

Kisspeptin and Vaspin: Indicators of Insulin Sensitivity Improvement before Weight Loss Following Sleeve Gastrectomy in Experimental Type 2 Diabetes Mellitus

Mohamad Yosof Rezk^{1,*}, Hany Ahmed Elkatawy²

^{1,2}Physiology Department Faculty of Medicine Zagazig University, Egypt

Abstract **Aim:** detect changes in serum kisspeptin level and possible associated mechanisms in diabetic rats following sleeve gastrectomy (SG) specially the relationship between body weight (BW) and serum kisspeptin (Kiss). **Animals and Methods:** 40 rats were divided into 4 groups; Control (C), Diabetic (D), Diabetic Sham Operated (DSO) and Diabetic with Sleeve Gastrectomy (DSG) Group. Body weight (BW), Food Intake (FI) and serum Kisspeptin (Kiss) and Vaspin (V) levels were analyzed. Also, some serum glucostatic parameters including serum Glucose, Insulin and Homeostasis model assessment of insulin resistance (HOMA-IR) were measured. **Results:** BW increased significantly in D group in relation to C group ($p=0.006$). Insignificant changes in BW are found in DSG after 2 weeks in comparison with D group ($p=0.8$) however, significant reductions in body weight were found in DSG group after 4 and 10 weeks in comparison with D group ($p=0.04$ and 0.008 respectively). Kiss decreased significantly in D group in comparison with C group ($p=0.04$), however insignificant changes were found in DSO group at 2 w, 4 w and 10 weeks in comparison with D group ($p=0.5$). Significant increases were found in Kiss in SG group after 2w, 4 w and 10 weeks in comparison with D group ($p=0.0001$). Significant increases were found in kiss DSG group after 2w, 4 w and 10 weeks in comparison with C group ($p=0.03$, 0.003 , 0.0006 respectively). Vaspin (V) was decreased significantly in D group in relation to C group ($p=0.0001$), however insignificant changes in V were found in DSO group at 2 w, 4 w and 10 weeks in comparison with D group ($p=0.9$). Significant increases in V were found in DSG group after 2w, 4 w and 10 weeks in comparison with D group ($p=0.0003$, 0.0001). **In Conclusion:** Significant increases were found in Kisspeptin and Vaspin serum levels in DSG after 2w, 4 w and 10 weeks in comparison with C and D groups ($p\leq 0.05$). A moderate negative correlation ($r=-0.688$) was found between serum Kiss and BW in all groups which means incomplete association and there is a tendency for Kiss to increase without corresponding reduction in body weight. Significant increases were found in Kiss in DSG groups after 2w, 4 w and 10 weeks in comparison with the D group ($p=0.0001$) without corresponding or associated significant reduction in BW at 2 weeks in comparison with D group ($p=0.8$). The significant increase in Kiss at week 2 with insignificant BW reduction indicates early changes in serum kisspeptin after sleeve gastrectomy preceding weight changes.

Keywords Kisspeptin, Sleeve gastrectomy, Type 2 diabetes mellitus, Vaspin, Body weight

1. Introduction

Kisspeptins (Kiss) has been detected in the nervous system as well as peripheral tissues like placenta, testes and pancreas [1]. The Kiss-1 gene encodes 54, 14, 13 or 10 amino-acid peptides known as kisspeptins [2]. Actions of kisspeptins are conducted through G protein-coupled receptor 54 (GPR54) [3]. Kiss-1/GPR54 system has been included in tumor progression and metastasis and potent antimetastasis actions of Kiss-1 peptide have been found in

thyroid and breast carcinoma [4]. Expression of Kiss-1/GPR54 system has been found in brain areas as hypothalamus, spinal cord, pituitary and human plasma [5] which strongly proves additional physiological functions of this peptide. It was found that there is a role for Kiss-1/GPR54 system in the neuroendocrine control of gonadotropin secretion, brain sex differentiation, puberty onset and fertility [6].

Hauge-Evans et al. (2006) have demonstrated the presence of kisspeptin and GPR54 mRNAs in both pancreatic B and A cells. Kisspeptin-54 has been shown to stimulate the late phase of glucose-induced insulin secretion in mouse and human islets [7].

A number of metabolic modulators have been found as regulators of kisspeptin like leptin, ghrelin,

* Corresponding author:

myr777777777@yahoo.com (Mohamad Yosof Rezk)

Published online at <http://journal.sapub.org/diabetes>

Copyright © 2017 Scientific & Academic Publishing. All Rights Reserved

pro-opiomelanocortin (POMC) and neuropeptide Y (NPY) [8]. De Bond JA and Smith JT. (2014) reported that kisspeptin neurons can directly excite anorexigenic POMC neurons and indirectly inhibit orexigenic NPY neurons. They suggested that kisspeptin may have a direct role in regulating energy balance. They concluded that kisspeptin signaling may also be a direct regulator of metabolism.

Expression of Kiss-1 in the hypothalamus is sensitive to nutritional status and it might contribute to the suppression of reproductive function in such conditions as negative energy balance periods [9]. Sagheb *et al.*, (2017) reported that Kisspeptin (Kiss1) and its G protein-coupled receptor (GPR-54) (Kiss1r) is an essential component of controlling ghrelin expression in the hypothalamus and pancreatic beta cells express Kiss-1 and kissR. They suggested that ghrelin may have a similar role in the transcription of kiss1-KissR signaling in the pancreas too [10].

Type 2 diabetes mellitus (T2DM) is a prevalent disease that endangers human health, and searching methods to control the disease in a long-term and efficient manner is a worldwide problem [11]. Bariatric surgery can not only effectively reduce body weight but also relieve insulin resistance rapidly and permanently, a finding that has been confirmed in clinical studies [12]. Furthermore, bariatric surgery has been considered in the guidelines for treatment of T2DM [13, 14]. Sleeve gastrectomy (SG), the most widely used bariatric surgery [15], can significantly alleviate T2DM [16].

Vaspin is highly expressed in visceral adipose tissue of obese Otsuka Long-Evans Tokushima Fatty rats, an animal model of type 2 diabetes [17]. Li *et al.* (2008) found that vaspin levels are usually high in diabetic or insulin-resistant individuals compared to normal individuals with low weight [18]. Additionally, Wada (2008) concluded that vaspin has a modulatory role in glucose metabolism [19]. Castro *et al.*, (2017) concluded that vaspin analogues or antagonists can modify insulin sensitivity in metabolic syndrome [20].

According to our resources, we found no available study demonstrated the effect of sleeve gastrectomy on serum levels of kisspeptin, so this study is designated to demonstrate this effect and search the timing of this effect in relation to weight loss.

2. Materials and Methods

Animals

40 male albino rats (weight 200–220 g), provided by the Laboratory Animal house from Faculty of Medicine Zagazig University, were housed in a 12-h light/dark cycle under constant temperature ($24 \pm 2^\circ\text{C}$) and humidity ($60 \pm 10\%$) in independent ventilated cages. After being acclimated for 2 weeks, the weight, food intake, fasting glucose, serum Kisspeptin, Vaspin, Insulin, HOMA-IR of the rats were measured. This study was done in physiology department, Faculty of Medicine, Zagazig University Egypt.

Chemicals

Kisspeptin commercial enzyme immunoassay (EIA) kits (Phoenix Pharmaceuticals Inc., Burlingame, California, USA) purchased from Sigma Aldrich Cairo Egypt.

Vaspin commercial enzyme immunoassay (EIA) kits purchased from Sigma Aldrich Cairo Egypt.

Induction of type 2 diabetic model

30 rats (out of 40) were given access to clean water and a high-fat diet (HFD, 40% fat, Huafukang Biotech, China) for 1 month to induce insulin resistance and then were injected with streptozotocin (STZ, 35 mg/kg) (Sigma, USA) intraperitoneally. After induction of diabetes (rats with random blood glucose ≥ 16.6 mmol/l (≥ 300 mg/dl) are divided into 3 groups:

2nd group Dioabetic (D) without procedures

3rd group Diabetic undergone Sham Operation (DSO).

4th group diabetic undergone Sleeve gastrectomy (DSG)

So as total we have four groups:

1st group: control (C) group (n=10).

2nd group: Diabetic (D) rats without procedures (n=10).

3rd group: Diabetic rats with Sham Operation (DSO) (n=10).

4th group: Diabetic rats with Sleeve Gastrectomy (DSG) (n=10)

Surgical Procedures: Before each procedure, rats were fed 10% Ensure (Abbott, USA) for 2 days and then fasted for 12 h.

Sleeve Gatrectomy [21, 22]: rats were anesthetized with intraperitoneal injection of 10% chloral hydrate (3 ml/kg) before procedure. An upper abdominal incision of approximately 5 cm was performed and the gastric omentum and lesser omentum were then dissected. After ligation and transection of the gastric omental vessels in the pylorus area, we used forceps to clamp the greater curvature in case of hemorrhage. The portion of the stomach outside the clamped area, which was approximately 70% of the whole stomach volume and included the gastric fundus, was resected. The stomach incision was sutured with 5-0 silk suture (Ningbo Medical Needle, China). The abdomen was closed after leakage and hemorrhage was prevented.

Sham Operation [23]: A laparotomy was performed to expose the stomach and esophagus and operative time was prolonged to mimic that experienced by the SG rats. Subsequently, the abdominal incision was closed.

Postoperative Care

At the end of the surgical procedures, all rats received sterile 0.9% NaCl 10 mL i.p. and 10 mL s.c. to maintain hydration during healing. The animals received ketoprofen 5 mg/kg as an analgesic. They were placed on a heated mat until they recovered and then were returned to their home cages. The rats were allowed to drink purified water for 12 h after surgery, and a liquid diet containing 5% glucose and 0.2% KCl was provided for the next 48 h. Thereafter, they received the HFD until 10 weeks after surgery.

Body Weight, fasting glucose, plasma insulin and HOMA-IR were measured in all groups and in SO and SG groups at the 2nd, 4th and 10th week after the surgery. Also, serum levels of Vaspin, Kisspeptin are measured in all groups. Food intake was calculated by the difference in weight between the offered diet and the weight of the rest of the diet. Blood glucose by samples taken from the rat tail vein.

Biochemical analyses

Serum was obtained by centrifugation of blood sample. Serum kisspeptin levels were measured by a commercial enzyme immunoassay (EIA) kit (Phoenix Pharmaceuticals Inc., Burlingame, California, USA). The range of kisspeptin was 0-100 and the minimum detectable concentrations were 0.06 ng/ml. Serum vaspin was measured using the enzyme-linked immunosorbent assay (ELISA) kit. Vaspin values were obtained with Rat EIA-VAP (RayBiotech, Norcross, GA, USA). Plasma glucose levels were analyzed by the glucose oxidase method (Glucose Analyzer II; Beckman Coulter, Fullerton, CA). Serum insulin was measured by a rat insulin ultrasensitive ELISA (BioVendor, Kassel, Germany). Homeostatic model assessment for insulin resistance (HOMA-IR) was calculated [24].

Statistical Analysis: GraphPad QuickCals software was used for calculation of unpaired t-test. The data obtained from serological examination of kisspeptin was expressed as mean values \pm standard Error of Mean (SEM). Statistical analysis was performed in unpaired t-tests. P value ≤ 0.05 was taken to indicate statistical significance. Pearson Correlation coefficient between Body weight and serum Kisspeptin was calculated by Social Science Statistics software.

3. Results

Table 1 showed that body weight and food intake increased significantly in diabetic rats in comparison with the control group (310 ± 30 , 200 ± 20 respectively, $p=0.006$) (200 ± 3 , 150 ± 6 respectively, $p=0.0001$). Insignificant changes in body weight are found in diabetic sham group at 2w, 4w and 10 weeks in comparison with diabetic group (307 ± 23 , 306 ± 22 , 306 ± 20 and 310 ± 30 respectively, $p=0.9$). Also, insignificant changes in body weight are found in diabetic rats with sleeve gastrectomy after 2 weeks in comparison with diabetic group (305 ± 25 and 310 ± 30 respectively, $p=0.8$) however, significant and highly significant reduction in body weight were found in diabetic rats with sleeve gastrectomy after 4 and 10 weeks in comparison with diabetic group (237 ± 16 , 201 ± 21 and 310 ± 30 respectively, $p=0.04$ and 0.008 at 4 and 10 W). Food intake was reduced significantly in Diabetic sham group at 2 w, 4 w and 10 weeks in comparison with the diabetic group (180 ± 5 , 179 ± 7 , 178 ± 9 and 200 ± 3 respectively, $p=0.008$). Highly significant reductions in food intake were found in diabetic group with sleeve gastrectomy at 2 w, 4 w and 10 weeks in comparison with the diabetic group (90 ± 4 , 105 ± 5 ,

130 ± 7 and 200 ± 3 respectively, $p=0.0001$).

Kisspeptin decreased significantly in diabetic rats in comparison with the control group (1.31 ± 0.21 , 2.11 ± 0.31 respectively, $p=0.04$), however insignificant changes were found in sham diabetic group at 2 w, 4 w and 10 weeks in comparison with the diabetic group (1.11 ± 0.29 , 1.10 ± 0.28 , 1.12 ± 0.27 and 1.31 ± 0.21 respectively, $p=0.5$). Extremely significant increases were found in Kisspeptin in diabetic rats with sleeve gastrectomy after 2w, 4 w and 10 weeks in comparison with the diabetic group (2.97 ± 0.22 , 3.43 ± 0.23 , 3.91 ± 0.30 and 1.31 ± 0.21 respectively, $p=0.0001$). significant, highly significant and extremely highly significant increases were found in kisspeptin serum levels in diabetic rats with sleeve gastrectomy after 2w, 4 w and 10 weeks in comparison with the control group (2.97 ± 0.22 , 3.43 ± 0.23 , 3.91 ± 0.30 and 2.11 ± 0.31 respectively, $p=0.03$, 0.003 , 0.0006 respectively).

Vaspin was decreased significantly in diabetic rats in relation to control (from 700 ± 40 to 300 ± 50 , $p=0.0001$), however insignificant changes in serum vaspin were found in sham diabetic group at 2 w, 4 w and 10 weeks in comparison with the diabetic group (295 ± 45 , 296 ± 44 , 294 ± 43 and 300 ± 50 respectively, $p=0.9$). Highly significant and Extremely highly significant increase in serum vaspin were found in diabetic rats with sleeve gastrectomy after 2w, 4 w and 10 weeks in comparison with the diabetic group (590 ± 43 , 670 ± 50 , 720 ± 40 and 300 ± 50 respectively, $p=0.0003$, 0.0001).

Table 2 shows moderate negative correlation between serum Kisspeptin and body weight in all groups.

Table 3 showed extremely significant increase in serum Glucose in diabetic, diabetic sham at 2w, 4w, and 10 weeks in comparison with the control group (329 ± 13 , 327 ± 12 , 326 ± 11 , 326 ± 13 and 100 ± 18 respectively, $p=0.0001$). Highly significant and very highly significant reductions was found in glucose levels in diabetic rats with sleeve gastrectomy after 2w, 4 w and 10 weeks in comparison with the diabetic group (258 ± 14 , 180 ± 13 , 115 ± 14 and 329 ± 13 respectively, $p=0.001$, 0.0001). Insignificant changes are found in glucose level in diabetic rats with sleeve gastrectomy after 10 weeks in comparison with the normal control group (115 ± 14 and 100 ± 18 respectively, $p=0.51$).

Serum insulin was decreased significantly in diabetic and diabetic sham groups (2w, 4w and 10 w) in relation to control group (4.54 ± 0.05 , 4.55 ± 0.07 , 4.53 ± 0.03 , 4.57 ± 0.05 and 8.83 ± 0.04 respectively, $p=0.0001$). insignificant changes in serum insulin were found in diabetic sham groups (2w, 4w and 10 w) in relation to diabetic group (4.55 ± 0.07 , 4.53 ± 0.03 , 4.57 ± 0.05 and 4.54 ± 0.05 respectively, $p=0.9$). Insignificant changes are found in serum insulin levels between diabetic rats with sleeve gastrectomy after 2 weeks in comparison with the diabetic group (4.65 ± 0.05 and 4.54 ± 0.05 respectively, $p=0.3$). Significant and highly significant reduction in serum insulin levels in diabetic rats with sleeve gastrectomy after 4 w and 10 weeks in comparison with the diabetic group (4.35 ± 0.07 , 4.15 ± 0.09 and 4.54 ± 0.05 respectively, $p=0.04$, 0.001 respectively). Significant

Homeostasis model assessment of insulin resistance: The study showed that the HOMA-IR was significantly increased in diabetic and diabetic sham (2w, 4w and 10 w) groups in comparison with the control group (4.09 ± 0.07 , 4.07 ± 0.09 , 3.97 ± 0.08 , 3.99 ± 0.06 and 2.09 ± 0.09 respectively, $p=0.0001$). No significant changes were found in diabetic sham (2w, 4w and 10 w) groups in comparison with the diabetic group (4.07 ± 0.09 , 3.97 ± 0.08 , 3.99 ± 0.06 and 4.09 ± 0.07 , $p=0.9$,

0.27 and 0.4 respectively). Highly significant and significant increase in HOMA-IR in diabetic rats with sleeve gastrectomy after 2w and 4 weeks in relation to control group (2.45 ± 0.08 , 2.33 ± 0.07 and 2.09 ± 0.09 respectively, $p=0.007$ and 0.04 respectively) however, no significant change was found between diabetic rats with sleeve gastrectomy after 10 w and the control group (2.11 ± 0.06 and 2.09 ± 0.09 respectively, $p=0.8$). very highly significant reductions in HOMA-IR in sleeve gastrectomy groups at 2w, 4 w and 10w in relation to diabetic group (2.45 ± 0.08 , 2.33 ± 0.07 , 2.11 ± 0.06 and 4.09 ± 0.07 , $p=0.0001$).

Table 1. Effect of Sleeve Gastrectomy on Body Weight, Food Intake, Serum Kisspeptin and Vaspin

	Control	Diabetic	Diabetic + Sham (2 W)	Diabetic + Sham (4 W)	Diabetic + Sham (10 W)	Diabetic rats with Sleeve Gastrectomy (SG) after 2 W	Diabetic rats with Sleeve Gastrectomy (SG) after 4 W	Diabetic rats with Sleeve Gastrectomy (SG) after 10 W
BW(Body Weight) gm	200± 20	310± 30** ^a (a, p=0.006)	307± 23** ^a (p=0.002) (b, p= 0.9)	306± 22** ^a (b, p= 0.9)	306± 20** ^a (b, p= 0.9)	305± 25** ^a (p=0.004) (b, p= 0.8)	237± 16* ^b (b, p= 0.04)	201± 21** ^b (b, p= 0.008) Insignificant in relation to control
Food Intake: gm/day	150± 6	200± 3** ^a (p=0.0001)	180± 5*** ^{a, **b} (pa=0.001) (pb=0.008)	179± 7*** ^{a, **b} (pa=0.001) (pb=0.008)	178± 9*** ^{a, **b} (pa=0.001) (pb=0.008)	90± 4*** ^{a, ****b} (pb=0.0001)	105± 5*** ^{a, ****b} (pb=0.0001)	130± 7* ^{a, ****b} (p=0.04) (pb=0.0001)
Kisspeptin (ng/ml)	2.11± 0.31	1.31± 0.21* ^a (p=0.04)	1.11± 0.29* ^a (p=0.03) (pb= 0.58)	1.10± 0.28* ^a (pb= 0.55)	1.12± 0.27* ^a (p=0.02) (pb= 0.59)	2.97± 0.22* ^{a, ****b} (pa=0.03) (pb=0.0001)	3.43± 0.23* ^{a, ****b} (p=0.003) (pb=0.0001)	3.91± 0.30*** ^{a, ****b} (p=0.0006) (pb=0.0001)
VASPIN (Pg/ml)	700± 40	300± 50*** ^a (p=0.0001)	295± 45*** ^a (p=0.0001) (pb= 0.95)	296± 44*** ^a (pb= 0.9)	294± 43*** ^a (pb= 0.9)	590± 43*** ^b (pb=0.0003) Insignificant in relation to control	670± 50*** ^b (pb=0.0001) Insignificant in relation to control	720± 40*** ^b (pb=0.0001) Insignificant in relation to control

*** = Extremely significant (P value ≤ 0.001)

Table 2. Correlation between Body Weight and serum Kisspeptin[illegible]

Table 3. Effect of Sleeve Gastrectomy on Glucostatic Parameters

	Control	Diabetic	Diabetic + Sham (2 W)	Diabetic + Sham (4 W)	Diabetic + Sham (10 W)	Diabetic rats with Sleeve Gastrectomy (SG) after 2 W	Diabetic rats with Sleeve Gastrectomy (SG) after 4 W	Diabetic rats with Sleeve Gastrectomy (SG) after 10 W
Glucose mg/dl	100±18	329±13*** (pa=0.0001)	327±12*** (pa=0.0001)	326±11*** (pa=0.0001)	326±13*** (pa=0.0001)	258±14*** ^{a, **b} (pa=0.0001), (pb=0.001)	180±13*** ^{a, ***b} (pa=0.002) (pb=0.0001)	115±14*** ^b (pa=0.51) (pb=0.0001)
Insulin (mIU/ml)	8.83±0.04	4.54±0.05*** (pa=0.0001)	4.55±0.07*** (pa=0.0001) (pb=0.9)	4.53±0.03*** (pa=0.0001) (pb=0.8)	4.57±0.05*** (pa=0.0001) (pb=0.9)	4.65±0.05*** (pa=0.0001)	4.35±0.07*** ^{a, **b} (pa=0.0001) (pb=0.04)	4.15±0.09*** ^{a, ***b} (pa=0.0001) (pb=0.001)
HOMA-IR	2.09±0.09	4.09±0.07*** (pa=0.0001)	4.07±0.09*** (pa=0.0001) (pb=0.9)	3.97±0.08*** (pa=0.0001) (pb=0.27)	3.99±0.06*** (pa=0.0001) (pb=0.4)	2.45±0.08*** ^{a, ***b} (pa=0.007) (pb=0.0001)	2.33±0.07*** ^{a, ***b} (pa=0.04) (pb=0.0001)	2.11±0.06*** ^b (pa=0.8) (pb=0.0001)

DATA represented as mean ± standard Error of Mean (SEM).

*pa= significant in comparison with the control group (P value ≤ 0.05)

*pb= significant in comparison with the Diabetic group.

**= highly significant (P value ≤ 0.01)

*** = Extremely significant (P value ≤ 0.001)

4. Discussion

Kisspeptin is now considered an important regulator of reproductive functions as it is involved in the direct activation of gonadotropin-releasing hormone (GnRH) [36]. Ohtaki et al., (2001) found that Kiss exhibited the ability to suppress tumor metastasis and they reported that high level of *Kiss1* expression was found in healthy and tumorous human tissues [37]. According to our resources, we found no available study demonstrated the effect of sleeve gastrectomy on serum levels of kisspeptin, so this study is designated to demonstrate this effect and search the timing of this effect in relation to weight loss.

In the present study, body weight and food intake increased significantly in diabetic rats in comparison with the control group (p=0.006 and 0.0001 respectively). Insignificant changes in body weight are found in DSG group at 2w, 4w and 10 weeks in comparison with D group (p=0.9). Also, insignificant changes in body weight are found in DSO group after 2 weeks in comparison with D group (p=0.8) however, significant and highly significant reductions in body weight were found in DSO group after 4 and 10 weeks in comparison with D group (p=0.04 and 0.008 at 4 and 10 W respectively). These findings are supported by Lombardo et al., (2010) who found that sleeve gastrectomy reduced body mass index from 58.2 to 44.5 Kg/m² and they concluded that sleeve gastrectomy is a safe and effective treatment for the high-risk and super-obese patient [15]. The findings of the present study suggest that body weight is affected late by sleeve gastrectomy after 4 and 10 weeks. Our suggestion is supported by Wang et al., (2017) who found that the SG group displayed significant weight loss 6 weeks postoperatively.

In the present study, food intake was reduced significantly

in DSO group at 2 w, 4 w and 10 weeks in comparison with D group (p=0.008). Highly significant reductions in food intake were found in DSG group at 2 w, 4 w and 10 weeks in comparison with D group (p=0.0001). These findings are in agreement with Wang et al., (2017) who found that the Sleeve Gastrectomy group displayed significant reduction in food intake 4 weeks postoperatively [25].

In the present study, Kisspeptin decreased significantly in D group in comparison with the C group (p=0.04), however insignificant changes were found in DSO group at 2 w, 4 w and 10 weeks in comparison with D group (p= 0.5). These findings are in agreement with Dudek et al., (2016) who found that diabetic rats have changes in Kiss1 and/or GPR54 mRNA levels in the hypothalamic-pituitary-gonadal axis as well as in peripheral tissues [26]. The findings of the present study are also supported by Zhou et al., (2014) who found A marked suppression of ovarian Kiss1 mRNA levels in high fat diet (HFD) rats compared with the normal chow diet controls. They concluded that exposure of female rats to a high-fat diet may involve down-regulation of ovarian Kiss1 mRNA and kisspeptin [27].

In this study, Significant, highly significant and extremely highly significant increases were found in kisspeptin serum levels in DSG after 2w, 4 w and 10 weeks in comparison with C group (2.97±0.22, 3.43±0.23, 3.91±0.30 and 2.11±0.31 respectively, p=0.03, 0.003, 0.0006 respectively). Extremely significant increases were found in Kisspeptin in DSG after 2w, 4 w and 10 weeks in comparison with the D group (2.97±0.22, 3.43±0.23, 3.91±0.30 and 1.31±0.21 respectively, p=0.0001). This significant increase in kisspeptin serum levels in DSG group might be due to sudden drop in serum Ghrelin levels after gastrectomy as ghrelin is a strong inhibitor of kisspeptin. This explanation is supported by Sagheb et al., (2017) who found that Ghrelin

(10^{-6} M) significantly decreased the transcription of Kiss-1 compared to the C group in the islets of Langerhans and this concentration of ghrelin significantly diminished KissR transcription in islet cells too. They also found that ghrelin has a significant inhibitory effect on KiSS-1 and KissR mRNA transcription in CRI-D2 cells (insulinoma Cell line) [10]. Our explanation was also supported by Zhu *et al.*, (2014) who found that ghrelin secretion of Sleeve Gastrectomy group was significantly decreased ($P < 0.005$) [31].

In the present study, Vaspin was decreased significantly in D rats in relation to C group (from 700 ± 40 to 300 ± 50 , $p=0.0001$), however insignificant changes in serum vaspin were found in DSO at 2 w, 4 w and 10 weeks in comparison with the D group (295 ± 45 , 296 ± 44 , 294 ± 43 and 300 ± 50 respectively, $p=0.9$). These findings are supported by the findings of Castro *et al.*, (2017) who found that Vaspin level was lower for the D group than for the Non-D group [20]. Also the present study in agreement with Li *et al.* (2008) [18] who suggested that vaspin might have an insulin-sensitizing effect mainly on white adipose tissue. However, these findings are in controversy with Li *et al.* (2008) in their report that vaspin levels are usually high in diabetic or insulin-resistant individuals compared to normal individuals with low weight [18], this controversy may be due to species differences where this study was carried out on albino rats and also study of Castro *et al.* (2017) on wister rats however Lie *et al.* (2008) study was conducted in human. The present study is also supported by Hida *et al.* (2005) [17] who found that vaspin levels were markedly reduced in OLETF rats, an animal model of type 2 diabetes, when they developed severe hyperglycaemia at 50 weeks.

In this study, highly significant and extremely highly significant increase in serum vaspin levels were found in DSG group after 2w, 4 w and 10 weeks in comparison with the D group (590 ± 43 , 670 ± 50 , 720 ± 40 and 300 ± 50 respectively, $p=0.0003$, 0.0001). These findings are in agreement with Tomasz., (2015) who found that vaspin serum level was significantly higher after ileal transposition in relation to normal control rats [28].

In the current study, moderate negative correlation ($r = -0.688$) was found between serum Kisspeptin and body weight in all groups which means that Kisspeptin increases associated with decrease in Body Weight however, this association is incomplete and there is a tendency for Kisspeptin to increase without corresponding decrease in body weight. Extremely significant increases were found in Kisspeptin in DSG groups after 2w, 4 w and 10 weeks in comparison with the D group (2.97 ± 0.22 , 3.43 ± 0.23 , 3.91 ± 0.30 and 1.31 ± 0.21 respectively, $p=0.0001$) without corresponding or associated significant decrease in body weight at 2 weeks in comparison with D group (305 ± 25 and 310 ± 30 respectively, $p=0.8$). Body weight reduction become significant after 4 weeks and highly significant after 10 weeks in comparison with D group (237 ± 16 , 201 ± 21 and 310 ± 30 respectively, $p=0.04$ and 0.008 at 4 and 10 W). The highly significant increase in Kisspeptin at week 2 with insignificant body weight reduction indicates early changes in

serum kisspeptin after sleeve gastrectomy preceding weight changes. Our findings and explanation were supported by Pories *et al.*, [33] who reported that the glycemic control often occurs within days before significant weight loss has been reached. Also, our explanation was supported by Zhu *et al.*, (2014) who suggested that the control of the glycemic status may be a direct effect of surgery rather than a secondary effect of weight loss [31].

In the present study, extremely significant increase in serum Glucose in D, DSO groups at 2w, 4w, and 10 weeks in comparison with the C group ($p=0.0001$). Highly significant and very highly significant reductions were found in glucose levels in DSG after 2w, 4 w and 10 weeks in comparison with the D group (258 ± 14 , 180 ± 13 , 115 ± 14 and 329 ± 13 respectively, $p=0.001$, 0.0001). The present study was supported by Nosso *et al.*, (2011) who found that sleeve gastrectomy is effective in producing a significant and sustained weight loss and improving glucose homeostasis in severely obese T2DM patients [30]. The present study was also supported by Zhu *et al.*, (2014) who found that fasting blood glucose of the rats in the SG group had significantly decreased with the improved glucose tolerance [31]. Insignificant changes are found in glucose level in DSG after 10 weeks in comparison with the C group (115 ± 14 and 100 ± 18 respectively, $p=0.51$) which suggest improved serum Glucose level and return to semi-normal. This finding and suggestion is supported by Liu *et al.*, (2017) who reported that sleeve gastrectomy resulted in better glucose tolerance, lower HOMA-IR, up-regulated hepatic insulin signaling [29].

In the current study, Serum insulin was decreased significantly in D and DSO groups (2w, 4w and 10 w) in relation to C group ($p=0.0001$). Insignificant changes in serum insulin were found in DSO groups (2w, 4w and 10 w) in relation to D group ($p=0.9$). Insignificant changes are also found in serum insulin levels between DSG after 2 weeks in comparison with the D group (4.65 ± 0.05 and 4.54 ± 0.05 respectively, $p=0.3$). Significant and highly significant reduction in serum insulin levels in DSG after 4 w and 10 weeks in comparison with the D group (4.35 ± 0.07 , 4.15 ± 0.09 and 4.54 ± 0.05 respectively, $p=0.04$, 0.001 respectively). Significant reductions are found in serum insulin levels between DSG after 2w, 4 w and 10 weeks in comparison with the C group (4.65 ± 0.05 , 4.35 ± 0.07 , 4.15 ± 0.09 and 8.83 ± 0.04 respectively, $p=0.0001$). These findings are supported by Lee *et al.*, (2010) who found that the mean fasting plasma insulin levels were reduced significantly from 16.8 ± 15.4 to 5.6 ± 3.2 uIU/mL at 1 week after operation [34]. The present study was also supported by Silvestre *et al.*, (2008) who found that Kisspeptin-13 reduced glucose-induced insulin secretion in a dose-dependent manner and inhibited the insulin responses to both carbachol and exendin-4 and they concluded that kisspeptins may be implicated in the regulation of B-cell [35].

The present study found that the HOMA-IR was significantly increased in D and DSO (2w, 4w and 10 w) groups in comparison with the C group ($p=0.0001$). No

significant changes were found in DSO (2w, 4w and 10 w) groups in comparison with the D group (4.07 ± 0.09 , 3.97 ± 0.08 , 3.99 ± 0.06 and 4.09 ± 0.07 , $p=0.9$, 0.27 and 0.4 respectively). Highly significant and significant increase in HOMA-IR in DSG groups after 2w and 4 weeks in relation to C group (2.45 ± 0.08 , 2.33 ± 0.07 and 2.09 ± 0.09 respectively, $p=0.007$ and 0.04 respectively) however, no significant change was found between DSG group after 10 w and the C group (2.11 ± 0.06 and 2.09 ± 0.09 respectively, $p=0.8$). Very highly significant reductions in HOMA-IR in DSG at 2w, 4 w and 10w in relation to D group (2.45 ± 0.08 , 2.33 ± 0.07 , 2.11 ± 0.06 and 4.09 ± 0.07 , $p=0.0001$). These findings are in agreement with Basso et al., (2016) who found that Insulin sensitivity was significantly improved in sleeve gastrectomy compared with Sham operated rats as demonstrated by HOMA-IR values, which were reduced by $\sim 50\%$ ($p<0.0001$) [32]. However, the present study is in controversy with Basso et al., (2016) in that they found significant reduction in insulin level rather than significant increase, this controversy may be explained by the model used in their study which is vertical sleeve gastrectomy in which the fundus of stomach is left intact.

5. Conclusions

In this study, Significant increases were found in kisspeptin and Vaspin serum levels in diabetic rats with sleeve gastrectomy after 2w, 4 w and 10 weeks in comparison with C and D groups ($p \leq 0.05$). This significant increase in kisspeptin serum levels in diabetic rats with sleeve gastrectomy might be due to sudden drop in serum ghrelin levels after gastrectomy as ghrelin is a strong inhibitor of kisspeptin. A moderate negative correlation ($r = -0.688$) was found between serum Kisspeptin and body weight in all groups which means incomplete association and there is a tendency for Kisspeptin to increase without corresponding decrease in body weight. Extremely significant increases were found in Kisspeptin in diabetic rats with sleeve gastrectomy after 2w, 4 w and 10 weeks in comparison with the D group ($p=0.0001$) without corresponding or associated significant reduction in body weight at 2 weeks in comparison with diabetic group ($p=0.8$). Body weight reduction became significant after 4 weeks and highly significant after 10 weeks in comparison with diabetic group ($p=0.04$ and 0.008 at 4 and 10 W). The highly significant increase in Kisspeptin at week 2 with insignificant body weight reduction indicates early changes in serum kisspeptin after sleeve gastrectomy preceding weight changes.

6. Recommendations

The role of kisspeptin in reducing weight should be investigated in humans and this role could serve in treatment of obesity as alternative to surgery. Drugs acting on kisspeptin receptors should be investigated in further studies.

REFERENCES

- [1] Mead EJ, Maguire JJ, Kuc RE, Davenport AP, (2007): Kisspeptins: a multifunctional peptide system with a role in reproduction, cancer and the cardiovascular system. *Br J Pharmacol*; 151:1143–1153.
- [2] Dungan HM, Clifton DK, Strainer R. (2006): Minireview: Kisspeptin Neurons as Central Processors in the Regulation of Gonadotropin-Releasing Hormone Secretion. *Endocrinology*; 147:1154–1158.
- [3] Muir AI, Chamberlain L and Elshourbagy NA et al. (2001): AXOR12, a novel human G protein-coupled receptor, activated by the peptide KiSS-1. *J Biol Chem* 276: 28969–28975.
- [4] Masui T, Doi R and Mori T et al. (2004): Metastin and its variant forms suppress migration of pancreatic cancer cells. *Biochem Biophys Res Commun* 315: 85–92.
- [5] Horikoshi Y, Matsumoto H and Takatsu Y et al. (2003): Dramatic elevation of plasma metastin concentrations in human pregnancy: metastin as a novel placental-derived hormone in humans. *J Clin Endocrinol Metab* 88:914–919.
- [6] Navarro VM, Castellano JM and Ferra'ndez-Ferra'ndez R et al. (2005): Effects of KiSS-1 Peptide, the Natural Ligand of GPR54, on Follicle-Stimulating Hormone Secretion in the Rat. *Endocrinology* 146(4):1689–1697.
- [7] Hauge-Evans AC, Richardson CC, Milne HM, Christie MR, Persaud SJ & Jones PM (2006): A role for kisspeptin in islet function. *Diabetologia* 49 2131–2135.
- [8] De Bond JA, Smith JT. (2014): Kisspeptin and energy balance in reproduction. *Reproduction*, 147(3):R53-63.
- [9] Fernandez-Fernandez R, Navarro VM, Castellano JM, Dieguez C, Aguilar E, Pinilla L, et al. (2006): Novel signals for the integration of energy balance and reproduction. *Mol Cell Endocrinol*; 254:127-132.
- [10] Sagheb MM, Azarpira N, Mokhtary M., (2017): The effect of ghrelin on Kiss-1 and KissR gene transcription and insulin secretion in rat islets of Langerhans and CRI-D2 cell line. *Iran J Basic Med Sci*, 20(1), 36-40.
- [11] Whiting DR, Guariguata L, Weil C, et al. (2011): IDF diabetes atlas: global estimates of the prevalence of diabetes for 2011 and 2030. *Diabetes Res Clin Pract.*; 94(3): 311–21.
- [12] Schauer PR, Burguera B, Ikramuddin S, et al. (2003): Effect of laparoscopic Roux-en Y gastric bypass on type 2 diabetes mellitus. *Ann Surg.*; 238(4): 467–84.
- [13] Standards of medical care in diabetes-(2016): summary of revisions. *Diabetes Care*. 2016; 39 Suppl 1:S4-5.
- [14] Rubino F, Nathan DM, Eckel RH, et al. (2016): Metabolic surgery in the treatment algorithm for type 2 diabetes: a joint statement by international diabetes organizations. *Diabetes Care.*; 39(6): 861–77.
- [15] Lombardo V, Baratta R, Giannone G. (2010): Laparoscopic sleeve gastrectomy for morbid obesity. Our initial experience. *Ann Ital Chir.*; 81(1):17-20.

- [16] Himpens J, Dobbeleir J, Peeters G. (2010): Long-term results of laparoscopic sleeve gastrectomy for obesity. *Ann Surg.*; 252(2): 319–24.
- [17] Hida K., Wada J., Eguchi J. et al. (2005): Visceral adipose tissue-derived serine protease inhibitor: a unique insulin-sensitizing adipocytokine in obesity. *Proc. Natl. Acad. Sci. U.S.A.* 102, 10610–10615.
- [18] Li Q., Chen R., Moriya J. et al. (2008): A novel adipocytokine, visceral adipose tissue-derived serine protease inhibitor (vaspin), and obesity. *J. Int. Med. Res.* 36, 625–629.
- [19] Wada J. (2008) Vaspin: a novel serpin with insulin-sensitizing effects. *Expert Opin. Investig. Drugs* 17, 327–333.
- [20] Castro CA, da Silva KA, Buffo MM, Pinto KNZ, Duarte FO, Nonaka KO, Anibal FF, Duarte ACGO. (2017): Experimental type 2 diabetes induction reduces serum vaspin, but not serum omentin, in Wistar rats. *Int J Exp Pathol.*; 98(1):26-33.
- [21] Sun D, Liu S, Zhang G, et al. (2014): Sub-sleeve gastrectomy achieves good diabetes control without weight loss in a non-obese diabetic rat model. *Surg Endosc.*; 28(3):1010–8.
- [22] Pereferrer FS, Gonzalez MH, Rovira AF, et al. (2008): Influence of sleeve gastrectomy on several experimental models of obesity: metabolic and hormonal implications. *Obes Surg.*; 18(1):97–108.
- [23] Bruinsma BG, Uygun K, Yarmush ML, et al. (2015): Surgical models of Roux-en-Y gastric bypass surgery and sleeve gastrectomy in rats and mice. *Nat Protoc.*; 10(3):495–507.
- [24] Margolis LM, Rivas DA, Ezzyat Y, Gaffneystomberg E, Young AJ, McClung JP, Fielding RA and Pasiakos SM. (2016): Calorie Restricted High Protein Diets Downregulate Lipogenesis and Lower Intrahepatic Triglyceride Concentrations in Male Rats. *Nutrients*; 8(9): pii: E571.
- [25] Wang M, Wu Q, Xie H, Shao Y, Zhong M, Zhang X, Liu S, He X, Hu S, Zhang G. (2017): Effects of Sleeve Gastrectomy on Serum 12 α -Hydroxylated Bile Acids in a Diabetic Rat Model. *Obes Surg.*; (27)11: 2912-2918.
- [26] Dudek M, Kołodziejewski PA, Pruszyńska-Oszmałek E, Sassek M, Ziarniak K, Nowak KW, Sliwowska JH. (2016): Effects of high-fat diet-induced obesity and diabetes on Kiss1 and GPR54 expression in the hypothalamic-pituitary-gonadal (HPG) axis and peripheral organs (fat, pancreas and liver) in male rats. *Neuropeptides*. 2016 Apr; 56: 41-9. doi: 10.1016/j.npep.2016.01.005. Epub 2016 Jan 22.
- [27] Zhou Q, Chen H, Yang S, Li Y, Wang B, Chen Y, Wu X. (2014): High-fat diet decreases the expression of Kiss1 mRNA and kisspeptin in the ovary, and increases ovulatory dysfunction in postpubertal female rats. *Reprod Biol Endocrinol*. 2014 Dec 26; 12: 127.
- [28] Tomasz S, Dominika S, Iwona KS, Jodok F, Bronisława SP, Marcin K, Bogdan D, Maria A, Agnieszka ZR, Michał K, Marek M, Aleksandra F, Krystyna ŻK, Kondrad KW. (2015): Long-term Effect of Ileal Transposition on Adipokine Serum Level in Zucker (Orl)-Lepr(fa) Fatty Rats. *Obes Surg.*; 25(10): 1848-57.
- [29] Liu T, Zhong MW, Liu Y, Huang X, Cheng YG, Wang KX, Liu SZ, Hu SY. (2017): Effects of sleeve gastrectomy plus trunk vagotomy compared with sleeve gastrectomy on glucose metabolism in diabetic rats. *World J Gastroenterol*. 2017 May 14; 23(18): 3269-3278.
- [30] Nosso G, Angrisani L, Saldalamacchia G, Cutolo PP, Cotugno M, Lupoli R, Vitolo G, Capaldo B. (2011): Impact of sleeve gastrectomy on weight loss, glucose homeostasis, and comorbidities in severely obese type 2 diabetic subjects. *J Obes*. 2011; 2011: 340867.
- [31] Zhu Z, Yang X, Wang K, Wang Z, Zhao Y, Yu M. (2014): The effects of sleeve gastrectomy on hormonal regulation of glucose metabolism in Goto-Kakizaki rats. *Eur Surg*. 2014; 46(5): 189-196. Epub 2014 Jul 10.
- [32] Basso N, Soricelli E, Castagneto-Gissey L, Casella G, Albanese D, Fava F, Donati C, Tuohy K, Angelini G, La Neve F, Severino A, Kamvissi-Lorenz V, Birkenfeld AL, Bornstein S, Manco M, Mingrone G. (2016): Insulin Resistance, Microbiota, and Fat Distribution Changes by a New Model of Vertical Sleeve Gastrectomy in Obese Rats. *Diabetes*. 2016 Oct; 65(10):2990-3001.
- [33] Pories WJ, Swanson MS, MacDonald KG, et al. (1995): Who would have thought it? An operation proves to be the most effective therapy for adult-onset diabetes mellitus. *Ann Surg*. 1995; 222(3):339–50.
- [34] Lee WJ, Ser KH, Chong K, Lee YC, Chen SC, Tsou JJ, Chen JC, Chen CM. (2010): Laparoscopic sleeve gastrectomy for diabetes treatment in nonmorbidly obese patients: efficacy and change of insulin secretion. *Surgery*; 147(5):664-9.
- [35] Silvestre RA, Egido EM, Hernández R, Marco J. (2008): Kisspeptin-13 inhibits insulin secretion without affecting glucagon or somatostatin release: study in the perfused rat pancreas. *J Endocrinol*. 2008 Feb; 196(2): 283-90. doi: 10.1677/JOE-07-0454.
- [36] Uenoyama Y, Pheng V, Tsukamura H, Maeda KI. (2016): The roles of kisspeptin revisited: inside and outside the hypothalamus. *J Reprod Dev*. 2016 Dec 20; 62(6): 537-545.
- [37] Ohtaki T, Shintani Y, Honda S, Matsumoto H, Hori A, Kanehashi K, Terao Y, Kumano S, Takatsu Y, Masuda Y, Ishibashi Y, Watanabe T, Asada M, Yamada T, Suenaga M, Kitada C, Usuki S, Kurokawa T, Onda H, Nishimura O, Fujino M. (2001): Metastasis suppressor gene KiSS-1 encodes peptide ligand of a G-protein-coupled receptor. *Nature*; 411: 613–617.