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Recombinant Irisin Ameliorates Insulin Resistance in Type 2 Diabetic Mice

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ABSTRACT

Introduction: An exercise-induced myokine, Irisin, is shown to cause the browning of white adipose tissue, promote glycogen storage, and inhibit glucose and cholesterol formation in the liver. Studies have, however, shown a reduction of circulating irisin in type 2 diabetes, suggesting altered secretion of myokines in the setting of insulin resistance.

Aims & Objectives: This study explored the effect of recombinant irisin on insulin resistance and oxidative strain in laboratory induced type 2 diabetic mice.

Place and Duration of Study: Animal House and Laboratory of Akhter Saeed Medical and Dental College, Lahore from March 2018 to December 2018.

Material & Methods: A randomized comparative trial was conducted. Type 2 diabetes mellitus was produced in 60 male albino mice by feeding them a fat rich diet (60% kcal fat) for 6 weeks, followed by low dose streptozotocin (40 mg/kg body weight). Thirty diabetic mice acted as diabetic controls and thirty mice were administered recombinant irisin intra-peritoneally, 1mg/kg body weight daily for 4 weeks. Serum fasting glucose, insulin, lipid profile, and malondialdehyde were estimated thereafter. Insulin resistance was predicted by employing the HOMA-IR score and triglyceride-HDL ratio. The data was analyzed by using SPSS (version 21), a p value less than 0.05 was regarded statistically significant

Results: Diabetic mice that received recombinant irisin showed significant (p=0.000) reduction in fasting blood glucose (249.03±66.17mg/dl) and serum insulin (4.61±0.08µU/L) as compared to the diabetic control mice (428.50±78.13 mg/dl, 5.95±0.72µU/L). The degree of insulin sensitivity as determined by HOMA-IR score and triglyceride HDL ratio was significantly increased in the diabetic irisin groups as compared to the diabetic control group. This correlated with reduction in the oxidative stress marker malondialdehyde.

Conclusion: Recombinant Irisin improved insulin sensitivity and reduced oxidative strain in type 2 diabetic mice.

Keywords: Irisin, insulin resistance, oxidative stress, diabetes mellitus type 2.

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is among the most common endocrine disorders globally. 'The International Diabetes Federation reported that, in 2022, 26.7% of adults in Pakistan were living with diabetes'[1]. This figure is gravely high and is expected to continue rising, paralleling the increase in obesity and sedentary lifestyles. The favorable results of exercise in diabetes are well documented and include improved glycemic control, insulin resistance and lipid profile; as well as delayed onset of complications[2].

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Irisin is an exercise mediated myokine, first discovered by Bostrom et al in 2012. It was shown to cause 'browning' of white adipose tissue through significant up regulation of uncoupling protein1 (UCP1) and several other mitochondrial genes, leading to an increase in oxygen consumption[3].

Studies have reported low circulating levels of irisin in T2DM patients[4]. Insulin resistance entirely alters the metabolic machinery of myocytes and exercise mediated up regulation of irisin is compromised in type 2 diabetics[5].

The primary goal of this study was to explore the impact of recombinant irisin administration on insulin response in the setting of altered myokine secretion in a type 2 diabetic mouse model.

MATERIAL AND METHODS

A randomized animal trial was undertaken at the Animal Lab of Akhtar Saeed Medical College, Physiology Department after review and approval by the Ethical Review Committee of the institute (890/AMDC, Dated: 01-11-17).

Sixty male albino mice of approximately 25 to 30gm, physically fit were purchased from the Veterinary Institute and Research Centre, Lahore. They were maintained in a controlled environment (adequate ventilation, 12 hour day night cycle, optimal temperature and humidity) at the department's animal laboratory facility. Mice were given a fat rich diet constituting 60% kcal fat for 6 weeks[6]. Subsequently, 35 mg/kg body weight of streptozotocin was administered[7]. Fasting glucose was checked one week after streptozotocin administration. Mice with levels more than 270mg/dl were tagged as diabetic[6].
Diabetic mice were randomly assigned into two groups. Thirty mice acted as diabetic controls and the remaining thirty administered recombinant irisin for 4 weeks.

Administration of recombinant irisin:

Recombinant irisin protein, manufactured from E.coli source, with -his tag at N terminal was obtained from Cusabio pharmaceuticals in lyophilized form. It was reconstituted by adding demineralized water to compose a stock solution of approximately 1 mg/ml. 1mg/kg was given daily to the diabetic irisin group by intra peritoneal injection for 4 weeks[8].

Blood sampling and biochemical assays:

Terminal blood sampling was done 28 days (4 weeks) after commencing the study by cardiac puncture, under ether anesthesia. Serum was extracted by centrifuging at 2000 revolutions per minute for 10 minutes and immediately transferred into clean Eppendorf containers. Fasting blood glucose was found by the glucose oxidase method and serum insulin was estimated by ELISA using the direct sandwich technique.

Estimation of insulin resistance by HOMA score and TG/HDL ratio:

HOMA-IR is an established *indirect* measure to estimate insulin resistance. Other recently-developed indirect measures of insulin resistance include HOMA2, QUICKI, and the triglyceride / HDL ratio.

Homeostasis Model Assessment - insulin resistance (HOMA-IR) score was determined using the formula:

$$\text{HOMA-IR}= \frac{glucose\left(\frac{mg}{dl}\right)\times insulin\left(\frac{mU}{L}\right)}{405}$$

HOMA-IR ≥ 2 was considered as a marker of insulin resistance[9].
TG/HDL ratio has also been considered as a practical marker of insulin resistance. A TG/HDL ratio >2.75 is considered a strong indicator of insulin resistance with a sensitivity of 80% and a specificity of 78%[10].

Estimation of oxidative stress marker MDA by TBARs method:

Thiobarbituric acid reactive substance (TBARS) assay technique was used to estimate the oxidative stress marker malondialdehyde (MDA) which is a byproduct of lipid peroxiation. This technique is based on the reaction of malondialdehyde with thiobarbituric acid in an acid medium at 194–212°F. The end product is a pink colored compound with maximum absorbance at 532 nm, the concentration of which is estimated by spectrophotometry[11].

RESULTS

The data was analyzed by using SPSS (version 21). Mann Whitney U test was used to ascertain the significance of difference of means between the two groups. Biochemical parameters were expressed as mean ± standard deviation and a p value less than 0.05 was regarded statistically significant with a confidence interval of 95%.
The diabetic mice that received recombinant irisin showed significant (p=0.000) reduction in serum fasting glucose (326.83±71.42l) and serum insulin (4.61±0.08µU/L) as compared to the diabetic control mice (434.96±92.45, 5.95±0.72µU/L) (Table 1)

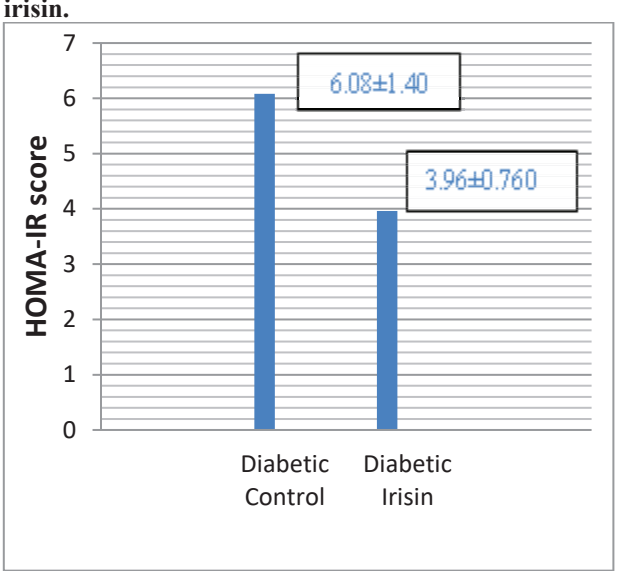
Table1: Effect of recombinant irisin on mean fasting blood glucose and serum insulin in diabetic control mice and diabetic mice administered recombinant irisin.

Biochemical Parameter	**Diabetic Control Mice** (mean±SD)	**Diabetic mice administered Irisin (mean±SD)**	**P - value**
Fasting blood glucose (mg/dl)	434.96±92.45	326.83±71.42	0.000*
Insulin (µU/ml)	5.95±0.72	4.61±0.08	0.000*

Figure 1 illustrates that the diabetic mice that were administered recombinant Irisin have a significantly lower mean HOMA-IR score compared to the

diabetic control mice, suggesting that treatment with irisin reduced insulin resistance.

Figure 1. Comparison of HOMA-IR score between diabetic control mice and diabetic mice administered irisin.



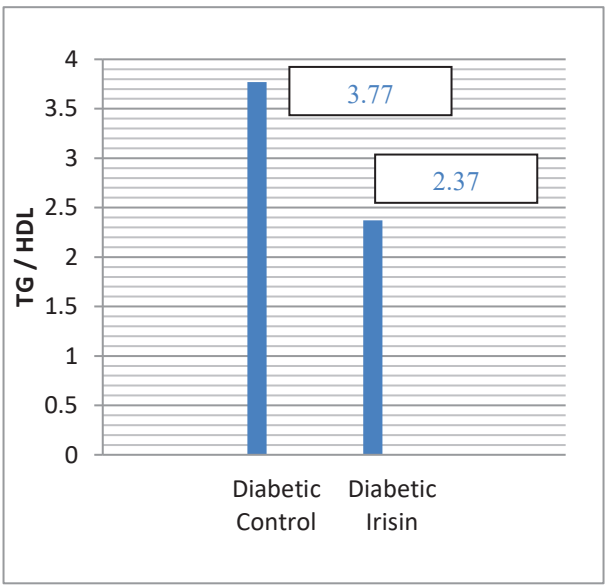
Furthermore, the diabetic mice that received recombinant irisin also showed a statistically significant (p=0.000) reduction in serum cholesterol (159.80±6.54mg/dl), triglycerides (137.16±8.87) ,low density(71.23±7.94mg/dl) and very low density (26.53±1.33) lipoproteins as compared to the diabetic control mice respectively. Furthermore, serum HDL was significantly (p= 0.000) higher in the diabetic irisin group(58.30±2.86mg/dl) as compared to the diabetic control group(40.61±5.41mg/dl) (Table 2).

Table 2. Effect of recombinant irisin on lipid profile in diabetic control mice and diabetic mice that were administered irisin.

Biochemical Parameter	Diabetic control mice (mean±SD)	Diabetic mice administered irisin (mean±SD)	P value
Cholesterol (mg/dl)	180.23±12.66	159.80±6.54	0.000*
Triglycerides (mg/dl)	149.73±9.66	137.16±8.87	0.000*
HDL (mg/dl)	40.61±5.41	58.30±2.86	0.000*
LDL (mg/dl)	89.96±9.87	71.23±7.94	0.000*
VLDL (mg/dl)	29.96±1.75	26.53±1.33	0.000*

Figure 2 illustrates that the Diabetic Irisin group has a significantly lower TG/HDL ratio compared to the Diabetic Control group, which implies that treatment with irisin may have a beneficial effect on lipid profiles and reduce cardiovascular risk.

Figure 2. Comparison of TG/HDL ratio between diabetic control mice and diabetic mice administered irisin.



Moreover, diabetic mice that received recombinant irisin showed highly significant (p=0.000) reduction in serum malondialdehyde (16.22±0.96 µmol/L) as compared to the diabetic control mice (19.94±1.31µmol/L) (Table 3). These findings suggest that the reduction in oxidative stress induced by irisin may have contributed to the improvement in insulin sensitivity in the Diabetic Irisin group.

Table 3: Effect of recombinant Irisin on serum Malondialdehyde in diabetic control mice and diabetic mice administered irisin.

Blood Parameter	Diabetic Control mice mean±SD	Diabetic mice administered irisin (mean±SD)	P value
Malondialdehyde (umol/L)	19.94 ± 1.31	16.22 ± 0.96	0.000*

DISCUSSION

The present study assessed the impact of recombinant irisin on insulin resistance in a type 2 diabetic mice model and sought to relate these

effects to reduction in oxidative stress and lipid profile.

Our study demonstrated statistically significant ($p<0.001$) amelioration of hyperglycemia and hyper-insulinemia after administration of recombinant irisin.

These results corroborate a related work by Wang J et al. that showed that irisin alleviated insulin intolerance ($p<0.05$) in homozygous db/db mice as compared to controls. When these mice were treated with irisin, hyperglycemia and hyperinsulinemia significantly decreased ($p<0.05$ and $p<0.01$ respectively)[12]. It is thought that irisin primarily targets muscle and adipose tissue in order to control glucose homeostasis. In a study conducted by Yano N et al, when recombinant irisin was applied to human skeletal muscle cells and C2C12 myoblast cell line for at least one hour, the absorption of glucose dramatically increased. In a similar vein, C2C12 cells in which the FNDC5 gene was overexpressed demonstrated enhanced glucose absorption and glycogen accumulation. Moreover, the basal insulin receptor (IR) phosphorylation level was considerably greater in the irisin-overexpressed C2C12 cells[13].

Furthermore, in glucose stimulated insulin secretion of insulinoma INS-1E cells, irisin opposed increases in lipogenic gene expression, phosphorylated acetyl-CoA-carboxylase (ACC), and reversed the intracellular buildup of triglycerides and unsaturated fatty acids. Irisin's protective effects on the capacity and survival of INS-1E cells to secrete insulin were also mediated by AMPK signaling. Furthermore, in INS-1E cells exposed to glucolipotoxic conditions, irisin reduced the expression of pro-inflammatory genes. In isolated mouse islets under glucolipotoxic circumstances, irisin also enhanced insulin production, prevented apoptosis, and restored gene expression linked to β-cell function[14].

The preservation of mitochondrial integrity is linked to cells' heightened resilience in situations of hyperglycemic stress. C2C12 myoblasts exposed to hyperglycemic and/or hyper-lipidemic conditions for two hours had increased mitochondrial disintegration and as well as higher oxygen free radicals. However, administration of recombinant irisin lessened these effects[15]. In another in vivo study, irisin administration ameliorated insulin resistance and oxidative stress in high fat diet fed mice. The underlying mechanisms were inhibition of β cell apoptosis and activation of the Trx2 gene that regulates the mitochondrial apoptotic signaling pathway[16].

CONCLUSION

Recombinant irisin may help improve insulin sensitivity in type 2 diabetic patients whose endogenous secretion of irisin is reduced.

LIMITATIONS OF STUDY

These findings warrant further investigation of the potential of recombinant irisin to improve blood glucose and insulin sensitivity in human therapeutic trials.

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