



# Effect of 8-Week Interval Training on Protein Tyrosine Phosphatase 1B Expression in Gastrocnemius Muscle and Insulin Resistance in Rats with Type 2 Diabetes

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## Abstract

**Background:** Insulin resistance induced by genetic and metabolic disorders is the main cause of the prevalence or severity of type 2 diabetes (T2D). Protein tyrosine phosphatase 1B (PTP1B) plays a key role in regulating glucose homeostasis as a negative regulator of insulin signaling pathway.

**Objectives:** This study aimed to assess the effect of interval training on PTP1B expression in gastrocnemius muscle and insulin resistance in male rats with T2D.

**Methods:** T2D was induced by high fat diet (HFD) and intraperitoneal injection of STZ in 14 male Wistar rats and then they were divided randomly into exercise (n=7) or control (n=7) groups. Exercise rats completed an 8 weeks interval training (5 days/week) and control rats remained without training. Fasting glucose, serum insulin, and PTP1B expression in gastrocnemius muscle were measured 48 hours after the last exercise session. Insulin resistance was assessed using homeostasis model assessment of insulin resistance (HOMA-IR) formula based on fasting insulin and glucose levels. An independent *t* test was used to compare each parameter between 2 groups. A *P* value less than 0.05 was considered statistically significant.

**Results:** Interval training resulted in a significant decrease in fasting glucose level ( $P < 0.0001$ ) and insulin resistance ( $P = 0.018$ ) as well as an increase in serum insulin level ( $P < 0.0001$ ). PTP1B expression in gastrocnemius muscle decreased significantly compared with control rats ( $P = 0.003$ ).

**Conclusion:** Interval training can improve insulin resistance in T2D rats. This improvement may be attributed to the decrease in PTP1B expression in gastrocnemius muscle by interval training.

**Keywords:** Interval training, Insulin resistance, PTP1B expression, Type 2 diabetes

## Background

The pathogenesis of type 2 diabetes (T2D) is associated with genetic mutations, heredity, obesity, and obesity-related abnormalities that predispose the individuals to insulin resistance (1). Although T2D is a multifactorial disease, one of its prominent risk factors is the prevalence of obesity. Consideration of obesity as one of the most important factors contributing to the development of T2D has been supported by some previous studies (1,2).

Apart from the effective role of metabolic, hormonal, and inflammatory disorders, current studies strongly emphasize the identification of the genetic factors contributing to obesity and T2D. In the recent decade, several genetic factors that are of particular importance in the prevalence or increased severity of type 1 and 2 diabetes have been recognized. Therefore, impairments in their expression or polymorphisms have contributed to the prevalence or severity of diabetes because of the damage to the insulin

secretion or damage to the release of some insulin stimuli and mechanisms responsible for insulin function at the target cell surface (3). Among them, protein tyrosine phosphatase 1B (PTP1B), which is the key member of the protein tyrosine phosphatase (PTP) family, has been regarded as not only a negative regulator of insulin signaling (4) but also a therapeutic target for T2D (5). The use of its antagonists has been considered a means of controlling and treating T2D. This 435 amino acid protein with a molecular weight of 50 kDa is encoded by protein tyrosine phosphatase non-receptor type 1 (PTPN1) (6). In fact, PTP1B interacts with insulin receptors and insulin receptor substrate-1 (IRS-1) and leads to the impairment of glucose uptake by affecting insulin signaling pathways (4).

In cases of insulin resistance caused by high-fat diets, leptin deficiency, hyperglycemia, or age-dependent insulin signaling impairments, especially in the presence of

obesity, PTP1B expression is increased in insulin-sensitive tissues (7,8). Clinical studies have revealed that increased endoplasmic reticulum (ER) stress in response to obesity leads to the insulin resistance in target cells (9,10). In addition, an increased PTP1B expression as one of the most important regulators of insulin signaling has been identified as one of the factors contributing to the increased ER stress as well as the subsequent insulin resistance (11). It has been suggested that PTP1B inhibitors are able to improve insulin resistance as well as insulin and blood glucose levels without causing hypoglycemia (12). Therefore, it seems that the decreased expression or protein levels in target tissues such as fatty or muscle tissues in gastrocnemius muscle induced by drug or non-drug stimuli are associated with an improved insulin function in the target tissue or decreased blood glucose levels. The gastrocnemius muscle is a superficial two-headed muscle that is in the back part of the lower leg of humans. It runs from its 2 heads just above the knee to the heel, a three joint muscle (knee, ankle and subtalar joints). In this regard, several studies have been conducted to determine the effect of exercise training alone or in combination with diet management on the hormonal and genetic factors affecting insulin function in diabetic or non-diabetic individuals. Furthermore, although some studies have pointed to the significant effect of exercise training on the expression or protein levels of genes affecting the secretion and function of insulin and blood glucose levels such as TCF7L2 (13), FOXO1 (14), and MTNR1B (15), the direct effect of exercise training on PTP1B expression in skeletal muscles of diabetic patients has not been thoroughly investigated. Therefore, the aim of the present study was to determine the effect of an interval training program on PTP1B gene expression in gastrocnemius muscle as well as insulin resistance and blood glucose levels in type 2 diabetic obese rats.

## Materials and Methods

In the present study, T2D was induced by high-fat diet (HFD) and intraperitoneal STZ in 14 male Wistar rats ( $220 \pm 10$  g) aged 10 weeks old. Then, they were randomly divided into exercise (interval training, 8 weeks, 5 days/week,  $n=7$ ) and control (no training,  $n=7$ ) groups. Animals were maintained under standardized conditions (12-hour light/dark cycle,  $25 \pm 2^\circ\text{C}$ , and 45-55 % humidity). The rats were acclimatized for 1 week prior to the commencement of the experiment. The study was approved by the Department of Exercise Physiology of Islamic Azad University, Saveh Branch, Iran and carried out in accordance with CPCSEA (Committee for the Purpose of Control and Supervision of Experiment on Animal) guidelines.

## Induction of Type 2 Diabetes

T2D was induced by an 8-week high fat diet (HFD) followed by a single intraperitoneal injection of 25 mg/

kg streptozotocin (dissolved in citrate buffer, pH 4.5) (16-18). It should be noted that a high-fat diet was continued to the end of the study for 2 groups. Hyperglycemia was confirmed by elevated blood glucose levels on day 7 after diabetes induction and only animals with fasting blood glucose level between 150 and 400 mg/dL were selected to serve as T2D rats and used in the study (13).

## Training Protocol

All rats in the exercise group completed an interval training program for 8 weeks, 5 sessions of 30 minutes per week in the form of treadmill running with 40-second repetitions and a 2-minute active rest period between each repetition (Table 1). Finally, all rats were dissected 48 hours after the last exercise session following 10 to 12 hours of overnight fasting. It should be noted that the diabetic control rats were not included in the training program during this period.

## Sample Collection and Biochemical Assays

Forty-eight hours after the last training session (10 to 12 hours of fasting), the rats in each group were anesthetized by intraperitoneal injection of 10% ketamine (50 mg/kg) and 2% xylazine (10 mg/kg). After the rats were anesthetized, blood samples were collected through cardiac puncture. Then, gastrocnemius muscle was removed and immersed in RNAlater™ (DNAbiotech Co., Iran) until gene analysis was performed to determine PTP1B expression. The blood samples were used to analyze glucose and serum insulin. The serum was separated by centrifugation (5 min, 3000 rpm) and was analyzed for glucose using a Cobas 6000 analyzer (Roche, Germany). Glucose was measured by enzymatic colorimetric assay using glucose oxidase technology (Pars Azmoon kit, Tehran). The intra- and inter-assay coefficients of variation for glucose were 1.74% and 1.19%, respectively. Insulin was measured by ELISA method (Demeditec, Germany). The intra-assay and inter-assay coefficients of variation for insulin were 2.6% and 2.88, respectively. Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated using the following formula:  $\text{HOMA-IR} = [\text{glucose (nmol/L)} * \text{insulin } (\mu\text{U/mL}) / 22.5]$ , using fasting values (19).

**Table 1.** Distribution Pattern of Exercise Intensity in Resistance Training Group Based on Percentage of Body Weight

Exercise Sessions (wk)	Activity Stage		Active Rest	
	Speed (m/min)	Time (s)	Speed (m/min)	Time (s)
1-2	20	40	14	120
3-4	25	40	14	120
5-6	30	40	14	120
7-8	35	40	14	120

### RNA Extraction/Real-Time PCR

To purify RNA, 20 mg of tissue (gastrocnemius muscle) was ground using a mortar and pestle, and the extraction was then performed employing the RNeasy Protect Mini Kit (manufactured by Qiagen Inc. in Germany) according to the manufacturer's protocol (20).

In this stage, the One Step SYBR PrimeScript RT-PCR Kit (manufactured by Takara Bio Inc., Japan) was employed according to the manufacturer's protocol to prepare the reaction product. The thermal cycle program used for the Rotor-Gene Q instrument was as follows: 42°C for 20 minutes, 95°C for 2 minutes, 40 cycles at 94°C for 10 seconds, and 60°C for 40 seconds. Temperatures used for the melting curve after the PCR to study the characteristics of the primers ranged from 50 to 99°C. The comparative  $\Delta\Delta\text{CT}$  method was used to quantify TCF mRNA expression. We used RNA Polymerase II as the control gene (Table 2)(13).

### Statistical Analysis

All statistical analyses were performed using a statistical software package (SPSS, version 15.0, SPSS Inc., IL, USA). Data were tested for normal distribution by the Kolmogorov-Smirnov test. Comparisons of the mean of each variable between groups were done using the independent *t* test. The differences between the groups were considered to be significant at a *P* value of  $\leq 0.05$ .

### Results

The pattern of body weight changes before and after exercise intervention in exercise and control groups is presented in Table 3. In the pre-training, the results of the independent *t*-test showed that there was no significant difference between the groups in the weight of rats ( $P=0.523$ ). A significant increase was observed with regard to body weight in the 2 groups at the end of the training program (post-training) compared to pre-training. In addition, the results of the independent *t* test showed a significant difference in body weight between groups at post-training ( $P=0.004$ ). In other words, interval training resulted in a significant decrease in body weight of exercise rats compared to control rats.

Based on statistical analysis, significant differences were observed between 2 groups with regard to glucose, serum insulin and insulin resistance (Table 4). In other words, interval training resulted in a significant increase in serum insulin level ( $P<0.0001$ ) as well as a decrease in fasting glucose level ( $P<0.0001$ ) in exercise rats when compared with control rats. A significant increase was also observed with regard to insulin resistance in the exercise group ( $P=0.018$ , Figure 1). In addition, PTP1B expression in gastrocnemius muscle decreased significantly by exercise compared with control rats ( $P=0.003$ , Figure 2).

**Table 2.** Primer Sequence

Genes	Primer Sequence
PTP1B	For: GCAGTTGGAGTTGGAGAACCTG Rev: CGTGCTCTGGGCTGAGTG
RNA Polymerase II	For: ACTTTGATGACGTGGAGGAGGAC Rev: GTTGGCCTGCGGTCGTTT

**Table 3:** Pre and Post-training of Body Weight (g) of Exercise and Control Groups (Mean  $\pm$  SD).

Group	Pre-training	Post-training	<i>P</i> Value
Control	294 $\pm$ 17	393 $\pm$ 15	<0.001
Exercise	289 $\pm$ 13	368 $\pm$ 10	<0.001
<i>P</i> value	0.523	0.004	-

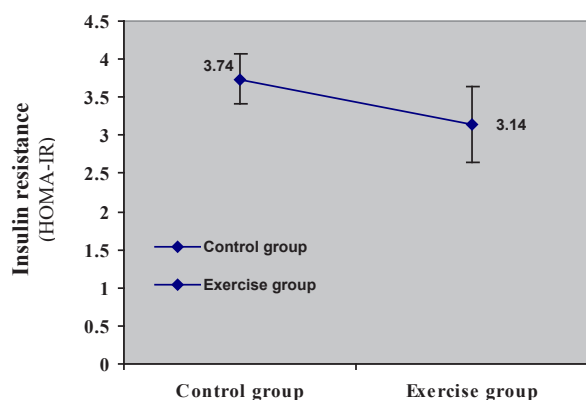
**Table 4:** Clinical Characteristic and Gene Expression of Exercise and Control Groups (Mean  $\pm$  SD) After the Intervention

Variable	Control Group	Exercise Group	<i>P</i> Value
Fasting glucose (mg/dL)	312 $\pm$ 21	195 $\pm$ 24	<0.001
Insulin ( $\mu\text{U/mL}$ )	4.87 $\pm$ 0.38	6.53 $\pm$ 0.47	<0.001
Insulin resistance (HOMA-IR)	3.74 $\pm$ 0.32	3.14 $\pm$ 0.49	0.018
Gene expression	1	0.56 $\pm$ 0.31	0.003

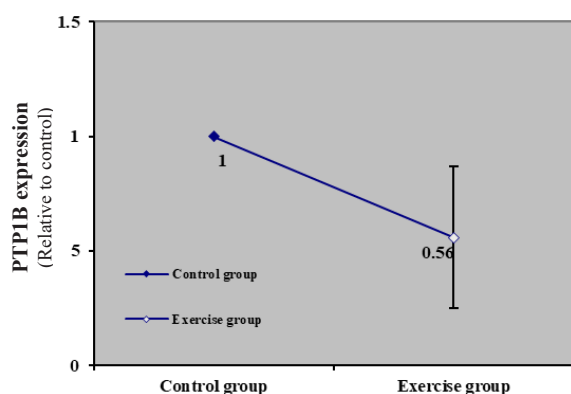
### Discussion

The decrease in PTP1B expression in gastrocnemius muscle in response to interval training was the main finding of the present study. In other words, 8 weeks of interval training with 5 sessions per week decreased PTP1B expression in the gastrocnemius muscle of T2D rats as compared to the control rats. In addition, the training intervention was associated with a significant decrease in fasting glucose and insulin resistance in the exercise group. Controversial findings were reported in previous studies in this regard. Contrary to the findings of the present study, another study revealed that 6 weeks of exercise training at 60%-80%  $\text{VO}_2\text{max}$  did not lead to a significant change in glucose levels (21). Moreover, another study indicated that 20 weeks of exercise training with 3 to 5 sessions per week at 70%  $\text{VO}_2\text{max}$  did not lead to a change in the glycated hemoglobin as an indication of a long-term glucose change (22). In a study by Maltais et al, 4 months of resistance training did not result in a change in the glucose and insulin levels in 26 overweight old men although their body fat mass decreased significantly (23).

In line with the findings of the present study, Moslehi et al noted a significant decrease in glucose and insulin levels along with an increase in GLUT4 in response to 8 weeks of aerobic training along with milk supplement in overweight immature boys (24). Eizadi et al also reported a decrease in the blood glucose level in response to long-term interval training in T2D rats (25). Decreased glucose levels have also been reported following 12 weeks of aerobic exercise in T2D rats by Rashidi et al (26). Based on the available evidence, improved blood glucose levels can be attributed to the decreased insulin resistance in response



**Figure 1.** Insulin Resistance after Interval Training in Exercise and Control Groups Interval training resulted in significant decrease in insulin resistance in exercise rats.



**Figure 2.** PTP1B Expressions in Gastrocnemius Muscle in Exercise Rats Compared to Control Group. Interval training resulted in significant decrease in PTP1B expressions in gastrocnemius muscle in exercise rats.

to the periodic exercise training. Abd El-Kader et al ascribed the improved fasting glucose and HbA1C in T2D patients after 12 weeks of aerobic exercise to a decreased insulin resistance level (27). Moreover, Steckling et al also attributed the improvement of glucose level following 12 weeks of HIIT with 3 sessions per week at an intensity of 70%-90% of maximum heart rate to a decreased insulin resistance (28). It is worth noting that decreased insulin resistance in response to interval training was another finding of the present study.

Clinical studies have also revealed that decreased insulin resistance is grounded in the change of the hormonal or metabolic factors affecting insulin function in target tissues such as fatty or muscle tissues. In this regard, Sheu et al attributed the decrease in insulin resistance following long-term aerobic exercise in non-diabetic women to a decrease in Tumor necrosis factor alpha (TNF- $\alpha$ ) and an increase in adiponectin or, in other words, an improvement in the pro-inflammatory markers affecting insulin function (29). The decrease in insulin resistance is also grounded in the

alteration of the genetic factors affecting insulin signaling pathways in the target tissue. The role of transcription factors such as FTO, FOXO1, IRS-1, PPAR $\gamma$ , and GLUT4 in insulin-dependent glucose transport has been previously reported (8,10,11,14). In this regard, the PTP1B gene is of particular importance (8) as some researchers have identified it as a regulator of insulin function in mammals and a pharmacological target in T2D (1). It is worth mentioning that the findings of the present study indicated a decrease in blood glucose and insulin resistance levels along with a decrease in PTP1B expression in gastrocnemius muscle in response to interval training in T2D rats. PTP1B antagonizes insulin action by catalyzing dephosphorylation of the insulin receptor (IR) and/or other key proteins in the insulin signaling pathway (30). Increased PTP1B expression due to alterations in PTKs activity (31) leads to loss of binding to insulin receptors at target tissues such as the skeletal muscle, the consequences of which are insulin resistance, leptin deficiency, obesity, and T2D (32).

Moreover, it has been indicated that knockout of PTP1B in mice is associated with lower levels of fatty tissue, increased insulin sensitivity, and increased energy uptake (33,34). Therefore, the presentation of non-invasive stimuli such as diet modifications or exercise training methods that causes changes in the expression or protein levels of genes affecting insulin function may be associated with a decreased insulin resistance level as well as an improved glycemic profile. Based on the available evidence and apart from other effective mechanisms, a decreased insulin resistance level and an improved glycemic profile may be somehow attributed to the decrease in the PTP1B expression in the gastrocnemius muscle in response to interval training in T2D rats in the present study. It is possible that the decrease of PTP1B expression in response to interval training may directly or indirectly affect insulin signaling pathways. In this respect, whole body PTP1B-deficient mice have been proven to have a decreased TNF- $\alpha$ -dependent insulin resistance and increased insulin sensitivity in the skeletal muscles. The muscle-specific deletion of PTP1B is associated with improved glucose uptake and insulin signaling in the skeletal muscle of rats receiving a high-fat diet (35).

It is also conceivable that the decrease of PTP1B in response to exercise training may lead to improved insulin function in the target tissues by inhibiting ER stress induced by high-fat diets and free radicals. It has been suggested that the increase of PTP1B through increased ER stress by activating ROS-NF- $\kappa$ B in the target tissues leads to an increased level of proteins stimulating insulin resistance in obesity (36). Besides, the skeletal muscle is one of the major target tissues for PTP1B function in glucose homeostasis (37,38). In this regard, Delibegovic et al reported improved glucose uptake and insulin signaling in mice with muscle-specific deletion of PTP1B based on his

laboratory findings (35). The mentioned process appears to be consistent with the inhibitory effect of interval training on PTP1B expression in the skeletal muscle observed in the present study. Furthermore, the knockdown of PTP1B leads to a decrease in high-fat diet-induced fat accumulation, TG, and hepatic cholesterol, which suggests the role of PTP1B in hepatic fat metabolism (36). In this regard, Shimizu et al have pointed out that PTP1B promotes hepatic lipogenesis by regulating the expression of sterol regulatory element-binding protein-1 that is obtained by activating protein phosphates 2A (39). In vitro studies have indicated a concomitant increase in PTP1B expression and inflammatory pathways in the skeletal muscle and liver in obese species (40). These findings all support the effect of PTP1B on insulin function and blood glucose levels in target tissues, especially in the presence of obesity.

### Conclusion

Eight weeks of interval training leads to decreased blood glucose levels in T2D rats. This decrease can be attributed to the improvement of PTP1B-dependent insulin function in the gastrocnemius muscle in response to interval training. However, further studies are required to shed light on the genetic mechanisms that influence insulin function in response to exercise training.

### Conflict of Interest Disclosures

The authors declare that they have no conflict of interests.

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