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BRIEF NOTE

# The response of insulin signaling proteins IRS1 and PTP-1B to endurance, HIIT and resistance training in rats with experimental diabetes

## *Réponse des protéines de signalisation de l'insuline IRS1 et PTP-1B à l'exercice en endurance, à l'intervalle training de haute intensité, et à l'entraînement en résistance chez des rats diabétiques*

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**KEYWORDS**
PTP-1B;
IRS1;
Aerobic;
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Resistance training;
Diabetes

**Summary**
*Objectives.* — Exercise is widely perceived to be an important component for diabetes. This study designed to evaluate the responses of different exercises (aerobic, HIIT, resistance) to PTP1B and IRS-1 expression of gastrocnemius muscle in experimental diabetes rats.
*Material and methods.* — 40 diabetic male Wistar rats were divided into: four groups: control group, aerobic, resistance, and HIIT group. The exercises protocols were carried out for 12 weeks. Blood sample was collected for serum glucose and insulin analysis. The animals were then sacrificed and Blood sample was collected for serum glucose and insulin analysis. Gastrocnemius muscle was also exposed for evaluation of *PTP-1B* and *IRS1* genes expression.
*Results.* — Fasting blood glucose decreased and fasting serum insulin level increased significantly in all trained groups compared to diabetic control group, whereas HOMA_IR index not significantly changed. The expression of *IRS1* gene upregulated significantly in aerobic and HIIT trained groups, but no significant difference observed the resistance group compared to the diabetic control group. Moreover, *PTP-1B* gene expression downregulated significantly in all aerobic, HIIT, and resistance groups compared to control group by different mechanisms.

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*Conclusion.* — The results provide new insights into the mechanism of insulin resistance linked to physical exercise and which exercise protocol restores insulin sensitivity.


**MOTS CLÉS**
PTP-1B ;
IRS1 ;
Aérobie ;
HIIT ;
Entraînement de résistance ;
Diabète

**Résumé**
*Objectifs.* — L'exercice est une composante importante du traitement du diabète. Cette étude a été conçue pour évaluer les réponses à différentes modalités d'exercice de l'expression de PTP1B et IRS-1 dans le muscle gastrocnémien de rats diabétiques.
*Matériels et méthodes.* — 40 rats Wistar mâles diabétiques ont été répartis en quatre groupes: groupe témoin, groupe exercice en endurance aérobie, groupe exercice en résistance, et groupe d'exercice intermittent de haute intensité (high intensity interval training [HIIT]) Le programme d'entraînement comprenait 3 séances par semaine sur une durée de 12 semaines. Les animaux ont été ensuite sacrifiés et un échantillon de sang a été recueilli pour l'analyse de la glycémie sanguin et de l'insulinémie. Le muscle gastrocnémien a été également prélevé pour évaluation de l'expression des gènes *PTP-1B* et *IRS1*.
*Résultats.* — La glycémie à jeun a diminué et l'insulinémie à jeun a augmenté significativement dans tous les groupes entraînés par rapport au groupe témoin diabétique, tandis que l'indice HOMA-IR n'a pas été significativement modifié. L'expression du gène *IRS1* a augmenté significativement dans les groupes aérobie et HIIT, mais aucune différence significative n'a pas été observée entre le groupe entraîné en résistance et le groupe témoin diabétique. De plus, l'expression du gène *PTP-1B* a été diminuée de manière significative dans tous les groupes aérobies, HIIT, et résistance par rapport au groupe témoin.
*Conclusion.* — Ces résultats fournissent de nouvelles informations sur l'action insulino-sensibilisatrice de l'exercice physique.


## 1. Introduction

Diabetes is characterized by increased level of blood glucose and impaired carbohydrate, fat, and protein metabolisms. It is a metabolic disorder characterized by high blood glucose level resulting from insufficient insulin production and/or action. Peroxidation of membrane lipids induced by hyperglycemia contributes to the pathophysiology of cardiovascular disease and atherosclerotic foam cell formation in the arterial wall. Diabetes, which is a silent disease, is the most prevalent disease in developed and underdeveloped countries, and its prevalence is increasing considerably. Protein tyrosine phosphatase 1B (PTP1B) induced by inflammation appeared as key phosphatase, which has been shown to be a negative regulator of the insulin transduction in resistant states. PTP1B is a highly considered purpose of the pharmaceutical industry in the treatment of diabetes and obesity disorders that protects against these two important metabolic diseases. PTP-1B can block or reduce insulin action through tyrosine dephosphorylation of the insulin receptor (IR). It has been shown that complete absence of PTP-1B caused an increased insulin sensitivity and improved glucose tolerance, establishing PTP-1B as a physiologically important IR and IRSs phosphatase [1].

Great efforts have been widely conducted to investigate the mechanism of insulin resistance related with exercise. Physical activity affects overall metabolism, especially of blood glucose and lipids. Therefore, it has a significant value in the treatment of diabetes, which is often managed by doing exercise modification. For instance, lipid profiles and insulin sensitivity in diabetes have improved by doing aerobic exercise. Moreover, exercise can also elevate Foxo1 expression, further reduce glycated hemoglobin (HbA1c). We speculated that different exercises may represent the expression of insulin signaling molecules in skeletal muscle in experimental diabetes. Therefore, the present study was aim to examine the responses of different exercises (aerobic, HIIT, resistance) to PTP1B and IRS-1 expression of gastrocnemius muscle in experimental diabetes rats.

## 2. Methods

All experiments involving the animals were conducted according to the policy of the Iranian Convention for the Protection of Vertebrate Animals Used for Experimental and other Scientific Purposes. Fourty healthy male Wistar rats (10 weeks old, average weight of $220 \pm 20$) were acquired and kept in the animal laboratory of physical education and exercise Science College of University of Tehran. The animals were housed 3 rats per cage under strictly controlled conditions at temperature $22 \pm 3\,^{\circ}C$, humidity 20% and light: dark cycle of 12:12 hours. The rats were fed standard rodent chow and water ad libitum with free access. After the induction of diabetes, the animals were randomly divided into four groups: the control group, the aerobic group, the resistance group and the HIIT group

($n = 10$). Diabetes was induced using administration of Streptozotocin (STZ) (60 mg/kg body weight). The elevated plasma glucose was determined 72 hours after STZ administrations and those rats with fasting glucose levels greater than 126 mg/dL were identified as diabetic rats and used in the study. All rats in the training groups began their respective training seven days after injections of STZ.

Aerobic training began with familiarization of rats with the apparatus for 1wk by placing them on the motorized-driven treadmill. In the first week, animals were exercised on treadmill at 18 m/min speed, 0% inclination and for 18 min/day. During the next 12 weeks, the load of training gradually increased up to 20—26 m/min, at a 0% inclination, 20—55 min/day, 5 days/week. The rats in the resistance trained group undertook one training session per day, 5 days/week by climbing a ladder and weights which were attached to the base of their tail with adhesive tape and a clip. Resistance training was conducted with the use of a 1-meter high ladder inclined at 85°. There were 26 rungs on the ladder, evenly spaced. The resistance training consisted of 3 sets of 6 repetitions climbing the ladder with a 3 min rest interval between the sets and 45 seconds between the reps for 12 weeks. The initial weight attached to tail was 10% of the rat's body weight in the first week, but gradually increased up to 100% of their body weight throughout the 12 weeks of training period. The rats were motivated to climb the ladder by the occasional grooming action to the tail. Rats assigned in the HIIT exercise training group were also acclimatized to the treadmill for 1 week. HIIT involved treadmill running 5 days per week for 12 weeks which entailed running in 1 min intervals followed by 2 min active rest intervals for a total of 30 minutes per session. The rats started running at the speed of 16 m/min for the 1 min interval. Throughout the 12 week period, this speed gradually increased by 3—5 min/min every two weeks until it reached 36 m/min in the final three weeks. The HOMA-R and HOMA-beta was calculated using the following formulas:

$$\text{HOMA-R} = \frac{\text{Fasting Insulin } (\mu\text{U/ml}) \times \text{Fasting Glucose (mmol/l)}}{22.5}$$

$$\text{HOMA-}\beta = \frac{360 \times \text{Insulin}}{\text{Glucose} - 63}\%$$

Gastrocnemius muscle of the animals was also exposed using standardized dissection methods, cleaned of excess fat and tendon/connective tissue and cut into 30—20 mg slices to kept in RNA stabilization reagent (RNA later TM, QIAGEN; Germany). A real-time quantitative polymerase chain reaction system was used to measure the abundance mRNA in muscle tissue and total RNA was isolated using the RNeasy protect mini kit (QIAGEN) following the manufacturer's instructions. The quantification of purified total RNA was performed using a Nano-Drop ND 1000 spectrophotometer. RT-PCR was performed using retro-gene 6000 system and a One Step SYBR TAKARA kit according to the manufacturer's instructions. The amplification reactions were carried out with the following cycles: 42 °C for 20 min, 95 °C for 2 min followed by 40 cycles at 94 °C for 10 seconds and 60 °C for 40 seconds. Temperatures between 50 to 95 °C were used for the generation of the melting curve. RNA Polymerase II was used as the Reference gene. The expression of target mRNA over the reference value was calculated by equation $2-\Delta\Delta CT$. The primers used for *IRS1* and *PTP-1B* gene expression analysis are shown in Table 1.

## 3. Results

The Changes of rat's body weight before and after the study period are shown in Table 2. The results showed that before exercise intervention, there were no significant differences in body weight between the groups ($P \geq 0.05$). However, after intervention, the rat's body weight increased significantly in the resistance and HIIT trained group ($d < 0.05$). The rat's body weight in the control and aerobic trained groups didn't change significantly after 12 weeks ($P \geq 0.05$).

Statistical analysis revealed that as a result of 12 weeks of exercise intervention, the level of fasting blood glucose decreased significantly in all aerobic, HIIT and resistance groups compared to the diabetic control group ($P \leq 0.0001$), while fasting serum insulin level increased significantly in all the exercised trained groups compared to the control group ($P \leq 0.0001$). The HOMA_IR index was also not significantly different between the groups ($P = 0.447$). In addition, it was also revealed that the expression of *IRS1* gene upregulated significantly in aerobic ($P = 0.018$) and HIIT ($P = 0.023$) trained groups, while no significant difference observed the resistance group ($P = 0.900$) compared to the control group (Table 2). In addition, the expression of *PTP-1B* gene downregulated significantly in all aerobic ($P = 0.012$), HIIT ($P = 0.019$), and resistance ($P = 0.022$) groups compared to control group (Table 2).

## 4. Discussion

Physical activities have been proven to link glucose homeostasis improved and raised insulin sensitivity after an acute bout of exercise in animals and humans. Impaired insulin action on body glucose uptake is a particular sign of diabetes. In the present study, we demonstrated that a 12 week of exercise intervention; fasting blood glucose decreased and fasting serum insulin level increased significantly in all trained groups compared to diabetic control group, whereas HOMA_IR index not significantly changed. The expression of *IRS1* gene upregulated significantly in aerobic and HIIT trained groups, but no significant difference observed the resistance group compared to the diabetic control group. Furthermore, *PTP-1B* gene expression downregulated significantly in all aerobic, HIIT, and resistance groups compared to control group by different mechanisms.

The findings suggest that fasting blood glucose levels had decreased and fasting serum insulin level increased in all trained groups. The beneficial effect of physical training on glucose reduction has been proved by multiple studies. Moreover, fluctuations in body weight can be the reasons for fasting blood glucose reduction, which is caused by metabolic marker improvement. Twelve week of exercise intervention had significant effects on insulin induction in diabetic rats, which is compatible with findings of several studies. The positive effects of exercise on improved insulin resistance can be achieved through insulin receptor enhancement in muscle cells or an increase in the number

**Table 1** IRS1 & PTP-1B primers used.

| Genes | Primer sequence | Product size | T m | Gene Bank |
|---|---|---|---|---|
| *IRS1* | Forward: GGCCATGAGC-GATGAGTTTC Reverse: GGCGGAG-GATTGTTGAGATG | 176bp | 59 | NM_012969.1 |
| *RNA PolymraseII* | Forward: ACTTTGAT-GACGTGGAGGAGGAC Reverse: GTTGGCCTGCGGTCGTTC | 164 bp | 60 | XM_008759265.1 |
| *PTP-1B* | Forward: GCAGTTG-GAGTTGGAGAACCTG Reverse: CGT-GCTCTGGGCTGAGTG | 159 bp | 60 | NM_001191052.1 |
| *RNA PolymraseII* | Forward: ACTTTGAT-GACGTGGAGGAGGAC Reverse: GTTGGCCTGCGGTCGTTC | 164 bp | 60 | XM_008759265.1 |

**Table 2** Changes in blood glucose, serum insulin, HOMA-IR, IRS1, and PTP-1B before and after intervention (mean ± SD).

| Variables | Diabetic control | Aerobic | HIIT | Resistance |
|---|---|---|---|---|
| Body weight (gr)Before intervention | 28 ± 219 | 19 ± 226 | 21 ± 218 | 25 ± 228 |
| Body weight (gr) After intervention | 42 ± 255 | 58 ± 241 | 61 ± 271 | 59 ± 281 |
| Blood glucose (mg/dL) | 293 ± 12 | 244 ± 15* | 230 ± 12* | 221 ± 16* |
| Serum insulin (μIU/mL) | 4.06 ± 0.25 | 5.08 ± 0.29* | 5.74 ± 0.53* | 6.10 ± 0.96* |
| HOMA-Index | 2.94 ± 0.26 | 3.07 ± 0.32 | 3.26 ± 0.37 | 3.32 ± 0.57 |
| HOMA2 parameters | 1.59 ± 0.85 | 0.86 ± 0.08 | 0.94 ± 0.11 | 1.01 ± 0.15 |
| HOMA-b cell indexes% | 6.35 ± 0.03* | 10.10 ± 0.05* | 12.37 ± 0.09* | 13.89 ± 0.08* |
| IRS1 | 1.00 | 4.00 ± 2.65* | 3.85 ± 2.36* | 0.85 ± 0.58 |
| PTP-1B | 1.00 | 0.42 ± 0.16* | 0.53 ± 0.13* | 0.69 ± 0.20* |

*: indication of significant difference and $P < 0.05$ as compared to diabetic control group.

of glucose transporter proteins in the skeletal muscle cells. With regard to insulin markers, the HOMA-IR has not changed after training, which is in accordance with the findings of other studies that were also unable to detect a change in the HOMA index despite an increase in IRS1 [2]. Such deviation can be ascribed to the subjects and diabetes induction process in rats. STZ is an alkylating agent which causes DNA damage in pancreatic beta-cells resulted in beta-cell injury and lower insulin production and prevent insulin sensitivity improvement.

As much of the molecular mechanism of the beneficial effects of different exercises in the insulin resistant state remains unknown. Thus, whether exercise contributes to insulin resistance improvement or streptozotocin reduction cannot be determined definitively. Aerobic and HIIT exercises were observed to lead to increase in the IRS1 level, but resistance training had no significant impact. These apparent contradictory results may be related to the various types of exercise and physiological or metabolic changes. IRS1 plays a key role in transmitting signals from the insulin and insulin-like growth factor-1 (IGF-1) receptors to intracellular pathways. The activation of this protein is believed to be a major mechanism contributes to insulin resistance. Importantly, previous studies have shown that HIIT can improve insulin sensitivity, independent of losing weight in obese or overweight people sedentary adults [3], or type 2 diabetes. The impact of physical training on IRS-1 expression is controversy; increase after 1 day of training, or decrease after 5 days of training, even unaffected by short-term exercise training. However, this controversy may depend on study subject, protocol of training, and the mechanism of different training. The mechanism of different energy system in exercise types might associate with glycolysis that linked to the ability of specific pathway to promote IRS1. In addition, physiological and metabolic parameters of resistance exercise may not able to stimulate IRS pathway. Our findings indicating that resistance training does not alter insulin sensitivity or IRS1 expression suggest that resistance exercise alone, its power or intensity is likely insufficient to stimulate insulin pathway. As the insulin resistance — IRS1 and 2 pathway is involved in glucose uptake and glycogen synthesis in muscle, we recommend that aerobic and HIIT exercises reverses insulin resistance through activation of this pathway.

Our finding also indicated that the expression of *PTP-1B* gene downregulated significantly in all trained groups and

expanded insulin-signalling network in gastrocnemius muscle of rats. In contrast to our finding, it has been reported that the level of PTP-1B is not changed in the normal rat's muscle after cessation of chronic exercise [4]. Multiple mechanisms may involve in the pathogenesis of insulin resistance in skeletal muscle. PTP1B is a major regulator of insulin pathway and its ability has been established at the molecular level, which is considered a promising potential therapeutic target for type 2 diabetes. In a study, erosion of the *PTP1B* gene indicated that inhibition of PTP1B function may be an effective tactics for diabetes or obesity treatment. In accordance with this, our results showed a reduction activity and expression of PTP1B after different exercises (aerobic, HIIT, resistance) in gastrocnemius muscle of diabetic rats. All 3 exercise protocol used in the present study were sufficient to inhibit PTP-1B mRNA in the gastrocnemius muscle. Different exercises protocol could suppress *PTP-1B* gene in gastrocnemius muscle which may act through protein level/activity in the hypothalamus. In this study, authors did not evaluate protein levels associated with PTP-1B production. Thus, it could be our research limitation that we do not provide any evidence to prove accurately. Nonetheless, these findings suggest that inhibition or downregulation of PTP-1B can be a useful method for improving insulin sensitivity. However, the signalling pathway or detail mechanism by which exercise protocol could completely inhibit PTP-1B production remains unclear and requires further investigation.

## 5. Conclusion

Collectively, our study demonstrates that 12 week of exercise intervention decreased fasting blood glucose, increased fasting serum insulin, and HOMA_IR index not significantly changed in gastrocnemius muscle of experimental diabetes rats. Furthermore, *PTP-1B* gene expression induced in all aerobic, HIIT, and resistance training protocol, while *IRS1* gene varied in an exercise protocol manner. Thus, the effects of exercise on PTP1B mRNA levels seems to depend on the protocol or type of training, and this phenomenon may help to reorganize the set of exercise to protect against insulin resistance. Overall, our data provide considerable progress in our understanding of the insulin resistance that link different physical exercise to improve muscle insulin sensitivity in diabetes.

## Disclosure of interest

The authors declare that they have no competing interest.

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