CHANGES IN IRISIN RELEASE IN RESPONSE TO PERIPHERAL KISSPEPTIN-10 ADMINISTRATION IN HEALTHY AND OBESE ADULT MEN

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Abstract

Context. Kisspeptin role in metabolism has been implicated recently. However, the nature of the signals that may connect body fat/muscle tissues with the central nervous system governing energy homeostasis remains to be elucidated.

Objective. The present study was designed to investigate the effects of peripheral kisspeptin-10 administration on irisin release in human males.

Subjects and methods. Kisspeptin-10 was administered to normal weight (n=8) and obese (n=8) men. Sequential blood sampling was performed for 30 minutes pre and 210 minutes post kisspeptin injection at 30 minutes interval. ELISA kit was used to detect plasma irisin levels.

Results. There is a significant ($P < 0.0001$) effect of Kisspeptin-10 administration on irisin release in both normal weight and obese participants. Mean irisin levels (96.24 ± 1.351 ng/mL) at 210 minutes were significantly ($P < 0.0001$) enhanced as compared to pre-kisspeptin (59.18 ± 4.815 ng/mL) in normal weight subjects. In obese subjects mean irisin levels (75.76 ± 4.06 ng/mL) were significantly ($P < 0.0001$) elevated at 180 minutes post-kisspeptin when compared with pre-kisspeptin irisin levels (41.28 ± 2.89 ng/mL).

Conclusion. Our findings suggest that kisspeptin may have a novel therapeutic potential to induce irisin release in humans which may have anti-obesity effects.

Key words: Obesity, Kisspeptin-10, Metabolism, Irisin.

INTRODUCTION

Kisspeptin is a group of peptides which belongs to RF amides and is named due to its C-terminal amino acids (1). RF amides and kisspeptin have the role in regulation of blood pressure and pain, reproduction and feeding mechanism (2).

Kiss1 and Kiss1r are not only expressed in the brain, but also in several peripheral tissues such as the pancreas, adipose tissue and liver (3) which are involved in the metabolism. The role of kisspeptin/GPR54 signalling in the regulation of metabolism is supported by many evidences. First, it is expressed in peripheral tissues. Second, kisspeptin/GPR54 signalling is impaired in metabolic disorders such as obesity, diabetes and under-nutrition (4). Third, kisspeptin signalling affects the lipid metabolism *in vitro* studies (5). Fourth, kisspeptin receptor knock-out female mice exhibit an obese phenotype (6, 7).

A recently recognized myokine secreted by skeletal muscle through physical activity is irisin. It is a myokine that changes the fat cells into brown cells and increases the burning of fat. This suggests that irisin may play a role in treating obesity. Bostrom *et al.* reported the anti-obesity effect of irisin. After exercise irisin was secreted from the skeletal muscle in mammals (8).

Kisspeptin system regulates gonadotropic axis. In rats, impairment in the kiss1 gene expression leads to metabolic disorder and hypogonadism (9).

All of the reported data have focused on the role of kisspeptin neuronal network in mediating energy balance to the reproductive axis. However, data about the function of kisspeptin signalling in controlling energy balance is very scarce. Kiss1r is also expressed in various brain regions which do not have GnRH neurons (10) and in peripheral tissues (11) such as pancreas and adipose tissue (3). In addition to this Kiss1r knock-out mice exhibit obese phenotype (6, 7). All of these findings suggest that kisspeptin signalling may have some additional roles other than reproduction. Moreover, kisspeptin signalling may

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also be implicated in energy homeostasis in white and brown adipose tissue. However, the nature of the signals that may connect body fat/muscle tissues with the central nervous system governing energy homeostasis remains to be elucidated. Consequently, exploring out such signals will really help in understanding the mechanism of energy homeostasis. Therefore, we hypothesize that kisspeptin signalling might serve as a possible pathway that connects the hypothalamic center with the body fat or muscle mass governing energy homeostasis. We propose that kisspeptin acts as a trigger for the stimulation of the irisin release monitoring the browning of white adipose tissue.

Hence, the present research work was designed to determine the effects of peripheral administration of kisspeptin-10 on the irisin release in normal and obese subjects.

MATERIALS AND METHODS

Participants

Eight adult obese men (115 ± 14 kg; BMI 34 ± 2 kg/m²) and eight adult healthy men (BW 75.7 ± 4.8 kg, having BMI 23 ± 0.7 kg/m²) participated in this study. BMI was selected as per Asian population BMI reported by WHO in 2004 (18.5 - 23 kg/m² is the BMI of normal weight persons and BMI for obese persons is >27 kg/m²) (12) and all of the volunteers belong to the middle class socioeconomic group and were residents of the Lutheran city, a district of Punjab province. Anthropometric data of the volunteers are given in Table 1. Experimental procedure was conducted according to the guidelines in the Declaration of Helsinki and ethical approval was obtained from a Departmental Ethical Committee of Quaid-I-Azam University Islamabad. Informed written consents were obtained from all the participants of this study as per WHO's Performa. The age of both obese and control participants was 20-40 years. Blood pressure recorded before the study was below the limit of 130/90 mmHg in every participant, blood glucose levels at the start of the sampling (four hours prior to breakfast) were not more than 109 mg/dL and mean pulse rate recorded before the experiment was from 74 ± 4 beats/min. All

individuals who participated in this study had normal kidney function, liver function, hemoglobin levels and normal blood count.

Experimental design

Cohorts of obese men ($n=8$) and normal men ($n=8$) were administered human kisspeptin-10 (metastin 45-54) to study the role of acute administration of kisspeptin-10 on plasma irisin levels. The sampling was done at Kamal surgical complex Lodhran. All the subjects had breakfast at 0700 hr. The volunteers were fixed with an infusion cannula (Farcocath; G/Ø/L: 22, 0.9, 25 mm; Farcomake for Advanced Medical Industries SAE (Alexandria, Egypt) in the cephalic vein for the ease of blood sampling and kisspeptin-10 administration. The blood samples were collected in heparinized syringes (BD 3 mL, Luer-Lok Tip with Precision Glide Needle, 23G×1TW (0.6×25mm), Becton Dickinson Pvt Ltd). Sampling was started at 10 a.m. to 12 a.m. and finished up at 2 p.m. to 4 p.m. daily. Serial blood samples (2 mL) were drawn before 30 minutes and after 210 minutes of kisspeptin injection at many intervals with a 30min gap (-30, 0, 30, 60, 90, 120, 150, 180, 210 min), whereas 0 min sample was taken within 5 min, after injection. Kisspeptin-10 was administered ($0.5 \mu\text{g/kg}$ BW) as an intravenous bolus, immediately after collecting -30 min sample.

Pharmacological agents

Heparin (Hepa Rotex; BP 5000 units/mL; Rotex Medica, Trittau, Germany) and normal saline (0.9% NaCl) used in this experiment was acquired from local pharmaceutical industries. Kisspeptin-10 with (metastin 45-54) was purchased from Calbiochem, Darmstadt, Germany.

Preparation of different doses of kisspeptin-10

Kisspeptin-10 (1mg vial) was originally obtained in lyophilized form. Stock solution of 1mg/mL was prepared by adding 1 mL normal saline (0.9% NaCl). Sixteen doses of different concentrations of kisspeptin-10, according to body weight of each individual ($0.5 \mu\text{g/kg}$ BW), were prepared in hygienic environment in sterile glass vials of 5 mL. The final volume of injection was 2 mL, containing kisspeptin-10 as per dose rate ($0.5 \mu\text{g/kg}$ BW) and normal saline.

Blood sampling

The blood samples were drawn by using heparinized syringes. After the collection of every sample, 2mL of normal saline was also administered.

Table 1. Anthropometric characteristics of the studied groups

Parameter	Normal Weight	Obese
Age (years)	20-40 years	20-40 years
Weight (kg)	68-83 kg	98-140 kg
Height (m)	1.65-1.82 meters	1.70-2 meters
BMI (kg/m ²)	22-24 kg/m ²	33-41 kg/m ²

Samples were then transferred to EDTA coated tubes and stored in a refrigerator at 4-6°C until centrifuged. All of the blood samples (within 4 hours) were centrifuged at 3000 rpm at 28°C for 15 minutes. Blood plasma was isolated in eppendorf vials of 1.5 mL capacity and stored at -20°C until hormonal analysis.

Blood pressure and pulse rate measurement

Blood pressure (mmHg) and pulse rate (beats/min) of every participant were measured at 30 minutes interval using a mercurial desk sphygmomanometer (Reister, Germany) from the brachial artery and by feeling from the radial artery, respectively, during sampling.

Blood glucose measurement

Blood glucose (mg/dL) of every participant was measured at 30 minute intervals by using a glucometer (Accu-check, Roche, Pakistan).

Hormonal analyses

Plasma levels of irisin were measured by using a Human Fibronectin Type III Domain Containing Protein 5 (FNDC5) ELISA Kit (Cat# RD-FNDC5-Hu). The whole assay was conducted by following the protocol and procedure provided with the kit. The sensitivity of the assay was 0.63 ng/mL. The intra-Assay variation was <10 %. After the completion of all steps the optical density was read at 450 nm by using a micro plate reader (680XR, BIO RAD, Tokyo, Japan) within 10 minutes.

Figure 1. Graphical representation of mean plasma irisin levels before and after kisspeptin-10 administration in (NW) group (A) in (OB) group (B) showing a significant increase ($P < 0.05$) in irisin release after kisspeptin-10 administration. Comparison of mean irisin levels before kp-10 administration in normal weight (C) and (OB) group (D) It indicating that normal weight subjects have significantly ($P < 0.05$) higher irisin levels as compared to obese subjects. Kisspeptin-10 has a profound effect on obese individuals as its irisin level increased as compared to normal subjects irisin levels before kp-10 administration.

Calculation of Results

A standard curve was drawn by taking standard concentration on X-axis and their absorbance on Y-axis. Irisin concentrations in samples and positive control were calculated by using the 4PL model given in microplate reader software version 5.2.1 (680XR, BIO RAD, Tokyo, Japan).

Figure 2. Graph shows that kisspeptin-10 administration significantly increased irisin release in normal weight participants ($r=0.61$; $P<0.05$).

Figure 3. Effect of kisspeptin-10 administration on irisin release in obese participants. Graph shows a significant effect of kisspeptin-10 administration on irisin release in obese subjects ($r=0.67$; $P<0.05$).

Figure 4. Comparison of Pre and Post Kisspeptin-10 administration in (NW) and (OB) participants. There is a significant increase ($P<0.001$) in irisin in both groups as determined by post hoc test.

Statistical Analysis

Pre and post kisspeptin mean irisin levels were compared by paired t test. Two-way ANOVA was used to determine the effect of single iv bolus administration of kisspeptin-10 on irisin release in obese and normal weight men. All data are presented as mean $\pm$ SEM. A $P<0.05$ indicated the significant difference. Analysis of data was done using Graph Pad Prism, version 5.01 (Graph Pad Software Inc., San Diego, CA, USA).

RESULTS

Baseline plasma irisin levels in study participants

In the present study, a significant ($P<0.0001$) increase in plasma irisin levels was observed after kisspeptin-10 administration in both normal and obese subjects (Fig.1 A and B). Plasma irisin levels were higher in normal weight subjects 59.18 ± 4.815 ng/mL as compared to obese subjects 41.28 ± 2.893 ng/mL (Fig.1 C).

Levels of plasma irisin during 3 hour intravenous infusion of kisspeptin-10 in normal weight subjects

Time-dependent dose effect of kp-10 was observed in normal weight subjects. Post-kisspeptin irisin levels are presented as Mean $\pm$ SEM (96.24 ± 1.351 ng/mL) as compared to pre-kisspeptin irisin levels (59.18 ± 4.815 ng/mL) in normal weight subjects. Graph (linear regression analysis) shows the significant effect of kisspeptin-10 administration on irisin release in normal weight subjects ($r=0.61$; $p<0.05$) Fig. 2. There was a positive correlation between kisspeptin-10 administration and irisin release.

Levels of plasma irisin during 3 hour intravenous infusion of kisspeptin-10 in obese subjects

Time profiles for plasma irisin release after kisspeptin-10 administration is presented in obese subjects Fig 3. Mean plasma irisin levels were significantly ($P<0.0001$) elevated at 180 minutes post-kisspeptin (75.76 ± 4.059 ng/mL) when compared with pre-kisspeptin irisin levels (41.28 ± 2.893 ng/mL) in obese subjects.

It is determined that there is a significant ($P<0.0001$) effect of kisspeptin-10 administration on release of irisin hormone. Post kisspeptin plasma irisin levels were significantly ($P<0.05$) high as compared to pre kisspeptin levels. There was a positive correlation

between time and plasma irisin levels in both groups. This may be the additional role of kisspeptin in energy homeostasis rather than reproduction.

Two way ANOVA results were significant ($P<0.05$), it shows the kisspeptin-10 administration has significant effects on irisin release. After two factorial ANOVA Bonferroni post test was used to measure differences among groups. Both groups have significant effect of Kp-10 dose on irisin release as shown in Fig. 4.

DISCUSSION

In our study, kisspeptin-10 administration resulted in the increase of plasma irisin concentrations in healthy and obese men. This effect was observed after 180 min of kisspeptin-10 administration. It has been known that irisin neurons play a key role in the regulation of GnRH release (13). Previous studies have demonstrated that irisin and NPY are co-localized in the paraventricular nucleus of the hypothalamus suggesting a possible role of irisin in energy balance also (14). Similarly, kisspeptin neurons also showed close proximity with NPY in ARC nucleus of hypothalamus (15), which may suggest a possible link between irisin and kisspeptin neurons. In addition to this KiSS-1R is also expressed in NPY neurons in hypothalamus (16). Previous studies showed that after irisin and kisspeptin-10 administration NPY levels increased (14, 17) which may depict that NPY play a role in mediating the effects of kisspeptin-10 administration on irisin release. The rise in irisin levels after kisspeptin-10 administration further support the hypothesis that kisspeptin stimulates irisin release either by activation of irisin neurons in the hypothalamus or through skeletal muscles. Recent findings showed possible relationships between irisin and kisspeptin neurons involved in reproduction and growth.

Although many studies reported an increase in irisin release after exercise, but in some this effect was not found. It means other factors may stimulate irisin release in addition to exercise. Irisin release is influenced by the day and night cycle (18), circadian rhythms (19) and season of the year (20). It has been reported that basal irisin levels also change with age (21). Hurdles and pitfalls faced during the measurements of irisin levels in the laboratory experiments have been recently deliberated extensively (22) but they are very far from being solved. In light of these observations our findings showed a potential relationship between kisspeptin-10 and irisin as kisspeptin-10 administration

has stimulated irisin release.

In our study, we have found the positive effect of kisspeptin-10 administration on irisin release in both obese and normal weight human males. This stimulation of irisin release may be from skeletal muscle or adipose tissues, either by peripheral or by central pathways such as hypothalamus. This finding suggests that kisspeptin hormone may have the potential to regulate energy homeostasis. It shows that there is a significant difference ($P<0.0001$) between pre kisspeptin and post kisspeptin irisin release in normal weight (NW) participants.

We are still unable to determine it precisely. We may only hypothesize that a kisspeptin-10 is one of the most important neuropeptides which stimulate irisin release. The effects of irisin in humans are not fully understood. To our knowledge, our study is the first to investigate associations between kisspeptin-10 and irisin concentration. As previously reported that Kiss1r knock-out mice exhibit obese phenotype (6, 7) which suggests that kisspeptin may have some roles in energy homeostasis in addition to reproduction. Our findings suggest that kisspeptin can be used to enhance the circulating levels of irisin in humans, which may have novel therapeutic roles that may in turn convert white adipose tissues to brown adipose tissues and may treat obesity. The actual mechanism underlying this process may be stimulation of uncoupling protein 1 (UCP1) expression and release of energy by burning these adipose tissues. This ATP energy may regulate the biochemical reaction occurring inside the body and improve the metabolic physiology of the individual.

In conclusion, our results found the stimulatory effects of kisspeptin-10 on irisin release in adult male humans. At the end, we conclude that our study will greatly help in the treatment of obesity and metabolic disorders in human males and regulate energy homeostasis.

Conflict of interest

The authors of this study have no conflict of interest.

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