ORIGINAL ARTICLE

Effects of Recombinant Irisin on Body Mass Index, Serum Insulin, Luteinizing Hormone and Testosterone Levels in Obese Female BALB/c Mice

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ABSTRACT

Objective: To determine the effects of recombinant irisin on body mass index (BMI), serum insulin, luteinizing hormone (LH) and testosterone levels, and to correlate the serum insulin levels with serum luteinizing hormone and testosterone levels and to correlate the body mass index with serum insulin levels in obese female BALB/c mice.

Study Design: Laboratory-based experimental study.

Place and Duration of Study: Department of Physiology, Shifa College of Medicine, Islamabad in collaboration with Research Laboratory of Shifa College of Medicine, National Institute of Health (NIH) and Reproductive Physiology Laboratory, Quaid-e-Azam University, Islamabad, from March 2015 to September 2016.

Methodology: Ninety female BALB/c mice were divided into three equal groups. Group A which was the control group was fed with normal chow diet. Group B and Group C were fed with high fat-high sucrose (HF-HS) diet for five weeks to induce obesity. After four weeks group C was divided into two subgroups. Group C-low dose (LD) was injected with low dose irisin and group C-High dose (HD) was injected with high dose irisin for one week. After five weeks, the BMI, serum insulin, LH and testosterone levels were measured in all the groups. Data was analysed by SPSS version 21. P-value of <0.05 was considered significant.

Results: Group B showed statistically significant elevation in BMI, serum insulin, LH and testosterone levels as compared to Group A (p <0.001, <0.001, 0.007 and 0.014, respectively). Group C-HD showed statistically significant decrease in BMI, serum insulin, and LH as compared to Group B (p <0.001, 0.013 and 0.028, respectively). Serum testosterone level was also decreased in group C-HD as compared to Group B, however the difference was not significant.

Conclusion: Obesity increases the serum insulin, LH and testosterone and irisin significantly lowers the elevated BMI, serum insulin and LH levels in female BALB/c mice. It also lowers the elevated testosterone levels, but not significantly.

Key Words: *Irisin, Myokine, Obesity, Infertility.*

INTRODUCTION

Obesity is a worldwide health problem, and its prevalence is increasing day-by-day. Obesity is associated with a number of complications, one of the most significant being insulin resistance,[1] According to a study conducted in 2006, the prevalence of obesity in Pakistan is 10.3%.[2]

Obesity is associated with insulin resistance due to overproduction of certain adipokines and cytokines.[3] Insulin resistance is associated with female infertility because pituitary and ovary remain sensitive to insulin in a setting of peripheral insulin resistance. Hyperinsulinemia associated with insulin resistance leads to elevated LH and testosterone levels resulting in female infertility.[4,5]

Lifestyle modification, especially changes in diet and exercise, presently continues to be the unsurpassed option for the management of obesity.[6] It has been discovered that skeletal muscle during or just after physical activity releases numerous hormones into the circulation. These hormones named myokines affect metabolism in various tissues.[7] One of the recently discovered myokines is irisin. Exercise induces the expression of a transcriptional coactivator, peroxisome proliferator-activated receptor γ (PPARγ) coactivator 1α (PGC1α) in the skeletal muscle, which in turn stimulates the expression of fibronectin type III domain containing 5 (FNDC5), a transmembrane protein, which is cleaved and released into the circulation as "irisin". Irisin induces the conversion of white adipose tissue (WAT) into brown adipose tissue (BAT) by mediating the expression of mitochondrial uncoupling protein 1 (UCP1) in WAT.[8] BAT is involved in energy expenditure leading to weight loss and improvement in high fat diet-induced insulin resistance.[9]

Thus irisin could generate key exercise-induced health benefits and could be a therapeutic agent for the management of obesity and related metabolic disorders,[10] including female infertility. This study was conducted to determine the effects of recombinant irisin on BMI, serum insulin, LH and testosterone levels in obese female BALB/c mice.

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METHODOLOGY

This study was approved by Institutional Review Board and Ethical Committee of Shifa College of Medicine, and carried out in the Department of Physiology, Shifa College of Medicine, Islamabad in collaboration with the Research Laboratory of Shifa College of Medicine and National Institute of Health (NIH) Islamabad. BALB/c is an albino, laboratory-bred strain of the house mouse. BALB/c is the strain bred and kept at NIH. Ninety female BALB/c mice, 6-8 weeks of age, with weights of 25 ±5 gms were housed in a spacious room at NIH with good ventilation. Twelve hours light and 12 hours dark cycles were maintained under a temperature of 23 ±5°C through a central temperature regulating system. Mice were divided into three equal groups. Each group was kept in a separate cage and the cages were labelled according to the grouping done. Group A was the control group and was supplied with NIH standardised laboratory diet and water *ad libitum*. Group B was induced with obesity by feeding them high fat-high sucrose (HF - HS) diet containing 21% fat and 34% sucrose for five weeks.[11] Group C was induced with obesity by feeding them HF-HS diet for five weeks. After four weeks of the start of experiment, Group C mice were divided into two subgroups C-Low dose (LD) and C-High dose (HD), which were injected respectively with intra-peritoneal injections of 2.8 μg and 4.3 μg of recombinant irisin (CUSABIO) daily for 7 days.[8]

At the end of five weeks, BMI of all groups was measured by applying the following formula.[12] Body weight in grams / (Crown-rump length in cm^2).

The mice having body weight of ≥40g and BMI of ≥0.39 g/cm^2 were considered obese.[13] Intra-cardiac sampling was done for the measurement of serum insulin, luteinizing hormone and testosterone. The blood was centrifuged. Serum was obtained and stored at a temperature of -70°C in the Research Lab of Shifa College of Medicine, Islamabad. Analysis of serum insulin, luteinizing hormone and testosterone was done by using ELISA kits at Reproductive Physiology Lab of Quid-e-Azam University, Islamabad.

Statistical Package for Social Sciences (SPSS) version 21 was used for data analysis. All the variables were expressed as mean ± standard deviation. One-way analysis of variance (ANOVA) was followed by post-Hoc Tukey's t-test to determine the difference among groups. P-value <0.05 was considered significant. Confidence intervals were kept at 95%. Correlation between the variables was studied by Pearson's Correlation Coefficient.

RESULTS

Mean ±SD of variables among all the groups and the comparison of variables among different groups after applying ANOVA (Analysis of variance) are presented in Table I. Comparison of variables among groups after applying post-Hoc Tukey's t-test is presented in Table II.

In group B, which was induced with obesity, all the variables were significantly raised as compared to group A (control) including BMI, serum insulin, LH, and testosterone ($p < 0.05$).

In group C-LD, which was injected with low dose of irisin, only BMI was significantly lowered as compared to the group B, which was induced with obesity ($p < 0.05$).

In group C-HD which was injected with high dose of irisin, there was significant decrease in BMI, serum insulin and serum LH as compared to group B which was induced with obesity ($p < 0.05$). Serum testosterone was also lowered, but the difference was not statistically significant.

The correlation among variables after applying Pearson's correlation coefficient is presented in Table III. BMI showed a significant positive correlation with serum insulin, serum LH and serum testosterone ($p < 0.05$). Serum insulin had a significant positive correlation with serum LH ($p < 0.05$). Serum insulin showed a positive

Table I: Mean ±SD and comparison of BMI, serum insulin, luteinizing hormone and testosterone among groups A, B, C-LD, C-HD by analysis of variance (ANOVA).

Groups / variables	Group A (Control)	Group B (Obesity)	Group C (Irisin - Low dose)	Group C (Irisin - High dose)	ANOVA (p-value)
BMI	0.311 ±0.02	0.418 ±0.02	0.381 ±0.02	0.300 ±0.02	<0.001*
Serum Insulin (uM/l)	3.986 ±0.92	5.915 ±2.23	4.897 ±1.52	4.341 ±0.76	<0.001*
Serum LH (ng/ml)	2.866 ±0.71	3.275 ±0.56	3.248 ±0.34	2.757 ±0.53	0.007*
Serum testosterone (ng/ml)	0.904 ±0.41	1.242 ±0.44	1.109 ±0.28	1.008 ±0.25	0.014*

Table II: Comparison of BMI, serum insulin, luteinizing hormone and testosterone among groups A, B, C-LD and C-HD by post-Hoc Tukey's t-test (p-values).

Variables	Group A (Control)			Group B (Obesity)		Group C (Irisin-low dose)
	Group B (Obesity)	Group C (Irisin - low dose)	Group C (Irisin - high dose)	Group C (Irisin - low dose)	Group C (Irisin - high dose)	Group C (Irisin - high dose)
BMI	<0.001*	<0.001*	0.448	<0.001*	<0.001*	<0.001*
Serum insulin (mU/l)	<0.001*	0.305	0.904	0.189	0.013*	0.775
Serum LH (ng/ml)	0.049*	0.183	0.937	0.183	0.028*	0.097
Serum testosterone (ng/ml)	0.008*	0.358	0.837	0.691	0.221	0.887

** Significance (p <0.05) at 95% Confidence Interval.*

Table III: Correlation between BMI, serum insulin, serum luteinizing hormone and serum testosterone.

Variables	p-value	Pearson's correlation
BMI		
Serum insulin (mU/l)	0.003*	+0.315
Serum LH (ng/ml)	0.033*	+0.231
Serum testosterone (ng/ml)	0.008*	+0.286
Serum insulin (mU/l)		
Serum LH(ng/ml)	0.001*	+0.359
Serum testosterone (ng/ml)	0.062	+0.203
Serum LH (ng/ml)		
Serum testosterone (ng/ml)	0.066	+0.200

**Significance (p < 0.05); - (Negative Correlation); + (Positive Correlation)*

correlation with serum testosterone which was statistically not significant. Serum LH had a positive correlation with serum testosterone, which was statistically not significant.

DISCUSSION

Obesity has received substantial attention as a major health hazard and its prevalence is increasing in most countries. Obesity is linked with an increased risk of various chronic diseases.[14] One of the metabolic complications of obesity is insulin resistance.[15] Obesity also plays an important role in reproductive disorders, predominantly in women.[16] Insulin responsiveness of the pituitary and ovary is exclusively preserved in the background of peripheral insulin resistance, resulting in elevated luteinizing hormone levels and ovarian overproduction of testosterone, both of which lead to anovulation and infertility.[17] Increasing energy expenditure is an accepted methodology to combat the worldwide epidemic of obesity. Exercise causes an increase in energy expenditure through augmentation in brown fat and the browning of white fat. Irisin, a newly discovered myokine, is thought to be the missing link as it induces the browning of white fat deposits.[18]

The present study was planned to determine the effects of recombinant irisin on body mass index, serum insulin, luteinizing hormone and testosterone levels, to correlate the serum insulin levels with serum luteinizing hormone and serum testosterone levels and to correlate the body mass index with serum insulin levels in obese female BALB/c mice.

The results of this study showed that the serum insulin levels were increased in the mice with diet-induced obesity. This was in consistence with the study conducted by Xu *et al*. in which the mice with diet-induced obesity showed a dramatic increase in serum insulin levels.[19]

Another study performed by Kappe and colleagues revealed similar results. In this study, after 10 weeks on a HFD, mice had significantly increased body weight, fasting blood glucose, HbA1c and fasting serum insulin levels.[20]

The results of my study also showed that the serum luteinizing hormone (LH) levels were increased in high fat diet-induced obese mice with hyperinsulinemia. This was in consistence with the study conducted by Akamine *et al*. Female rats submitted to high-fat diet for 120 days showed greater body weight, retroperitoneal and gonadal fat pad, serum insulin levels, HOMA index, progesterone levels and LH levels.[21]

Another study conducted by Di Vall and co-researchers showed similar results. Diet-induced obese (DIO) female mice exhibited weight gain, increased fasting plasma insulin and basal LH levels.[22]

The results of this study also depicted that the serum testosterone levels were increased in high fat diet-induced obese female mice with hyperinsulinemia. These results were in consistence with another study conducted by Brothers *et al*. In this study, wild type (WT) female mice placed on a high fat diet for 12 weeks, which showed statistically significant increase in serum testosterone in relation to lean WT females.[23]

A study conducted by Radavelli-Bagatini *et al*. showed positive correlation between body weight and serum insulin levels, the results of which are similar to this study. However, the results regarding serum LH and testosterone levels in obese female mice are not consistent with this study. Female New Zealand obese (NZO) mice showed higher body weights, increased basal plasma glucose and insulin levels as well as insulin resistance, compared with control mice.[24] These findings were consistent with this study. However, in contrast to this study, there was no difference between testosterone levels of NZO and control mice.[24] Additionally, LH levels were lesser in young NZO compared with control mice, but no differences were appreciated at the older ages,[24] which was not consistent with this study, where obese mice showed increased LH levels.

The results of this study also revealed that the treatment of obese female mice with recombinant irisin reduced their body mass indices and serum insulin levels. This was in consistence with the study conducted by Bostrom *et al*. They delivered FNDC5-expressing adenovirus to mice induced with obesity and insulin-resistance, by feeding a high fat diet, which moderately increased blood irisin levels of the mice three-fold. They observed that the body weights of the irisin expressing mice were fairly reduced and they showed a substantial improvement in glucose tolerance and reduced fasting insulin levels as compared to the control mice after 10 days.[8]

Another study which showed similar results was conducted by Zhang *et al*. Mice fed with high fat diet for 10 weeks and treated with intraperitoneal injection of purified recombinant irisin for 14 days showed significant decrease in body weight and improvement in glucose tolerance as compared to the control fat mice.[9]

Since it has been demonstrated that obesity is linked with female infertility; and amongst the possible causes

responsible for obesity-induced infertility in females, are the effects of hyperinsulinemia (resulting from insulin resistance) on the pituitary and ovarian tissues as these tissues retain their insulin sensitivity in a setting of peripheral insulin resistance, we considered that a potential therapeutic agent that is beneficial in lowering the body mass index and serum insulin levels; and at the same time, is a candidate to be an alternative to the exercise, can be equally effective in treating the obesity linked infertility by lowering the raised serum luteinizing hormone levels and raised serum testosterone levels.

Brothers *et al*. demonstrated that the obesity-induced infertility in female mice can be rescued by pituitary specific knock out of insulin receptor.[23] And in another study conducted in 2014, Wu *et al*. showed that obesity-induced infertility and hyperandrogenemia can be corrected by deletion of insulin receptor in ovarian theca cell.[5] Evidence is lacking regarding the potential therapeutic role of irisin in treatment of obesity-induced infertility in females. This study suggests a possible role of irisin in the management of obesity-linked infertility because it has reduced the elevated levels of luteinizing hormone and testosterone levels in insulin-resistant obese female mice. Data presented here show that the reduction in serum luteinizing hormone levels after short treatments of obese insulin resistant mice with recombinant irisin is statistically significant. Although the same dose of irisin has reduced the raised serum testosterone levels but the results are not statistically significant, suggesting the probability of existence of some additional pathway linking obesity, hyper-insulinemia and hyperandrogenemia in obese female mice. Further, it remains to be determined whether longer treatments with irisin and/or higher doses would cause more weight loss, further reduction in insulin and luteinizing hormone levels and statistically significant reduction in serum testosterone levels.

A study conducted by Bastu *et al*. revealed similar results regarding the effects of irisin on body weight and serum insulin levels. However, their findings of the effect of irisin on increasing the serum LH levels are not consistent with my study. In this study, high-fat diet-induced obese female mice were intravenously injected with 10^{10} FNDC5-expressing adenovirus. The body weight, glucose and insulin levels were significantly greater in the obese controls. They also observed significant negative correlations between serum irisin levels and weight and insulin.[25] These findings are consistent with this study. In contrast to the present study, the serum luteinizing hormone levels were significantly higher in the irisin group.[25]

The therapeutic potential of irisin is evident. The world-wide alarming increase in obesity and related metabolic disorders strongly suggest exploring the clinical efficacy of irisin in these disorders. These findings have provided initial experimental evidence in supporting possible therapeutic role of recombinant irisin for the treatment of obesity, insulin resistance as well as associated disorders like obesity-induced infertility.

CONCLUSION

Obesity increases the serum insulin, luteinizing hormone, and testosterone levels; whereas, irisin significantly lowers the elevated BMI, serum insulin and luteinizing hormone levels in the obese female BALB/c mice. It also lowers the elevated testosterone levels, but not significantly.

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