilibrium solution, take out the glass plate and put it into the transfer solution, excise the concentrated gel, use the wet transfer method to transfer the gel proteins uniformly onto the PVDF membrane with a pore size of 0.22 μm (Constant Pressure 100V, 75min), and close it for 90min at room temperature using 5% milk (TBST preparation) closure solution.

2.5.5 Incubation of Antibodies

Cut out the desired protein bands at the corresponding positions according to the molecular weight suggested by the Marker. The primary antibody was diluted using 5% milk, AKT protein, insulin receptor protein at a ratio of 1:1000 and GAPDH at a ratio of 1:5000, and incubated at 4 ℃ overnight. The following morning, the strips were washed 3 times (10 min/time) with sufficient washing buffer (TBST) at 10 min/time standard, and then incubated by 5% milk dilution of the secondary antibody (goat anti-rabbit) (the ratio of secondary antibody dilution is 1:10,000), and incubated at room temperature for 60 min. at the end of the incubation of the secondary antibody, the strips were washed again with a sufficient amount of TBST 3 times (10 min/time), and washed clean of residual membrane Antibody.

2.5.6 Luminescence

Configure the ECL ultrasensitive chemical in a 1:1 ratio and expose the protein strips in the instrument after the strips are in full contact with the luminescent solution.

2.6 Data Statistics and Analysis

The data of OGTT and ITT were analyzed by SPSS, and the expression results of AKT protein and insulin receptor protein from Western blotting were analyzed by Image Lab Software 5.2, an image analysis software of Bio-Rad multicolor gel imaging system. All data were plotted with GraphPad Prism 5.01 software, and the one-way ANOVA significance level was analyzed by SPSS software to determine whether the differences among WT, C, and E groups met $P<0.05$ and $P<0.01$.

3. Research Results

3.1 Basic Indicators

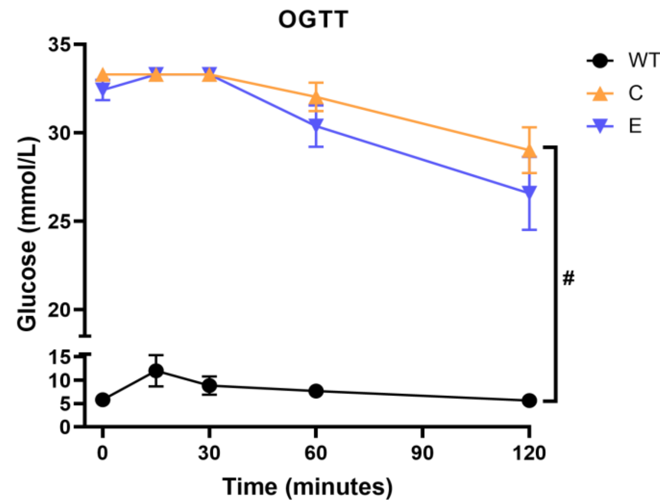

Figure 2. Glucose Tolerance

Note. # indicates a significant difference between Group C and WT, P < 0.05.

The glucose tolerance of the quiet control group (WT), diabetic quiet control group (C group), and diabetic simple exercise group (E group) was measured at the end of eight weeks of aerobic exercise (Figure 2), and it was found that the glucose tolerance of mice in C group was significantly higher, and the difference was significant (C vs. WT, P<0.05), but C group showed a decreasing trend in E group compared with E group, but the difference was not significant (C vs E, P > 0.05).

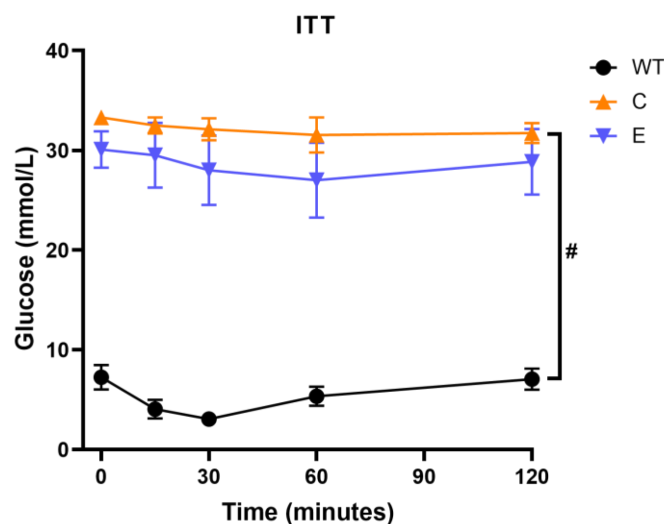

Figure 3. Insulin Tolerance

Note. # indicates a significant difference between Group C and WT, P < 0.05.

Meanwhile, as shown in the graph (Figure 3), among the WT, C, and E groups, the insulin tolerance level of the mice in the C group increased significantly and the difference was significant (C vs WT, P<0.05), but when the C group was compared with the E group, the E group showed a downward trend

(before 60 minutes) followed by an upward trend, and there was no significant difference between the C group and the E group (C vs E, P>0.05).

3.2 Renal Insulin Signaling Pathway Related Protein Detection

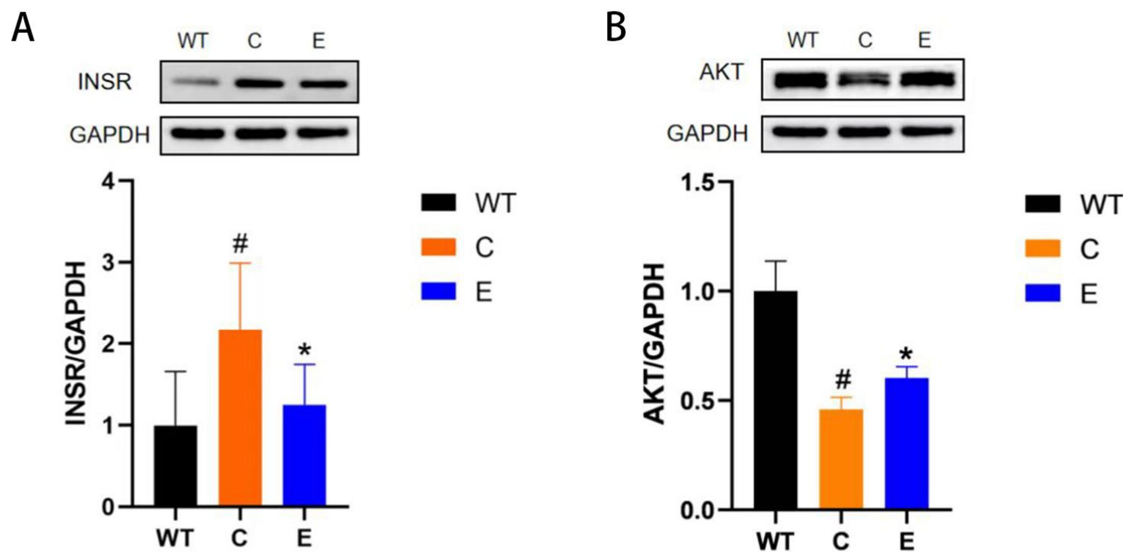

Figure 4. Kidney Insulin-related Protein Bands and Protein Level Expression Changes

Note. A is the result of INSR protein and GAPDH protein blotting assay and quantitative analysis of protein level in each group; B is the result of AKT protein and GAPDH protein blotting assay and quantitative analysis of protein level; # indicates that there is a significant difference between WT group and C group, P < 0.05; * indicates that there is a significant difference between C group and E group, P < 0.05.

Using protein immunoblotting test, the level of insulin receptor protein was detected and the results (Figure 4) showed that compared with the WT group, the level of insulin receptor protein in the C group showed an increasing trend, with a significant difference (C vs WT, P < 0.05); compared with the C group, the level of insulin receptor protein of the mice in the E group showed a decreasing trend, with a significant difference (C vs E, P < 0.05). Detecting the level of AKT protein, the results (Figure 4) found that compared with the WT group, the level of AKT protein in group C showed a decreasing trend, and the difference was significant (C vs. WT, P < 0.05); compared with group C, the level of AKT protein in the mice in group E showed an increasing trend, and there was a significant difference (C vs. E, P < 0.05).

In recent years, exercise therapy has gained a lot of attention as an adjunctive clinical treatment due to its better therapeutic effects on diabetic patients. Previous studies have shown that the main risk reason for close to 90% of chronic type 2 diabetic patients is the long-term unhealthy tendency related to their original lifestyle, and it can be advocated that the purpose of reducing the risk of diabetes mellitus can

be achieved through moderate exercise and weight loss exercise (Edith & Pittas, 2017). In addition, it has been shown that both resistance and aerobic exercise are likely to control blood glucose levels by improving cellular metabolism in vivo (e.g., multiple pathways such as insulin resistance, glycosylated hemoglobin production, etc.) (Buresh & Berg, 2018), and that the mechanism of action is also likely to be that of insulin through the modulation of glucose metabolism-related conductive signaling pathway function in diabetic cells and the natural mitigating effect of inflammatory effects, among other aspects (Karstoft & Pedersen, 2016). AKT protein, as a direct target protein of PI3K, is a key molecule in the PI3K/AKT signaling pathway (Hengchuan & Fukang, 2012), and it has been shown that in the skeletal muscle of IR rats, the activity and expression of AKT show a decreasing trend, and there is an inhibition of the activity of GSK-3, which increases the synthesis of glycogen, leading to the emergence of glucose anabolism; it also leads to the reduction of the amount of GLUT4 transported to the cell membrane, and the utilization of glucose uptake and absorption is reduced (Bi et al., 2005).

The study of Coffey et al. (2007) indicated an increase in AKT protein content in Wister rats after swimming exercise. Liu Jun et al. (2020) further found that the process of aerobic exercise could effectively inhibit the relatively rapid rate of mass gain in db/db mice, improve the eating behavior of db/db mice, and improve the symptoms of glucose tolerance deficiency and insulin resistance in db/db mice, correcting and improving the PI3K/AKT protein levels in db/db mice. In addition, it corrects and improves the functional imbalance between the PI3K/AKT signaling pathway in db/db mice, thereby regulating the utilization of sugar by cells in the mice, which indicates and confirms that the effective maintenance of the relative stability of the PI3K/AKT pathway function in the mice is one of the important goals and key targets in the current clinical intervention for diabetes mellitus.

In a normal organism, insulin binds to insulin receptors on cell membranes thereby creating a stable blood glucose microenvironment, and prolonged exercise enhances insulin sensitivity of cells in the surrounding tissues. In the early stages of insulin resistance, however, defects in the receptor are thought to be the main cause of impaired insulin action (Coffey & Hawley, 2013), and the body compensates for the pathological defects resulting from its peripheral insulin action by increasing the level of insulin in the circulation. At this stage of national research it is generally accepted that aerobic exercise (AT) leads to an increase in insulin action in the skeletal muscle of healthy individuals. The effect of AT on insulin sensitivity has been noted in cross-sectional comparisons of trained versus untrained individuals and in untrained subjects after a period of training. One of the earliest demonstrations of increased insulin action was a cross-sectional study of trained and untrained men, which found that trained subjects had lower insulin and glucose levels during the OGTT (B öjRntorp et al., 1972). In this experiment, after eight weeks of aerobic exercise on the running platform, the glucose tolerance and insulin tolerance of the exercise-only group (Group E) showed a decreasing trend, indicating that aerobic exercise can improve gluconeogenesis; at the same time, the level of insulin receptor protein in Group E decreased, which may be due to an increase in the activation of insulin receptor in the kidneys, which leads to an increase in the binding of receptor protein to insulin protein.

Through long-term aerobic exercise, the body consumes more energy from the body, improves the renal glucose metabolism, and achieves the purpose of improving insulin sensitivity by improving the phosphorylation level of insulin signaling pathways such as PI3K-AKT-GLUT4 in the kidneys, which is able to effectively control the body's blood glucose (Yang, 2020), and plays an effective clinical prevention mechanism and drug Therapeutic adjuvant role. Comparing with this experiment, the level of AKT protein in kidney tissues of diabetic db/db mice increased after exercise, which suggests that aerobic exercise can improve the relevant pathway of AKT protein in kidney cells, thus achieving the purpose of lowering blood glucose.

In this experiment, the insulin receptor protein level and AKT protein level showed an opposite trend, which may be presumed to be related to the transduction mode of insulin signaling pathway. Previous studies have shown that there are two main pathways for insulin signaling in the kidney, and one of the most important signaling pathways is the IRS-1/PI3K/AKT signaling pathway (Zhou, Yang, & Shi, 2016). In this pathway, blood circulation delivers insulin to the corresponding target cells and/or target tissues and binds to its surface receptor, which activates the activity of tyrosine kinase (PTK), thus phosphorylating the tyrosine site of IRS-1 as well as the insulin receptor (INSR) (Chen et al., 2014), and the IRS is able to bind to molecular structures on the PI3K after phosphorylation to activate the p110 subunit of PI3K (Wu & Chang, 2016). p110 subunit of PI3K (Zhang et al., 2014), which then binds directly to its downstream molecules and transmits insulin signals to the downstream signaling molecules through a variety of ways and mechanisms, thus enabling the downstream molecules to activate AKT (Jing et al., 2014) and inhibit gluconeogenesis. In insulin resistance, there are many molecular mechanisms, such as a decrease in the amount of insulin, a decrease in the effect of insulin on downstream tissue cells, etc. In this experiment, the level of insulin receptor protein in group C increased, which can be speculated that the insulin resistance in the diabetic db/db mice in this experiment is due to a weakening of the effect of the insulin receptor protein, i.e., a decrease in the sensitivity to insulin, and a decrease in the amount of insulin bound per unit concentration. The decrease in insulin resistance is due to the weakened effect of the insulin receptor protein, i.e., the reduced sensitivity to insulin, which leads to the blockage of the activation of PTK and the weakened phosphorylation of the tyrosine site of IRS-1, which prevents the activation of p110 for insulin signaling; a series of chain reactions reduces the effect of this signaling pathway, and prevents it from activating the normal amount of AKT as in normal organisms, therefore, the insulin receptor level of Group C in the present experiment increases, and at the same time, the level of the AKT protein is decreasing; and after the eight-week aerobic running After eight weeks of aerobic running, the insulin-conducting signaling pathway was corrected, so the insulin receptor protein level in group E was lower than that in group C, while the AKT protein level was on the rise.

In this experiment, the glucose tolerance as well as insulin tolerance indexes of the overall organism of diabetic db/db mice did not change significantly after eight weeks of aerobic exercise, whereas in the kidney tissues, the levels of AKT proteins as well as insulin receptor proteins were significantly altered

after eight weeks of aerobic exercise, which may be related to the experimental intervention as a purely exercise intervention, e.g., the dietary habits of the db/db mice of drinking and eating more than one meal were This may be related to the fact that the experimental intervention was purely exercise intervention, such as the dietary habits of the db/db mice, which were characterized by excessive drinking and eating, were not controlled; at the same time, it may also be related to the organ tissues and exercise modalities, such as the presence of heart tissues, adipose tissues, and skeletal muscle tissues in the organism; and that there may have been differences in the duration of the exercise, the intensity of the exercise, or the modalities of the exercise, which led to the absence of relief of the IR in the rest of the organs and tissues other than kidneys, and thus to the overall pre- and post-exercise changes in the diabetic db/db mice.

4. Conclusion

Exercise intervention alone did not significantly improve insulin resistance in the organism of db/db mice. Eight weeks of aerobic exercise can improve the insulin receptor sensitivity in the kidneys of db/db mice, leading to an increase in insulin binding to the insulin receptor, and improve the renal insulin signaling pathway and thus insulin resistance in db/db mice.

References

Bi, H. M., Ouyang, J. P., Deng, X. Q., Chen, X. L., Zhang, Y., & Song, C. Y. (2005). Alterations of protein kinase B expression and glucose transporter protein 4 translocation in skeletal muscle of insulin-resistant rats. *Chinese Journal of Diabetes*, *2005*(04), 262-264.

BöjRntorp, P., Fahlén, M., Grimby, G., et al. (1972). Carbohydrate and lipid metabolism in middle-aged, physically well-trained men. *Metabolism*, *21*(11), 1037-1044. https://doi.org/10.1016/0026-0495(72)90034-0

Buresh, R., & Berg, K. (2018).Exercise for the management of type 2 diabetes mellitus: factors to consider with current guidelines. *J Sports Med Phys Fitness*, *58*(4), 510-524. https://doi.org/10.23736/S0022-4707.17.06969-9

Chen, J., Zhang, X. D., Chi, M., et al. (2014). Insulin induces drug resistance in melanoma through activation of the PI3K/Akt pathway. *Drug Design Development & Therapy*, *2014*(2), 255-262. https://doi.org/10.2147/DDDT.S53568

Coffey, V. G., & Hawley, J. A. (2007). The Molecular Bases of Training Adaptation. *Sports Medicine*, *37*(9), 737-763. https://doi.org/10.2165/00007256-200737090-00001

Cullen, M. T., Brice, E., & Kahn, C. R. (2006). Critical nodes in signalling pathways: Insights into insulin action. *Nature Reviews Molecular Cell Biology*, *7*, 85-96. https://doi.org/10.1038/nrm1837

Edith, A., & Pittas, A. G. (2017). The Role of Vitamin D in the Prevention of Type 2 Diabetes: To D or Not to D? *Endocrinology*, *158*(7), 7. https://doi.org/10.1210/en.2017-00265

Jing, Yang, Takayuki, et al. (2014). Bcr-Abl activates AURKA and AURKB in chronic myeloid leukemia cells via AKT signaling. *International Journal of Cancer*, *134*(5). 1183-1194. https://doi.org/10.1002/ijc.28434

Karstoft, K., & Pedersen, B. K. (2016). Exercise and type 2 diabetes: focus on metabolism and inflammation. *Immunol Cell Biol*, *94*(2), 146-150. https://doi.org/10.1038/icb.2015.101

Liu, J., Zhao, K., Ma, Y., Han, S. K., Chen, X. H., Gao, X. Y., & Sun, X. M. (2020). Vitamin D combined with aerobic exercise improves insulin resistance in db/db mice by regulating hepatic PI3K/Akt signaling pathway. *Journal of Xi'an College of Physical Education*, *37*(06), 742-749.

Niu, Y. M., Yuan, H., Liu, Y. H., Su, Z. P., Li, H. G., & Fu, L. (2009). Effects of aerobic exercise and dietary intervention on the signaling system of lipocalin-adenylate-activated protein kinase in skeletal muscle of insulin-resistant mice. *Chinese Journal of Sports Medicine*, *28*(04), 402-406

Roberts, C. K., Hevener, A. L., & Barnard, R. J. (2013). *Metabolic Syndrome and Insulin Resistance: Underlying Causes and Modification by Exercise Training*. American Cancer Society. https://doi.org/10.1002/cphy.c110062

Su, H. C., & Sun, F. K. (2012). Progress of PI3K/Akt/mTOR signaling pathway and mTOR inhibitors in urological tumors. *Journal of Shanghai Jiao Tong University (Medical Edition)*, *32*(05), 674-678.

Wang, Y. K. (2017). *Research progress of insulin signaling and insulin resistance*. Hebei Medical University.

Whyte, L. J., Gill, J., & Cathcart, A. J. (2010). Effect of 2 weeks of sprint interval training on health-related outcomes in sedentary overweight/obese men. *Metabolism-clinical & Experimental*, *59*(10), 1421. https://doi.org/10.1016/j.metabol.2010.01.002

Wu, Y. L., & Chang, X. T. (2016). Interaction of insulin signaling pathway, inflammatory signaling pathway and ubiquitin-proteasome system. *Chinese Journal of Biochemistry and Molecular Biology*, *32*(11), 1177-1184.

Yang, Y. N. (2020). *Mechanism study on the improvement of hepatic insulin resistance in db/db mice by electroacupuncture based on PI3K/Akt/GSK-3β signaling pathway*. Changchun: Changchun University of Traditional Chinese Medicine.

Zhang, Y., Wang, X., Yang, H., et al. (2014). Kinase AKT controls innate immune cell development and function. *Immunology*, *140*(2), 143-152. https://doi.org/10.1111/imm.12123

Zhou, F. Y., Yang, Y. F., & Shi, Y. (2016). Progress of insulin and insulin resistance signaling pathway in type 2 diabetes. *Journal of Liaoning University of Traditional Chinese Medicine*, *18*(11), 71-73.

Zick, Y. (2004). Uncoupling insulin signalling by serine/threonine phosphorylation: a molecular basis for insulin resistance. *Biochemical Society Transactions*, *32*(5), 812. https://doi.org/10.1042/BST0320812

Zick, Y. (2005). Ser/Thr Phosphorylation of IRS Proteins: A Molecular Basis for Insulin Resistance. *Stke*, *2005*(268), pe4. https://doi.org/10.1126/stke.2682005pe4