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CHANGES IN SERUM VASPIN AND OMENTIN AFTER SLEEVE GASTRECTOMY IN TYPE 2 DIABETIC MODEL

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ABSTRACT

Background: Vaspin and Omentin showed different serum levels in diabetes and obesity. **Aim:** No available data demonstrating the effect of sleeve gastrectomy on Omentin and Vaspin serum levels in a model of diabetic obese rats. **Materials and Methods:** 32 male albino rats divided into four groups: control (C) group, Diabetic (D), sham operated (SO) and Sleeve Gastrectomy (SG). Serum levels of Vaspin and Omentin, Body Weight, fasting glucose, plasma insulin and HOMA-IR are estimated in all groups and in SO and SG groups at the 2nd, 4th and 12th week after surgery. **Results:** Omentin increased significantly in diabetic and diabetic sham operated at 2nd, 4th, 12th weeks and diabetic sleeve gastrectomy at 2nd week (p=0.03, p=0.04, p=0.03, p=0.04 and p=0.04 respectively) compared with control group. Insignificant differences were found in diabetic sleeve gastrectomy at 4th and 12th week groups compared with control group. Significant differences are found in sleeve gastrectomy at 4 and 12 weeks (p=0.04 and p=0.009 respectively) compared with the Diabetic group. Significant reduction in Vaspin (V) was found in D group in relation to control group (p=0.0001). Highly significant increases in Vaspin were found in SG group at 2w, 4 w and 12 weeks compared with D group (p< 0.001, 0.0001). Insignificant reductions in BW are found in SG after 2 weeks compared with D group however, significant reductions in body weight were found in SG group at 4th and 12th weeks compared with D group. Highly significant reductions (p< 0.0001) in insulin levels were found between diabetic and sham operated (2, 4 and 12 w) groups compared with control group. No significant differences in insulin level between SG group at 12 w and the control group. Insulin significantly increased in SG group at week 2, 4 and 12 (p<0.01, p< 0.0001 and p< 0.0001 respectively) in comparison with diabetic group. **In Conclusion,** Sleeve gastrectomy improved serum Omentin and Vaspin levels in obese diabetic rats and returned these levels to values similar to normal control non-diabetic non-obese rats.

KEYWORDS: Omentin; Vaspin; Sleeve gastrectomy; Type 2 diabetes; Obesity.

INTRODUCTION

Vaspin was first described as a member of the serpin family and highly expressed in visceral adipose tissue (VAT) of obese rats (Hida et al. 2005).[1] Vaspin levels were high in diabetics in comparison with low weight normal individuals (Li et al. 2008).[2] Also, Escoté et al., (2017)[3] reported that Vaspin levels were higher in obese subjects, as well as in subjects showing insulin resistance or type 2 diabetes. So, Vaspin agonists and/or antagonists can be used for improvement of insulin sensitivity in metabolic syndrome (Castro et al., 2017).[4]

Omentin is an adipokine which was expressed in VAT and associated with obesity and insulin resistance (Castro et al., 2017).[4] Omentin enhances the effect of insulin on glucose metabolism (Yang et al. 2006).[5] Controversial studies about the level of Omentin in diabetics as well as obesity were found. Moreno-Navarrete et al. (2010)[6] found that omentin-1 significantly increased in obese subjects when they are submitted to a daily energy reduction by 500–1000 kcal/day for 120 days. Escoté et al., (2017)[3] stated that obesity reduced Omentin levels and adipose tissue secretion in both adults and adolescents. Also, De Souza Batista et al. (2007)[7] reported that serum omentin-1 concentration, as well as visceral adipose tissue omentin expression, were significantly lower in overweight and obese subjects than in lean ones. Additionally, Akbarzadeh et al. (2014)[8] found that Omentin was increased in streptozotocin induced diabetic rats. In another study by Urbanová et al. (2014)[9] obesity or diabetes type 2 has no significant effect on serum omentin-1 concentrations in any group studied.

Type 2 diabetes mellitus (T2DM) is a famous disease that endangers human life.[10] Bariatric surgery can effectively reduce body weight and relieve insulin resistance quickly and efficiently.[11] In addition, bariatric surgery is now considered in the management lines of T2DM.[12,13] Sleeve gastrectomy (SG), the commonly

used bariatric surgery[14], can significantly improve T2DM.[15]

Induction of diabetes by streptozotocin (STZ) was commonly used to study the pathophysiology of this disease. The experimental model combining a high-fat diet with a low dose STZ is used considering its ability to produce insulin resistance (Srinivasan et al. 2005). This model simulates the metabolic characteristics of type 2 diabetes observed in humans.

According to available resources, no available data investigating the effect of sleeve gastrectomy on serum Omentin and Vaspin, so this study was designated to demonstrate this effect and search the timing of this effect. Therefore, in this study we investigated the serum levels of omentin and vaspin after sleeve gastrectomy in a diabetic type 2 model associated with a high-fat diet.

MATERIALS AND METHODS

Animals

32 male albino rats (weighting 200–220 g), provided by the Laboratory Animal house, Faculty of Medicine, Zagazig University, were housed in a 12-h light/dark cycle in a constant temperature (24 ± 3°C) and humidity (50 ± 10%) in an independent ventilated cages. After acclimatization for 2 weeks; body weight, food intake, blood glucose, serum Vaspin, Omentin, Insulin, HOMA-IR were measured.

Induction of type 2 diabetes Mellitus

24 rats (out of 32) were allowed to water and a high-fat diet (HFD, 40% fat, Huafukang Biotech, China) for 1 month to induce insulin resistance and then injected with streptozotocin (STZ, 35 mg/kg) (Sigma, USA) intra-peritoneally. After induction of diabetes, rats with blood glucose ≥16.6 mmol/l (≥ 300 mg/dl), were divided into 3 groups.

2nd group Diabetic (D): No procedures
3rd group Diabetic: Diabetic Sham Operated (DSO).
4th group diabetic: Diabetic Sleeve gastrectomised (DSG)

So as total we have now four groups

1st group: control (C) group (n=8).
2nd group: Diabetic (D) rats (n=8).
3rd group: Diabetic rats Sham operated (SO) (n=8).
4th group: Diabetic Sleeve gastrectomised (DSG) (n=8)

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

Sleeve Gatrectomy[17, 18]: rats were injected with 10% chloral hydrate anesthetic (3 ml/kg) i.p. before procedure. An upper abdominal incision of approximately 5 cm was performed and the gastric and lesser omenta were dissected. After ligation and transection of the gastric omental vessels in pyloric area, forceps was used to clamp the greater curvature in case of hemorrhage. The gastric part outside clamped area (approximately 70% of stomach volume including the gastric fundus) was resected. The gastric incision was sutured with 5-0 silk suture (Ningbo Medical Needle, China). Leakage and hemorrhage were prevented then the abdomen was closed.

Sham Operation[19]: A laparotomy was performed to expose the stomach and esophagus and operative time was equal to that taken by sleeve gastrectomy. Then, the abdominal incision was closed.

Postoperative Care

At the end of the surgical procedures, all rats received sterile saline 10 mL i.p. and 10 mL s.c. maintaining hydration in healing process. Rats were received analgesic ketoprofen 5 mg/kg. Rats were placed on a heated mat till recovery then returned to home cages. They were allowed to drink water for 12 h postoperatively and a solution of 5% glucose and 0.2% KCl was provided to them for the next 48 hours. Thereafter, they received the HFD until 12 weeks after surgery. Serum levels of Vaspin and Omentin, body weight, food intake, fasting glucose, insulin and HOMA-IR were measured in all groups. These parameters are also measured in sham operated and sleeve gastrectomised groups at the 2nd, 4th and 12th week after surgery.

Analytical Methods

Food intake was calculated by the difference in weight between the offered diet and weight of the rest of diet. Blood glucose samples are taken from the rat tail vein and analyzed by accucheck glucose analyzer. Serum was obtained by centrifugation of blood sample. The omentin and vaspin values were obtained with Rat commercial enzyme immunoassay (EIA-OME) and EIA-VAP kits (Phoenix Pharmaceuticals Inc., Burlingame, California, USA) purchased from Sigma Co. Cairo Egypt. Serum insulin was measured by a rat insulin ELISA (BioVendor, Kassel, Germany). HOMA-IR was calculated.[20]

Statistical Analysis: GraphPad Quick-Calcs program was used for calculation of unpaired t-test. The data obtained was expressed as mean ± standard Error of Mean (SEM). Unpaired t-tests were used. P value ≤ 0.05 indicate statistical significance.

RESULTS

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

	BW(Body Weight) gm	Food Intake: gm/day	Omentin(ng/ml)	Vaspin (Pg/ml)
Control	210± 18	140±5	400±50	710±30
Diabetic	300± 20 **a a(p=0.004)	190±4***a a(p< 0.0001)	550±40*a a(p=0.03)	300± 40***a a(p<0.0001)
Diabetic + Sham (2 W)	308± 21**a a(p=0.003)	170± 5***a**b a(p=0.0008) b(p=0.007)	530±30*a a(p=0.04)	297± 35***a a(p<0.0001)
Diabetic + Sham (4 W)	307±23**a a(p=0.005)	175±9**a a(p=0.004)	540±35 *a a(p=0.03)	299±41***a a(p<0.0001)
Diabetic + Sham (12 W)	305±21**a a(p=0.004)	177±7***a a(p=0.0007) b(p=0.12)	545±40 *a a(p=0.04)	310±43***a a(p<0.0001)
Sleeve Gastrectomy (SG) after 2 W	300± 23**a a(p=0.008)	95±7***a***b a(p=0.0001) b(p<0.0001)	530±30*a a(p=0.04)	590±43*a a(p=0.03) ***b b(p<0.0001)
Sleeve Gastrectomy (SG) after 4 W	233±15*b a(p=0.3) b(p=0.01)	107±5***a***b a(p=0.0004) b(p<0.0001)	420±45*b b(p=0.04)	650± 40***b b(p<0.0001)
Sleeve Gastrectomy (SG) after 12 W	211±17**b a(p=0.9) b(p=0.004)	122±5*a***b a(p=0.02) b(p<0.0001)	400±30**b b(p=0.009)	705± 30***b b(p<0.0001)

DATA represented as mean ± standard Error of Mean (SEM).
*a= significant compared control group (P value ≤ 0.05)
*b= significant in comparison with the Diabetic group.
**= highly significant (P value ≤ 0.01)
*** = Extremely significant (P value ≤ 0.001)

Table 2: Effect of Sleeve Gastrectomy on Glucostatic Parameters.

	Glucose mg/dl	Insulin (mIU/ml)	HOMA-IR
Control	110±17	8.85±0.05	2.07± 0.05
Diabetic	330±15***[a] (pa<0.0001)	4.55±0.03***[a] (pa< 0.0001)	4.08±0.05***[a] (pa< 0.0001)
Diabetic Sham (2 W)	325±13***[a] (pa< 0.0001)	4.51±0.04***[a] (pa< 0.0001)	4.05±0.07***[a] (pa< 0.0001)
Diabetic Sham (4 W)	320±15***[a] (pa< 0.0001)	4.54±0.05***[a] (pa< 0.0001)	3.95±0.07***[a] (pa< 0.0001)
Diabetic Sham (12 W)	317±17***[a] (pa< 0.0001)	4.55±0.06***[a] (pa< 0.0001)	3.97±0.05***[a] (pa< 0.0001)
Diabetic+ Sleeve Gastrectomy (SG) after 2 W	259±15***[a, **b] (pa=0.0001), (pb=0.004)	4.75± 0.05***[a,**b] (pa< 0.0001) (pb=0.004)	3.75± 0.07 ***[a, **b] (pa< 0.0001)(pb=0.001)
Diabetic+ Sleeve Gastrectomy (SG) after 4 W	181±13**[a, ***b] (pa=0.005)(pb< 0.0001)	8.69± 0.05*[a, ***b] (pa=0.04)(pb< 0.0001)	2.91± 0.06***[a, ***b] (pa< 0.0001)(pb< 0.0001)
Diabetic+ Sleeve Gastrectomy (SG) after 12 W	113±14 ***[b] (pb< 0.0001)	8.83± 0.07,***[b] (pb< 0.0001)	2.19± 0.07***[b] (pb< 0.0001)

DATA represented as mean ± standard Error of Mean (SEM).
*a= significant compared with the control group (P value ≤ 0.05)
*b= significant compared with the Diabetic group.
**= highly significant (P value ≤ 0.01)
*** = Extremely significant (P value ≤ 0.001)

DISCUSSION

Omentin enhances the effect of insulin on glucose metabolism (Yang et al. 2006).[5] In diabetic or insulin-resistant individuals, Vaspin level is usually high, when compared to low weight normal individuals (Li et al. 2008).[2] Sleeve gastrectomy (SG), the commonest bariatric surgery[11], can significantly alleviate T2DM.[12] According to available resources, no available data demonstrating effect of sleeve gastrectomy on serum Omentin and Vaspin, so the aim of this study was to demonstrate this effect and search the timing of this effect. Therefore, in this study we investigated the serum

levels of Omentin and Vaspin after sleeve gastrectomy in an experimental model for type 2, diabetes associated with a high-fat diet.

In the present study, Body Weight increased significantly in diabetic, diabetic sham at 2w, 4 and 12 weeks (p=0.004, p=0.003, p=0.005 and p=0.004 respectively) compared with control group. Also, Body weight was increased significantly in Sleeve Gastrectomised (SG) after 2 W group (p=0.008) however, insignificant differences were found in Sleeve Gastrectomised groups (SG) after 4 and 12 weeks compared with control group. Insignificant differences were found in sham operated rats (2, 4th 12th weeks) and SG group at 2nd week compared with the diabetic group. Significant differences were found between sleeve gastrectomised at 4th and 12th weeks (p< 0.05 and p< 0.005 respectively) compared with the diabetic group. These findings confirmed that sleeve gastrectomy has a significant effect on body weight in obese diabetics.

This finding in agreement with Lombardo et al., (2010) who found that body mass index decreased from 58.2 to 44.5 Kg/m^2 after sleeve gastrectomy and they concluded that sleeve gastrectomy is a safe and efficient management for the high-risk and super-obese patient.[14] The findings of this study suggested that body weight is reduced lately by sleeve gastrectomy after 4 and 12 weeks. This suggestion is supported by Wang et al., (2017)[21] who found that the SG group has a significant weight loss 6 weeks postoperatively.

Significant increase in food intake was found in Diabetic and sham operated groups at 2nd, 4th and 12th weeks (p< 0.0001, p=0.0008, p=0.004 and p=0.0007 respectively) compared with control group. Highly significant reductions in food intake were found in sleeve gastrectomised at 2nd and 4th weeks (p=0.0001, p=0.0004) compared with control group. Significant reduction in food intake was found (p=0.02) in diabetic sleeve gastrectomy at 12th week compared with the control group. Food intake was decreased significantly in sham operated groups at 2nd and 4th weeks compared with D group but no significant difference was found in sham operated at 12th week. Food intake was reduced significantly in sleeve gastrectomy at 2nd, 4th and 12th weeks (p< 0.0001) compared with the diabetic group. These findings were supported by Wang et al., (2017) who found that the Sleeve Gastrectomy decreased food intake significant 4 weeks postoperatively.[21]

Omentin increased significantly (p=0.03, p=0.04, p=0.03, p=0.04 and p=0.04) in diabetic and diabetic sham operated at 2nd, 4th, 12th weeks and diabetic sleeve gastrectomy at 2nd week groups compared with control group. These findings are supported by Akbarzadeh et al. (2014)[8] who found that Omentin was increased in streptozotocin induced diabetic rats. However our findings are in controversy with Feng et al. (2013)[22] who showed that omentin-1 significantly reduced in obese mice when compared to non-obese ones. This controversy may be due to the model used in their study where they used high fat diet model without induction of diabetes. Also, the current study was in controversy with Castro et al., (2017)[4] who found insignificant differences in omentin level between diabetic and non-diabetic groups. This controversy might be due to species differences in the last study, where they used Wister rats and in the current study Albino rats were used. Also, duration of study and time for collection of samples were different where they collected samples for seven weeks and in this study for 12 weeks. These findings were also in controversy with Tan et al. who reported that the expression of mRNA for omentin-1 in adipose tissue and omentin-1 serum concentration correlated negatively with 17β-estradiol[23] and significant negative correlations were found between plasma omentin-1 concentration and BMI, waist circumference and HOMA-IR. However, the findings of this study were in agreement with Prats-Puig et al., (2011)[24] who found a positive correlation between serum omentin-1 and bad metabolic profile including; high insulin resistance, detected markers of adipose tissue metabolism, increased blood pressure and a positive family history of metabolic syndrome, in asymptomatic 7 years old children. The authors concluded that this effect can represent a compensatory mechanism whereby omentin-1 attenuates these metabolic abnormalities.

In the current study, insignificant differences in serum Omentin were found in sleeve gastrectomised at 4th and 12th week groups compared with control group. Insignificant differences were also found in sham operated at 2, 4, 12 weeks and sleeve gastrectomised at 2 week groups compared with diabetic group. However, significant differences were found in sleeve gastrectomised at 4 and 12 weeks (p=0.04 and p=0.009 respectively) compared with the diabetic group. In this study, serum Omentin returned to normal control 12 weeks after sleeve gastrectomy (400±30 and 400±50 ng/ml) which prove the significant effect of sleeve gastrectomy on Omentin serum level. This experimental evidence is supported by previous study of Sdralis et al., (2013)[25] who found significant reduction of omentin mRNA expression in subcutaneous adipose tissue after laparoscopic sleeve gastrectomy.

Highly significant reductions in Vaspin were found (p< 0.0001) in diabetic, diabetic sham operated at 2nd, 4th and 12th week groups in relation to control group and significant reduction was found in diabetic sleeve gastrectomy at 2weeks compared with control group. However insignificant changes in serum Vaspin were found in diabetic sleeve gastrectomy at 4 w and 12 weeks in comparison with the control group. These results in agreement with Castro et al., (2017)[4] who found that Vaspin level was lower in diabetic than non-diabetic group. Also these findings were supported by Li et al. (2008)[2] who suggested an insulin-sensitizing effect for Vaspin on white adipose tissue. However,

these results are in controversy with Li et al. (2008) who reported that Vaspin levels were found high in diabetic or insulin-resistant individuals compared to low weight normal individuals, this controversy might be due to species differences, male albino rats were used in this study, however Lie et al (2008)[2] study was conducted in human. The present study is in agreement with Hida et al. (2005)[1] who found that Vaspin levels were significantly reduced in OLETF rats (a rat model of type 2 diabetes) when hyperglycaemia becomes severe at 50 weeks. In this study, highly significant increases in serum vaspin were found in sleeve gastrectomised groups after 2w, 4 w and 12 weeks compared with diabetic group ($p<0.0001$). These findings are supported by Tomasz., (2015)[26] who found significant increase in serum Vaspin after ileal transposition compared with control rats.

In the present study, serum Glucose increased significantly in diabetic, sham operated groups at 2w, 4w, and 12 weeks and sleeve gastrectomised group at 2 and 4 weeks compared with control group ($p< 0.0001$). Insignificant differences in serum glucose were found between diabetic group and sham operated groups at 2, 4 and 12 weeks. However, highly significant reductions were found in glucose levels in SG at 2w, 4 w and 12 weeks compared with diabetic group ($p=0.004$, $p < 0.0001$ and $p < 0.0001$ respectively). This study was in agreement with Nosso et al., (2011) who reported that sleeve gastrectomy is efficient in producing a significant and long lasting weight loss and improving glucose homeostasis in severely obese T2DM patients.[27] The current study was also supported by Zhu et al., (2014) who found a significant reduction in blood glucose in sleeve gastrectomised group with the improved glucose tolerance.[28] In this study, insignificant differences in serum glucose were found in sleeve gastrectomised group at week 12 compared with control group suggesting improved blood glucose and return to normal control. This suggestion is supported by Liu et al., (2017) who reported a better glucose tolerance after sleeve gastrectomy as well as lower HOMA-IR and up-regulation of hepatic insulin signaling.[29]

The current study found that the HOMA-IR was highly significantly increased in diabetic, diabetic sham operated (2w, 4w and 12 w) and sleeve gastrectomy (2w, 4w) groups compared with control group ($p< 0.0001$) however, insignificant change was found in sleeve gastrectomised group at (12 w) compared with control group. Very highly significant reductions in HOMA-IR in SG groups at 2w, 4 w and 12w in relation to diabetic group ($p=0.001$, $p< 0.0001$ and $p< 0.0001$ respectively) however, no significant changes were found in SO (2, 4 and 12 w) groups compared with diabetic group. These findings are supported by Basso et al., (2016) who found significant improvement in insulin sensitivity in SG group compared with SO group demonstrated by HOMA-IR values, which were reduced by ~50% ($p<0.0001$).[30]

In the current study, highly significant reductions ($pa< 0.0001$) in insulin levels were found between diabetic and sham operated (2, 4 and 12 w) groups compared with control group. These results are supported by Basso et al., (2016) who found significant insulin reduction. No significant differences in insulin level between the SG group at 12 w and the control group (8.83± 0.07 and 8.85±0.05 respectively). No significant differences in insulin level between sham operated (2, 4 and 12 w) group and diabetic group. Insulin significantly increased in SG group at week 2, 4 and 12 ($p< 0.05$, $p< 0.0001$ and $p< 0.0001$ respectively) in comparison with diabetic group. Our findings are in agreement with Lho et al., (2015)[31] who found that random insulin significantly increased after vertical sleeve gastrectomy (VSG) (sham, 0.45 ± 0.16 µg/L; VSG, 1.05 ± 0.18 µg/L). Our findings are also supported by Eickhoff et al., (2015)[32] who reported that insulin level improved significantly as early as 3 months after sleeve gastrectomy and they concluded that insulin resistance was reduced after sleeve gastrectomy in obese patients with T2D.

IN CONCLUSION

Omentin increased significantly in diabetic and diabetic sham operated at 2nd, 4th, 12th weeks and diabetic sleeve gastrectomy at 2nd week groups. Insignificant differences in serum Omentin were found in sleeve gastrectomy at 4th and 12th week groups compared with the control group. Serum Omentin returned to normal control 12 weeks after sleeve gastrectomy. Highly significant reductions in Vaspin were found in diabetic, diabetic sham operated at 2nd, 4th and 12th week and diabetic sleeve gastrectomy at 2weeks groups in relation to control group. However insignificant changes in serum Vaspin were found in sleeve gastrectomy at 4 w and 12 weeks compared with control group. In this study, serum vaspin was significantly increased in sleeve gastrectomy groups after 2w, 4 w and 12 weeks compared with diabetic group. These findings proved by experimental evidence that sleeve gastrectomy improved serum Omentin and Vaspin levels in diabetic obese rat model and returned these levels to normal control values.

Conflict of Interest: No conflict.

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