

# The Effect of Training at Different Temperatures on The Gene Expression of GLUT4 and Insulin Receptor in the Brown Adipose Tissue of Streptozotocin-Induced Diabetic Rats

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## Abstract

**Background and Objective:** Diabetes is one of the health problems in all societies. The aim of the present study was to investigate the effect of a period of training at different temperatures on the gene expression of GLUT4 and insulin receptor in the brown adipose tissue of diabetic rats.

**Material and Methods:** In this experimental study, 21 diabetic rats were randomly divided into 3 groups of 7 animals, including: (1) control (C), (2) swimming training at 5° C (S5° C), and (3) swimming training at 36° C (S36° C). Water swimming training was performed at 5±2° C and 36±2° C for six weeks, 5 sessions per week and 2-4 minutes per session. Data were analyzed using one-way analysis of variance and Tukey's post hoc test at the significance level of p≤0.05.

**Results:** The gene expression of GLUT4 and insulin receptor in the S5° C and S36° C groups was significantly (P = 0.0001) higher than the control group. Also, the gene expression of GLUT4 in the S36° C group was higher than the S5° C group (p = 0.001), and the expression of insulin receptor in the S5° C group was significantly (p = 0.001) higher than the S36° C group.

**Conclusion:** Swimming training at 5° C and 36° C significantly increased the gene expression of GLUT4 and insulin receptor in the brown adipose tissue of diabetic rats.

**Keywords:** Physical Education and Training; GLUT4; Insulin Receptor; Diabetes

**Highlights**

Type 1 diabetes is characterized by high blood sugar due to the deficiency or lack of insulin secretion from the pancreas.

Swimming training in both temperate and cold temperatures increases the expression of insulin and GLUT4 receptor genes in diabetic rats, which leads to tissue glucose uptake and improved blood glucose levels.

**Introduction**

Diabetes embraces a group of metabolic diseases characterized by hyperglycemia due to a lack of insulin secretion, insulin resistance, or a combination of both (1). Research findings indicate that adipose-derived mediators are effective factors in insulin sensitivity and therefore adipose tissue is recognized as one of the tissues associated with diabetes (2). With the activation of brown adipose tissue, cyclic adenosine monophosphate (cAMP) levels increase rapidly, leading to lipolysis and high upregulation of Uncoupling Protein-1 (UCP1) and increased mitochondrial density and heat production. (3) Active brown adipose tissue consumes a significant amount of metabolic substrate and leads to strong anti-obesity and anti-diabetic effects. In this regard, brown adipose tissue is potentially an important target for the treatment of obesity and metabolic diseases (4). There are several mechanisms including cold and exercise, that affect the increase of metabolism in brown adipose tissue. Exposure to cold activates the sympathetic nerve and activates norepinephrine and subsequently activates the adrenergic receptor, which stimulates cyclic adenosine monophosphate (cAMP) and its signaling pathways, and leads to increased fuel uptake and oxidation to produce heat by UCP1 (5). On the other hand, the study of the role of exercise at different temperatures

shows different effects on glucose metabolism, so that in a study, researchers investigated the effect of exercise and cold pressure on glucose metabolism and the results showed that exercise at 4° C and 25° C reduced insulin and serum blood glucose levels. Although the effect of exercise was greater at 4° C (6), glucose uptake and blood sugar regulation is a complex process, and defects in the process have consequences such as increased insulin resistance, diabetes, and defects in the energy metabolism process and decreased athletic performance (7). Glucose transporters play an essential role in the uptake of glucose into the cell (8) GLUTs are one of the glucose transporters and so far 5 isoforms have been identified. A dependent transporter is insulin that is mainly expressed in adipose tissue and skeletal muscle.

Insulin and exercise stimulate rapid, vigorous movement to cell membranes to induce glucose uptake into muscle cells and brown adipose tissue (9). Lehnen et al. (2010) in their research on rats showed that exercise can increase GLUT4 in adipose and muscle tissue (10). However, some studies have shown that this transporter does not change as a result of exercise, for example in Gurley et al.'s (2016) study, although four-weeks of running improved plasma fasting insulin in high-fat diet rats, it had no effect on muscle GLUT4 mRNA (11). Since muscle is the main site of glucose uptake following insulin-mediated stimulation, impairment of whole-body insulin sensitivity or decreased amount or availability of protein can lead to decreased glucose uptake and subsequent increase in blood sugar (12). It has also been shown that in people with insulin resistance, GLUT4 transportation occurs naturally through exercise, so exercise has a therapeutic role in controlling blood sugar in diabetic patients (13). In another study, short-term cold

pressure caused insulin sensitivity in type 2 diabetic patients (6). However, the effect of exercise in cold conditions and environments with different temperatures on the health of diabetic patients is not well known and due to the small number of studies reported on the effects of exercise at different temperatures, this study attempts to investigate the effect of swimming training at water temperatures of 5° C and 36° C on changes in the gene expression of GLUT4 and insulin receptor in the brown tissue of diabetic rats.

## Materials and Methods

### Subjects

This experimental study was conducted on 21 rats aged eight weeks, with the mean weight of 155±35 grams. The animals were purchased from the reproductive center and animal house of Islamic Azad University, Marvdasht Branch, Iran. Afterwards, they were transferred to the animal sport physiology laboratory in standard conditions and kept at the temperature of 22-24°C, relative humidity of 55%, and controlled light (12-hour light/dark cycle) for the seven-day adaptation period. The animals had ad libitum access to water and food during this period. For induction of diabetes, 60 milligram streptozotocin medicine for kg weight of the body dissolved in buffer citrate (4.5=ph) in single dose and peritoneal. For assuring the induction of diabetes, after 4 days an examination of blood density was conducted. Glucose more than 250 milligrams deciliter was supposed as diabetes case. Based on fasting blood sugar, diabetic rats were divided into (1) control (2) swimming training at 5° C and (3) swimming training at 36° C groups for homogenization. Then rats in the training groups swam for six weeks based on the training protocol.

At the end of the sixth week and 48 hours after the last training session, rats in the study groups were anesthetized with ketamine and xylazine in a ratio of 3 to 1 by intraperitoneal injection following 16 hours of fasting. After diagnosis of complete anesthesia with pain reflex tests by squeezing the tail and ensuring complete analgesia, brown adipose tissue was extracted by laboratory specialists and immediately kept at -80° C.

### Training Protocol

To familiarize rats with the process of swimming training in a special pool for rats, the rats first swam in water at 5° C and all their activities were accurately observed for two minutes and recorded as long as the rats swam and attempted to get rid of the situation. This was performed for 6 sessions to familiarize the rats with the training conditions. Then, swimming training was performed in water at a temperature of 5 ± 2° C to 36 ± 2. ° C based on Lubkawasa et al.'s (2019) study for two minutes in the first week, 5 days a week, and 30 seconds were added to each workout until the duration reached four minutes. Following that, the rats trained at 5° C for 4 minutes until the end of the eighth week. Rat swimming pool contained a special rat swimming tank with dimensions of 100 cm in length, 50 cm in width and 50 cm in depth (14, 15).

### Measurement of the research variables

To do molecular studies at the level of gene expression, first RNA extraction of brown adipose tissue was performed according to the protocol of the manufacturer (Sinagen, Iran), then using light absorption at 260 nm wavelength, the concentration and purity of RNA samples were quantitatively obtained using the following formula:

$$C (\mu\text{g}/\mu\text{l}) = A_{260} \times \epsilon \times d / 1000$$

After extracting RNA with very high purity and concentration from all the studied samples, the instructions for cDNA synthesis in the fermentase kit (K1621) were followed and then the synthesized cDNA was used for reverse transcription reaction. First, the

designed primers related to genes were examined, and then the expression of genes was evaluated by the quantitative q-RT PCR method and using the formula  $2^{-\Delta\Delta Ct}$ , their relative expression was calculated ([Table 1](#)).

**Table 1.** Sequence of Forward-Reverse Primers of Genes in Real-time Polymerase Chain Reaction

Genes	Primer Sequences	Sizes (bp)
<b>Actin, Beta</b>	Forward: 5'-TCTATCCTGGCCTCACTGTC-3'	<b>122</b>
	Reverse: 5'-AACGCAGCTCAGTAACAGTCC-3'	
<b>GLUT 4</b>	Forward: 5'-GCCGGGACACTATACCCT-3'	<b>231</b>
	Reverse: 5'-TGTAGCTCTGTTCAATCACTTTCT -3'	
<b>IR</b>	Forward: 5'-CTTCTTCCGACATGTGGTCCT-3'	<b>131</b>
	Reverse: 5'-AGGGGGATCCAGATAGCCTC -3'	

### Statistical analysis

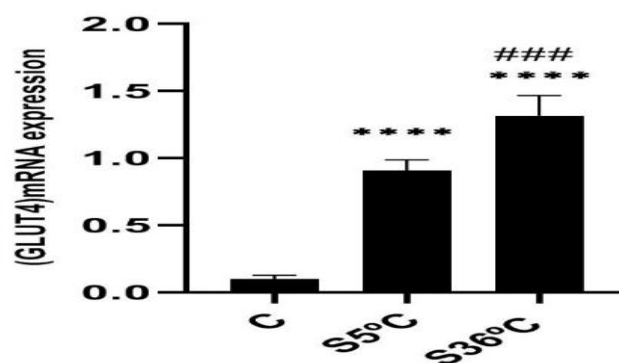
To obtain the results, the data were reported using descriptive statistics as mean and standard deviation. Then, the Shapiro-Wilk test was used to investigate the normality of data distribution. One-way analysis of variance (ANOVA) and Tukey's post hoc test were used to compare the study groups. All statistical analyses were performed using SPSS software (version 24) and a significance level of  $p < 0.05$  was considered.

### Result

The results of one-way analysis of variance showed that six weeks of swimming training at different temperatures ( $P = 0.0001$ ,  $F = 1531.531$ ) had a significant effect on increasing GLUT4 gene expression in the brown adipose tissue of diabetic rats.

The results of Tukey's post hoc test showed that GLUT4 gene expression in brown adipose tissue in the S5° C, S36° C groups ( $p = 0.0001$ ) was significantly higher than the C group. Also, GLUT4 gene expression in the

brown adipose tissue in the S36° C group ( $p = 0.001$ ) was significantly higher than the S5° C group ([Figure 1](#)).



**Figure 1:** Levels of GLUT4 gene expression in the brown adipose tissue of rats in the Three research groups

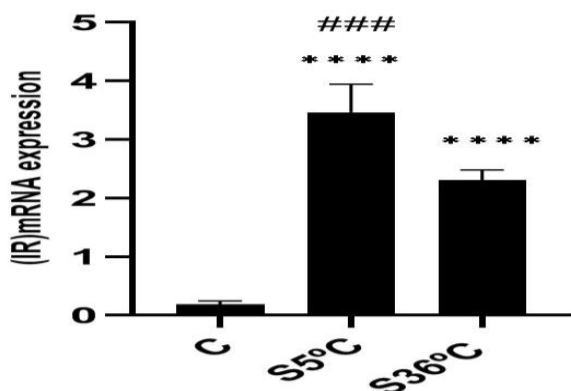
\*\*\*\* ( $p=0.0001$ ) Significant increase compared to the C group

#### ( $p=0.001$ ) Significant increase compared to the S5°C

Insulin R gene expression in the brown adipose tissue of diabetic rats.

The results of Tukey's post hoc test showed that Insulin R gene expression in the brown adipose tissue in the S5° C, S36° C groups ( $p$

= 0.0001) was significantly higher than the C group. Also, Insulin R gene expression in the brown adipose tissue in the S5° C group ( $p = 0.001$ ) was significantly higher than the S36° C group (Figure 2).



**Figure 2:** Levels of IR gene expression in the brown adipose tissue of rats in the Three research groups  
 \*\*\*\* ( $p=0.0001$ ) Significant increase compared to the C group  
 ### ( $p=0.001$ ) Significant increase compared to the S35°C

## Discussion

The present study investigated the effect of training environment temperature on GLUT4 and insulin receptor gene expression in the brown adipose tissue of streptozotocin-induced diabetic rats. Studies have shown that exercise employs brown adipose tissue through the sympathetic nervous system, heart muscle and skeletal muscle. The duration, intensity and type of exercise activity are effective in activating brown adipose tissue. Increased expression of GLUT4 and insulin receptor is one of the most important findings of the present study. In other words, 6 weeks of swimming training, 5 sessions per week in 5 and 36° C water increases the gene expression of GLUT4 and insulin receptor in the brown adipose tissue of streptozotocin-poisoned diabetic rats, which is consistent with the results of Jorge et al. and Hussey (2011) (16, 17), and is inconsistent with the results of Zarekar M et al. (2014) and Gurley et al. (11,

18). One of the reasons for the inconsistency of the results of these studies with the present study is the difference in the statistical population, intensity and type of training. Researchers have shown that exercise increases aerobic capacity by increasing fat metabolism, increasing mitochondrial biogenesis, increasing energy levels, and increasing mitogenesis following exercise in hot and cold weather (19). In a study, Hou et al. (2003) evaluated the interactive effect of swimming training and growth hormone consumption on the expression of GLUT4 protein in the soleus muscle of rats. Although the protocol performed in Hou research comprised 2 hours of swimming training, the results showed that training significantly increased GLUT4 protein levels. In this study, increasing the glucose transporter in the training group prevented the increase in insulin resistance (12). The mechanisms of increased GLUT4 and exercise-induced muscle glucose uptake are not well understood. Molecular signaling from contraction is complex and involves a set of signaling molecules including AMPK, calcium and NOS in the upstream signaling cascade as well as GTPases, Rab, SNARE proteins and cytoskeletal components in the downstream signaling cascade. Muscle glucose uptake is dependent not only on GLUT4 transportation but also on increasing the expression of this protein. AMPK and CaMKLL are key signaling kinases that appear to increase GLUT4 protein expression through the HDAC4 / 5-MEF2 axis and interaction with MEF2-GEF (20).

Increased transcription of GLUT4 gene in humans in response to exercise is mediated by the response of the promoter factors of this gene, and region I and MEF2 region and GEF and MEF2 transcription factors are also involved (20). Exercise increases histone

acetylation at the MEF2 site and binds MEF2A to the promoter portion of the GLUT4 gene, and these responses are dependent on CaMk activity (21, 20). In addition to these factors, there are other signaling pathways that are activated following exercise and move GLUT4 from inside the cell to the surface of the cell membrane. These pathways include activation of calcium-dependent pathways, mitogen-activated protein kinases (MAPK), cytoskeleton actin, nitric oxide (NO), reactive oxygen species (ROSs) (2016), intracellular energy expenditure signals such as AMPK and other downstream proteins that affect muscle contractions (20). Overall, exercise is the most important stimulant to increase the expression of GLUT4 in adipose tissue and skeletal muscle, and this effect is attributed to the improvement of insulin action and glucose consumption and increased glycogen storage in trained muscles. In this regard, with the intervention mechanisms in this adaptability, it seems that the upstream signaling pathways that ultimately lead to GLUT4 shift include AMPK; CaMKII, NOS and ROS. AMPK and CaMKII are key signaling kinases that regulate GLUT4 expression via the HDAC4 / 5-MEF2 axis and MEF2-GEF interactions.

Another problem in the diabetic groups is the lack of inhibition of the two key enzymes gluconeogenesis PEPCK and glucose 6-phosphate, and there is evidence that these two enzymes are involved in insulin resistance. Exercise seems to reduce fasting glucose by affecting the expression of these two proteins and inhibiting the key enzyme gluconeogenesis PEPCK and catalytic units of glucose 6-phosphate. Research also shows that exercise increases the substrate of insulin receptors in adipose tissue and muscle (22). Exercise by increasing the function and signaling of insulin, increasing glucose

transporters from inside to the cell membrane, increasing the rate of glucose uptake, increasing capillary density, increasing the expression of genes or activities of various proteins involved in insulin messaging, increasing glycogen synthetase activity and finally, increasing glycogen storage affects glucose homeostasis and increases insulin sensitivity (23). Regarding the limitations of the present study, we can point to the small sample size of this study, which is considered a typical limitation.

## Conclusion

In general, the results of the present study showed that swimming training at two temperatures of 5° and 36° C increases the gene expression of GLUT4 and insulin receptor in the brown adipose tissue of streptozotocin-poisoned diabetic rats.

## Authors' contributions

All authors contributed equally to this work.

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