EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 11
Issue: 10
1-6
Year: 2024

ASPROSIN LEVELS AFTER SLEEVE GASTRECTOMY IN A RAT MODEL OF DIABETES MELLITES TYPE 2

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Article Received on 01/08/2024 **Article Revised on 22/08/2024** **Article Accepted on 11/09/2024**

ABSTRACT

Background: Asprosin is a novel glucokine produced mainly by the white adipose tissue. Many studies investigated the role of asprosin in diabetes type-2 and confirmed the relationship of asprosin to body weight. **Aim:** According to available resources, no data demonstrating the effect of sleeve gastrectomy on serum asprosin, so the aim of this study was to demonstrate this effect and search the timing of this effect after sleeve gastrectomy in an experimental model for type 2, diabetes. **Materials and Methods:** 40 adult male albino rats were divided into four groups; (10 rats each); 1st: Control (C) group, 2nd: Diabetic (D) rats, 3rd: Diabetic Sham operated (SO), and 4th: Diabetic with Sleeve gastrectomy (DSG). Body weight, food intake, blood glucose, serum asprosin, Insulin, HOMA-IR and HOMA-B were measured. **Results:** asprosin level was significantly increased in diabetic group (19.03 ± 2.5) compared with the control group (14.05 ± 1.2). Asprosin is significantly decreased in diabetic with sleeve gastrectomy group (17.23 ± 2.6) compared with the diabetic group (18.07 ± 2.4) and this reduction is higher 6 weeks and highest 14 weeks after sleeve gastrectomy (15.03 ± 3.5, 14.06 ± 1.1 respectively) compared with diabetic group (39.05 ± 5.7, 53.03 ± 6.5 respectively). Asprosin is positively correlated with the body mass index (P-Value is < .00001). **Conclusion:** Asprosin is significantly reduced after sleeve gastrectomy and this reduction is time related. Asprosin has a potential therapeutic role in weight reduction.

INTRODUCTION

Asprosin is a novel adipokine synthesized by cleaving the C-terminus of profibrillin-1 protein by furin (a proteolytic enzyme) (Basu et al., 2021). Asprosin was named after the Greek word for white (ασπροσ), because the white adipose tissue appears to be the main source of plasma asprosin (Romere et al., 2016). Asprosin was first discovered by Romere et al. 2016, who identified the fasting-induced protein hormone that modulates hepatic glucose release and named it asprosin. They also detected *Fibrillin-1* (FBN1) mRNA in white adipose tissue in higher concentrations and suggested that white adipose tissue is the dominant producer of asprosin.

Asprosin is also produced by the heart, liver, pancreas, stomach, skeletal muscles, lungs, and brain (Kocaman and Kuloğlu., 2020). Measurable amounts of asprosin were found in different body fluids like saliva, breast milk, urine, serum, and plasma (Morcos et al., 2022; Gozel and Kilinc 2021). Asprosin was also expressed in the ovaries, placenta, and cartilage (Kerslake et al., 2021; Hoffmann et al., 2022).

Asprosin is secreted by white adipose tissue then circulates at nanomolar levels, and is recruited to the liver activating the G protein-cAMP-PKA pathway and releasing glucose into the circulation which in turn inhibits asprosin secretion *via* a negative feedback loop (Romere et al., 2016).

Asprosin exerts peripheral and central effects (Li et al., 2019; Mishra et al., 2022). Its peripheral effect is a glucogenic and appetite stimulant hormone through binding with the olfactory receptor 4M1 (OR4M1 rhodopsin family member) and a cell surface receptor termed protein tyrosine phosphatase receptor δ (Ptprd), respectively (Li et al., 2019; Mishra et al., 2022).

OR4M1 is considered the primary asprosin receptor in humans, while OLFR734 is the mouse ortholog. OLFR734 is markedly distributed in the testis, liver, kidney, olfactory epithelium tissue, and olfactory bulb (Li et al., 2019). Circulating asprosin binds to the OLFR734 on the surface of hepatocytes promoting glucose production by activating the cAMP-dependent-protein kinase A (PKA) pathway (Luís et al., 2020).

Asprosin central effect is mediated by stimulating the hypothalamic feeding center. In the hypothalamic arcuate nucleus (ARH), asprosin acts directly on hunger-stimulating neurons known as agouti-related peptide neurons (AgRP) *via* the G protein-cAMP-PKA pathway and inhibits pro-opiomelanocortin (POMC) neurons through γ-Aminobutyric acid (GABA) (Duerrschmid et al., 2017).

Asprosin has a high binding affinity to the protein tyrosine phosphatase receptor δ (Ptprd) in the hypothalamic AgRP neurons (Mishra et al., 2022). Asprosin mRNA and protein are highly expressed in the hypothalamus's paraventricular nucleus (PVN) where asprosin stimulates the sympathetic outflow (Wang et al., 2022).

Diabetes mellitus is the collective term for heterogeneous metabolic disorders whose main finding is chronic hyperglycemia. The cause is either a disturbed insulin secretion, a disturbed insulin effect, or usually both **(**Petersmann et al., 2019).

Many studies investigated the role of asprosin in diabetes type-2 like the study conducted by Zhang et al., (2019) who found a significant increase in asprosin levels in diabetic adults with (Diabetes mellites Type-2) T2DM. These findings are supported by Farrag et al., (2023) who reported that asprosin acts as an orexigenic and glucogenic hormone. Another study by Timurkaan and Timurkaan 2022, found a significant increase in serum asprosin levels in the diabetic compared with the control group. They also found that asprosin correlates positively with HOMA-IR, insulin, BMI, and triglyceride levels in the diabetic group.

In an older study by Wang et al., (2018) 143 participants were chosen and categorized into 3 groups, the 1st group was the normal control (n; 52) and the 2nd group was impaired glucose regulation (IGR) (n; 40), the 3rd group was T2DM (N; 51). They found a significant increase in plasma asprosin levels in IGR and T2DM compared with the control group.

Wang et al., (2018) also found a significant positive correlation between asprosin levels and fasting plasma glucose (FPG), post-challenge plasma glucose (2hPG), HbA1c, and HOMA-IR and negatively correlated with HOMA-*β*. This was also confirmed by Zhang et al., 2020 who found a significant increase in asprosin levels in T2DM patients compared with the normal control subjects. In addition, Gozel and Kilinc, (2021) found a significant increase in serum and saliva asprosin in the newly diagnosed T2DM. They suggested that asprosin could represent a risk factor for T2DM and concluded that asprosin could be a potential therapeutic target in the treatment of diabetes.

Sleeve gastrectomy (SG), the commonest bariatric surgery, can significantly alleviate T2DM (Rezk and Ibrahim 2018). According to available resources, no available data demonstrating effect of sleeve gastrectomy on serum asprosin, so the aim of this study was to demonstrate this effect and search the timing of this effect after sleeve gastrectomy in an experimental model for type 2, diabetes.

MATERIALS AND METHODS

ANIMALS

40 adult 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 independent ventilated cages. After acclimatization for 2 weeks; body weight, food intake, blood glucose, serum asprosin, Insulin, HOMA-IR and HOMA-B were measured.

Induction of type 2 diabetes Mellitus

30 rats (out of 40) 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, this small dose induces diabetes mellitus type-2.

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 gastrectomy (DSG)

So, we have four groups (10 rats each)

1st group Control (C) group.
2nd group Diabetic (D) rats.
3rd group Diabetic Sham operated (SO).
4th group Diabetic with Sleeve gastrectomy (DSG)

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

Sleeve Gastrectomy [(Sun, et al. 2014), (Rezk and Ibrahim., 2018)] 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 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 (Bruinsma et al. 2015).

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 asprosin, body weight, food intake, fasting glucose and insulin were measured in all groups. HOMA-IR and HOMA-B were calculated. These parameters are also measured in sham operated and sleeve gastrectomized groups at the 2nd, 6th and 14th 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. Asprosin 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). [Margolis et al., 2016] HOMA-IR and HOMA-B are calculated according to the following formulae: HOMA-IR = [fasting insulin (μU/ml) × fasting plasma glucose (mg/dl)]/405; HOMA-B = 20 × fasting insulin (μU/ml)/[fasting plasma glucose (mg/dl) – 63].

[Matthews et al., 1985]

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). One-Way ANOVA test was used to compare between the four groups. P value ≤ 0.05 indicate statistical significance

RESULTS

Table 1: Effect of Sleeve Gastrectomy on BMI, Food Intake, Serum asprosin.

		Body Mass Index (BMI) (gm/cm^2)	Food Intake: gm/day	Asprosin (ng/ml)
C	Control	0.39 ± 0.02	140±5	14.05 ± 1.2
D	Diabetic	0.45 ± 0.03	190±4	19.03 ± 2.5
Diabetic Sham (DS)	After (2 W)	0.44 ± 0.01	171± 5	18.07 ± 2.4
	After (6 W)	0.52 ± 0.02	173±9	39.05 ± 5.7
	After (14 W)	0.63 ± 0.02	184±7	53.03 ± 6.5
Diabetic with Sleeve Gastrectomy (DSG)	After 2 W	0.43 ± 0.02	91±5	17.23 ± 2.6
	After 6 W	0.41 ± 0.03	97±4	15.03 ± 3.5
	After 14 W	0.39 ± 0.03	103±5	14.06 ± 1.1
One way ANOVA		F= 11.9870* (p=0.0000)	F= 52.3441* (p=0.0000)	F= 15.3118* (p=0.0000)

*= significant (P value ≤ 0.05)

Table 2: Effect of Sleeve Gastrectomy on glucostatic parameters.

		Glucose mg/dl	Insulin (mIU/ml)	HOMA-IR	HOMA-B
Control	C	110±17	8.75±0.05	2.37± 0.05	3.72± 0.03
Diabetic	D	335±12	4.65±0.03	3.84±0.05	0.34± 0.02
Diabetic Sham	(2 W)	311±11	4.53±0.04	3.47±0.07	0.36± 0.01
	(6 W)	323±13	4.54±0.05	3.62±0.07	0.35± 0.02
	(14 W)	327±15	5.25±0.06	4.23±0.05	0.38± 0.03
Diabetic + Sleeve Gastrectomy (DSG)	After 2 W	245±15	6.35± 0.05	3.84± 0.07	0.70± 0.03
	After 8 W	182±13	7.89± 0.05	3.54± 0.06	1.33± 0.02
	After 16 W	112±14	8.74± 0.07	2.41± 0.07	3.56± 0.04
ANOVA		F= 483.8854* (p=0.0000)	F= 13350.1769* (p=0.0000)	F= 1186.8590* (p=0.0000)	F= 30335.8163* (p=0.0000)

*= significant (P value ≤ 0.05)

Data are represented as means ± Standard Deviation.

Table 3: Pearson correlation between asprosin levels and body mass index.

		Body Mass Index (BMI) (gm/cm^2)	Asprosin (ng/ml)
C	Control	0.39 ± 0.02	14.05 ± 1.2
D	Diabetic	0.45 ± 0.03	19.03 ± 2.5
Diabetic Sham (DS)	After (2 W)	0.44 ± 0.01	18.07 ± 2.4
	After (6 W)	0.52 ± 0.02	39.05 ± 5.7
	After (14 W)	0.63 ± 0.02	53.03 ± 6.5
Diabetic with Sleeve Gastrectomy (DSG)	After 2 W	0.43 ± 0.02	17.23 ± 2.6
	After 6 W	0.41 ± 0.03	15.03 ± 3.5
	After 14 W	0.39 ± 0.03	14.06 ± 1.1
Pearson correlation		R is: 0.9825 *	

DISCUSSION

Asprosin is a newly discovered glucogenic adipokine (Wiecek, Magdalena, et al. 2019) that increases with insulin resistance (Li, Xing, et al. 2018). Ceylan et al., (2020) found a significant reduction in plasma asprosin and insulin levels after aerobic exercise in overweight, obese, and normal-weight subjects. The same findings were reported by Kantorowicz et al., (2021) who found a significant reduction in asprosin levels and waist-hip ratio in obese women after a training program for two months. From the previous studies, we could suggest that asprosin has a potential role in weight reduction so we designed this study to investigate the effect of sleeve gastrectomy on asprosin levels and demonstrate the relationship between weight reduction after sleeve gastrectomy and asprosin levels.

In this study, we found that asprosin level was significantly increased in diabetic group (19.03 ± 2.5) compared with the control group (14.05 ± 1.2). These findings are supported by many studies investigated the role of asprosin in diabetes type-2 like the study conducted by Zhang et al., (2019) who found a significant increase in asprosin levels in diabetic adults with type-2 diabetes. We are also supported by another study by Timurkaan and Timurkaan (2022) who found a significant increase in serum asprosin levels in the diabetic compared with the control group.

We also in agreement with Wang et al., (2018) who found a significant increase in plasma asprosin levels in impaired glucose tolerance group (IGR) and type-2 diabetic group (T2DM) compared with the control group.

We also found a significant reduction in asprosin in diabetic with sleeve gastrectomy group (17.23 ± 2.6) compared with the diabetic group (18.07 ± 2.4) and this reduction is higher 6 weeks and highest 14 weeks after gastrectomy (15.03 ± 3.5, 14.06 ± 1.1 respectively) compared with diabetic group (39.05 ± 5.7, 53.03 ± 6.5 respectively).

Our findings are 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. The findings of our study suggested that body weight is reduced lately by sleeve gastrectomy after 6 and 14 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.

Our findings are confirmed by Wang et al., 2019 and Cantay et al., (2022), who found a significant reduction in asprosin levels 6 months after bariatric surgery and sleeve gastrectomy associated with weight loss. We found a significant correlation between asprosin levels and body mass index (P-Value is < .00001). Our finding is supported by Farrag et al., (2023) who reported that asprosin acts as an orexigenic and glucogenic hormone that increases body wight and body mass index. We are also supported by Timurkaan and Timurkaan (2022) who reported that asprosin correlates positively with HOMA-IR, insulin, BMI, and triglyceride levels in the diabetic group. Also, our finding is supported by Mishra et al., 2021) who found that asprosin neutralization decreased food intake and body weight in a mouse model of metabolic syndrome.

CONCLUSION Asprosin is significantly reduced after sleeve gastrectomy and this reduction is time related. Asprosin has a potential therapeutic role in weight reduction.

Conflict of interest None.

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