

EFFECTS OF DIABETES MELLITUS ON GASTRIC MOTILITY IN RATS

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OBJECTIVES: Diabetes mellitus is one of the most common endocrine diseases that affects most body organs. Peristaltic disorders and gastric distension have also been observed in diabetes. Because the effect of diabetes on gastric motility has not been fully examined, we decided to determine if gastric motility is also affected by diabetes in rat. **METHODS:** This study was carried out at Kerman University of Medical Science, Kerman, Iran from October 2004 to February 2005. Three groups of male wistar rats (control, vehicle, diabetic) weighing 200–250 g were used. Diabetic state was induced by intraperitoneal injection of 45 mg/kg streptozotocin. Animals were anesthetized by intraperitoneal (IP) injection of 50 mg/kg thiopental sodium. After tracheostomy and laparotomy, a balloon was inserted into the stomach, which was attached to a pressure transducer system via a cannula and this whole system was connected to a physiograph. Acetylcholine (ACh) was the stimulant agent which was used intraperitoneally. **RESULTS:** There was no significant difference between basal intragastric pressures in three groups. Also there was no significant difference in the basal and ACh-stimulated intragastric pressure among the three groups. But ACh-stimulated intragastric pressure was more than the basal state in each group (control 28.3 ± 1.77 vs 14 ± 1.4 , vehicle 30.8 ± 2.03 vs 15.9 ± 1.56 and diabetic 30.6 ± 0.05 vs 13.7 ± 0.84 mmHg). **CONCLUSION:** Although it has been shown that diabetes can change gastric acid and pepsin secretion in rats, no significant change in gastric motility could be shown.

Keywords: Diabetes mellitus, Gastric motility, Rat

INTRODUCTION

Diabetes mellitus (DM), a complicated disorder caused by genetic or environmental factors,^{1,2} is one of the most common endocrine diseases with a prevalence of 3% around the world^{3,4}. Diabetes has various effects on body organs, including alimentary tract. For example in oesophagus it causes motility disorders and decreases the tonicity of lower sphincter.⁵ In liver it causes steatosis and sometimes increases liver enzymes.⁵ Steatosis leads to hepatitis (steatohepatitis) which both can be suppressed after the controlling of diabetes. Cirrhosis, too has been observed in diabetic patients, but the reason is still unknown.⁵

Diabetes can also cause motility disorders and stasis in gall bladder and consequently increases the incidence of gall stone.^{6,7} Moreover, it has been observed that in long-standing diabetes in mice nor-epinephrine reserve of intestine is decreased that is evident of adrenergic disorders of intestine nerves.⁸ Diabetes also causes abnormal release of intestinal peptides and other intestinal-regulating substances.⁹ In stomach it causes achlorhydria in 0.2–5% of cases and in some diabetic patients the atrophy of gastric mucosa has been observed which is related to the antibodies affecting parietal cells.⁵ Peristaltic disorders and gastric distension have also been observed in diabetes, which cause hiccup, flatulence and pain and sometimes splashing stomach.⁵ Gastric emptying disorders occur in 30–50% of patients with diabetes mellitus.^{10,11}

Gastric emptying, especially of solid food, is affected in diabetes and it is believed that pyloric dysfunction has the main role in this regard. We have shown in another study that gastric acid and pepsin secretion is markedly less in diabetic rats than normal ones.¹² Therefore, we decided to determine if gastric motility is also affected by diabetes in rats.

MATERIALS AND METHODS

This study was carried out at College of Medicine, Kerman university of Medical science, Kerman-Iran from October 2004 to February 2005. Male Wistar rat (200–250 g) purchased from animal house of Medical School of Kerman University of Medical science, Kerman-Iran were used in experiment. The rats were maintained in a temperature-controlled environment and on a 12:12-h light-dark cycle with free access to water and standard chow. Rats were divided into the following 3 groups (n=10).

- 1-**Control group:** had access to normal food and water
- 2-**Diabetic group:** received streptozotocin (STZ 45 mg/kg weight, IP) prepared in 20 mM citrate buffer in the fed state at ~1300, and each rat was studied 35 days after STZ injection.¹³
- 3-**Vehicle group:** received normal saline (equal volume of STZ) IP in the fed state at ~1300, and each rat was studied 35 days after normal saline injection (normal saline is as STZ solvent).

Blood glucose was measured in tail blood using auto analyzer (Alcyon-300, USA), 1, 2, 3, 4 and 5 week after STZ injection. Rats with blood

glucose 300 mg/dl were considered as diabetic.¹⁴ All procedures were approved by the Institutional Animal Care and Research Committee at the Kerman University of Medical Science.

Rats were deprived of food but not of water for 24 hours before the experiment.¹⁵ Anaesthesia was induced by IP injection 50 mg/kg body weight of thiopental (Biochem GmbH, Vienna, Austria).¹⁵ The diabetic rats were weak and had high mortality rate during surgery, therefore these animals were anesthetized by IP injection of 30 mg/kg body weight experimentally. After tracheostomy, cervical oesophagus was ligated. A midline incision was made on abdomen and celiotomy was down.¹⁵ A silicon tube 2.5 mm in external diameter and 10 cm length, attached to a balloon, passed through an incision in the duodenum into the stomach and tied in the pyloric region. After waiting for 30 minutes to reach a steady state, gastric motility was measured.¹⁶

The internal end of intragastric tube was attached to balloon and its external end was connected to a 3-way valve and pressure transducer system. The pressure transducer was connected to physiograph (Beckman, USA). We knew that injection of 1.5 ml/100 g of body weight solution or more into the stomach causes this organ to distend.¹⁵ Therefore to measure basal gastric pressure we introduced different volumes of saline (less than 1.5 ml/100 g body weight) into stomach via balloon and then chose 0.5 ml/100 g of body weight. Basal gastric motility was measured for 15 minutes. To measure stimulated-gastric motility, 1 ml of 10^{-1} M Ach was used IP and then gastric motility was measured by physiograph for 15 minutes. (To find an appropriate concentration for Ach to use, we performed a dose-response study starting from the 10^{-5} M concentration which was shown to be effective *in vitro*.¹⁷ In this study intragastric pressure recording, using physiograph, is an indicator of gastric motility.^{18,19} The characteristics of physiograph system in this study were: velocity 0.5 mm/sec and sensitivity 0.5 mV/mm, also the physiograph was calibrated by standard mercury sphygmomanometer. Finally, the stomach of all the animals were removed and fixed in the formalin 10% for histological study.

Values were reported as Mean \pm SE. The differences between groups were assessed by ANOVA and post hoc Tukey test. Value of $p < 0.05$ was considered to be statistically significant.

RESULTS

The mean blood sugar in diabetic group on days one and 35 after STZ administration were 176.5 ± 6.22 and 564.3 ± 74.46 mg/dl, respectively, while in

vehicle group they were 167.9 ± 11.44 , 166.2 ± 10.24 and in control group, they were 142.7 ± 7.64 and 155.4 ± 8.05 mg/dl, respectively (Figure-1). Based on the mentioned levels there is no significant difference between the vehicle and control groups in the mean blood sugar on day 1 and day 35. But mean blood sugar on day 1 and day 35 show significant increase in diabetic animals in comparison to those in the another two groups ($p < 0.05$) (Figure-1). In regard to mean of body weight there was a significant decrease in diabetic rats on day 35 after STZ administration in comparison on day 1 (292.9 ± 8.43 , 246.9 ± 6.29 g respectively, $p < 0.05$, Figure-2), but there was no significant difference in the body weight of the other two groups on days 1 and 35 after STZ administration (vehicle group, 264 ± 12.73 , 279.5 ± 9.65 g respectively), (Control group, 257.6 ± 10.20 , 257 ± 10.12 g respectively) (Figure-2).

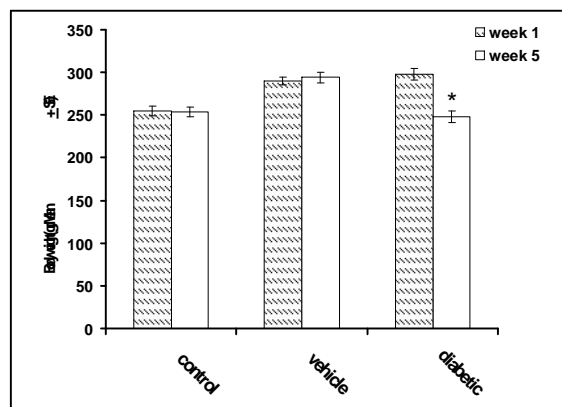


Figure-1: Comparing of body weight in control, vehicle and diabetic groups on day 1 and day 35, (n=10 in each group), * $p < 0.05$

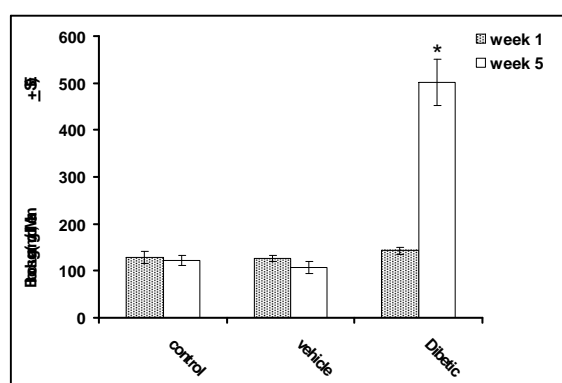


Figure-2: Comparing of blood sugar in control, vehicle and diabetic groups on day 1 and day 35, (n=10 in each group), * $p < 0.05$

The mean basal intragastric pressure in control, vehicle and diabetic groups were 14 ± 1.4 , 15.9 ± 1.56 and 13.7 ± 0.84 mmHg, respectively. The

mean Ach-stimulated intragastric pressure in control, vehicle and diabetic groups were 28.3 ± 1.77 , 30.8 ± 2.03 and 30.6 ± 0.05 mmHg, respectively. There was no significant difference in the basal and stimulated intragastric pressure between all of the three groups. But Ach-stimulated intragastric pressure was more than the basal state in each group ($p < 0.05$, Table-1).

Table-1: Mean variation of basal and Ach-stimulated gastric pressure in control, vehicle and diabetic groups, (n=10 in each group)

State	Control Group	Vehicle group	Diabetic group
Basal gastric pressure (mmHg)	14 ± 1.4	15.9 ± 1.56	13.7 ± 0.84
Ach-stimulated gastric pressure (mmHg)	$28.3 \pm 1.77^*$	$30.8 \pm 2.03^*$	$30.6 \pm 0.05^*$

* $p < 0.05$

Histological examination of the stomach specimens of the three groups showed no difference between the smooth muscle layers in the gastric fundus, body and antrum of these animals. Also the myenteric plexus was similar in all three groups. (Figures-3, 4, 5).

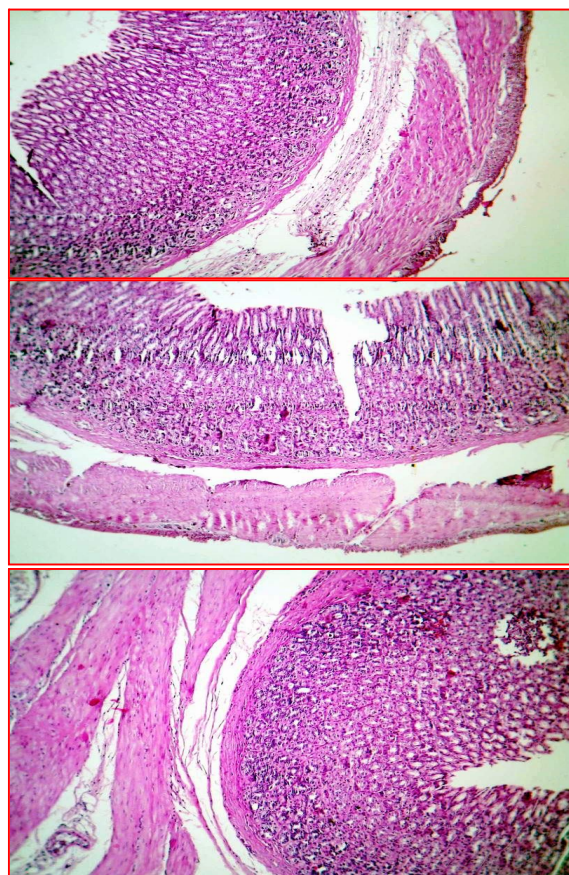


Figure-3: Gastric cross section of control group (fundus, antrum and body)

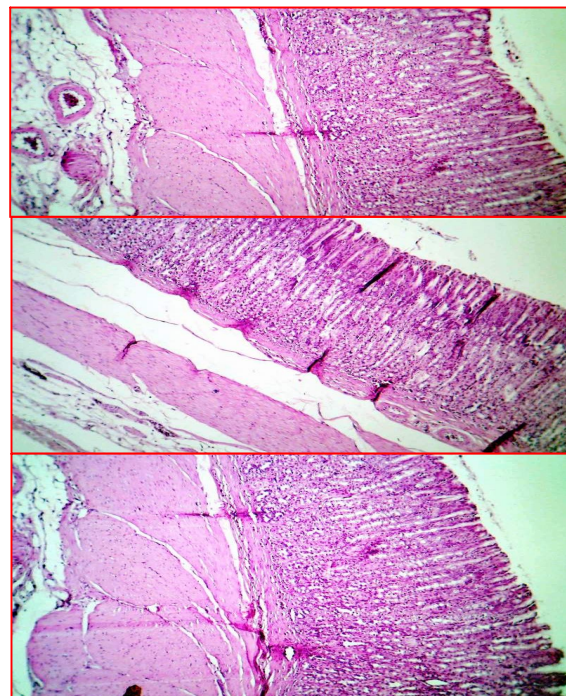


Figure-4: Gastric cross section of vehicle group (fundus, antrum and body)

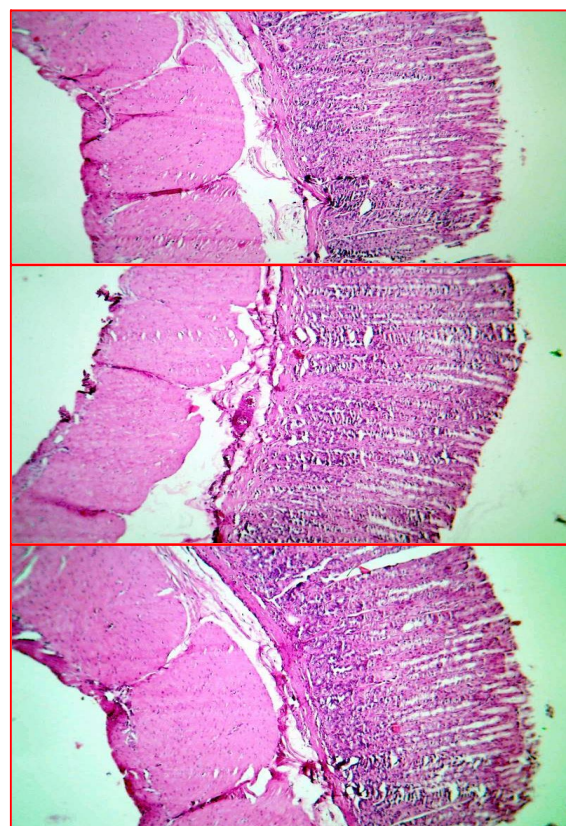


Figure 5: Gastric cross section of diabetic group (fundus, antrum and body)

DISCUSSION

STZ increases blood sugar and induces diabetes via destroying pancreatic B-cells. High blood sugar and body weight loss are two important criteria for diabetes.²⁰⁻²² In this study the blood sugar of the STZ treated group was significantly more than the other ones (Figure-1) also, their body weight was less than the other two groups (Figure-2).

There was no significant difference between the basal intragastric pressures of control, Vehicle and diabetes. Also, Ach as a stimulator increased intragastric pressure in comparison to the basal state in the control, vehicle and diabetes group (Table-1).

It has been observed that Ach activates M₁ and M₃ receptors and Ca⁺² channels, and therefore increases the intracellular Ca⁺² concentration in the smooth muscle cells.²³ Because Ca⁺² is the principal ion of contraction, Ach causes the gastric smooth muscles to contract and gastric pressure to increase. A well recognized impact of long standing DM on gastrointestinal tract of human being is delayed, gastric emptying and decreased antrum contractility.²⁴ These effects are attributed to diabetes induced gastroparesia and neuropathy.^{24,25} Although it has been shown that diabetes can change gastric acid and pepsin secretion in rats,¹² but there is no report about the gastric pressure changes in diabetic rats in literature.

Therefore we designed this study to show the effects of diabetes mellitus on basal and Ach-stimulated gastric motility (intragastric pressure) in rats. There was no difference in basal and Ach-stimulated intragastric pressure between the diabetic animals and vehicle or control ones. Also histological studies showed that the gastric muscles layers of these groups are the same.

It is probable that there is more time needed (>35 day) for diabetes to affect gastric pressure, but, as we know the life span of rats is so much shorter than human beings and taking care of diabetic rats is difficult. Therefore maybe gastric parietal and chief cells are more sensitive to blood glucose changes than smooth muscle cells and hence gastric acid and pepsin secretion change short term diabetes, but gastric motility will not. These possibilities need to be examined.

CONCLUSION

Although it has been shown that diabetes can change gastric acid and pepsin secretion in rats, no significant change in gastric motility could be shown. We suggest using genetically diabetic rats for these purposes. Also studying the effects of type

II diabetes mellitus on gastric motility would be helpful.

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