Assessment of the Metabolic Response After Echo-endoscopic Gastric Bypass

An experimental animal study

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The increasingly higher incidence of obesity and type 2 diabetes witnessed in today's society led to a continuous research for innovative medical and surgical solutions to this pathology. Our aim is to evaluate the metabolic effects of endoscopic ultrasound-guided gastric bypass (EUGB) in an experimental pig model. Eight female pigs were equally distributed into two groups: the endoscopic ultrasound-guided gastrojejunostomy group and a control group. Body weight and blood glucose concentration were assessed before the procedure and weekly in the postoperative setting for 6 months.

Keywords: obesity, gastric bypass, endoscopic ultrasound

Last decades witnessed a rapid change in lifestyle and nutritional habits in modern society, which fundamentally transformed the way obesity and its direct complications, such as diabetes and heart disease, were perceived, from a peripheric metabolic condition to a real, undisputed pandemic. Type II diabetes mellitus closely follows the trend towards today's dramatic levels, as more than 8% of the adult population worldwide is currently affected [1]. Moreover, an increasingly higher incidence in children and young adults makes this pathology even more troublesome.

Even if laparoscopic bariatric procedures gain a prominent position in the armamentarium of therapeutic solutions for obesity and diabetes, new ultra-minimally invasive approaches are currently tested, such as endoscopic or natural orifice transluminal endoscopic (NOTES) procedures, aiming to provide a less traumatic but equally safe and efficient solution for the ever-growing number of overweight and obese patients [2].

The aim of this study is to assess the long-term metabolic effects after endoscopic ultrasound-guided gastric bypass (EUGB) in an experimental pig model.

Experimental part

A prospective, experimental study involving 9 female pigs (sus scrofus domesticus) was conducted in two Surgical Centres (University of Medicine and Pharmacy of Craiova and Delta (Ponderas) Hospital Bucharest) in Romania. The procedures were performed by a joint team of endoscopists and surgeons with adequate experience in endoscopic ultrasonography and minimal invasive surgery, respectively.

Legal and Ethical Issues

All the procedures performed in this experimental study were reviewed and approved by the Ethical Committee of the University of Medicine and Pharmacy of Craiova (protocol number: 56 / 28.03.2014). The experimental protocol followed the national regulations for animal use and care, as well as the directives of the European Council (n° 2010/63/EU).

Animal care and groups

The study included two groups of Landrace pigs, with an weight of 29-36 kg and size of 60-70 cm. The animals were distributed as 5 experimental animals were allocated to the EUGB and 4 pigs were used as controls.

Prior to the operation, the animals were individually housed in pens in the Animal Facility of the University of Medicine and Pharmacy of Craiova with 20-24°C thermoneutral, temperature-controlled room and 12/12h light-darkness cycles.

The animals in both groups (control and EUGB group) were fed twice per day, with standard chow, providing an average daily caloric intake of approximately 3000 kcal during the first month, and constant caloric increase according to weight and age. Water was provided ad libitum. The feed portions were individually measured. As the wastages were negligible their assessment was not considered necessary.

Each animal was acclimated to the new environment and feed for 72 h before the initial blood samples were taken.

One dose of Ceftriaxone® (Sandoz - Austria) was administered prior to the induction of anaesthesia, as antimicrobial prophylaxis.

Anaesthesia and surgical technique

All cases were operated under the same general anesthesia protocol. Prior to the intervention, two peripheral venous catheters were placed at the level of the auricular vein. Propofol 5mg/kg was used for induction of anesthesia. For maintaining the general anesthesia, continuous perfusion of Propofol 0.5 mg/kg/h, along with Fentanyl 3 mg/kg as a bolus every 45 min, and Pavulon 0.1 mg/kg in 0.9% Sodium Chloride Solution, under endotracheal intubation.

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The EUGB procedure consisted in the endoscopic exploration of the stomach and duodenum, followed by the advancement of a 60 mm in diameter enteric balloon distal to the duodeno-jejunal junction which was subsequently filled with saline solution under direct ultrasonographic view. Then the self electrocautery-enhanced delivery system of the stent allowed the puncture of the endoscopic balloon, thus enabling the saline solution to effuse. The last step of the intervention consisted in the deployment of the stent, which was dilated with a 15-mm dilating balloon. All the above-mentioned steps were performed under EUS control.

Weight gain and blood glucose

After the endoscopic procedure, the clinical and nutritional status and feed consumption were observed by a mixed team comprising the veterinarian and surgeon.

The same housing and feeding conditions as in the preoperative interval were used. Food intake was permitted after 48 h.

Body weight and blood glucose concentration were determined 6 h prior to the procedure and every two weeks during the postoperative setting, after a 12 h fasting. The first postoperative determination was performed 30 days after the procedure, in order to allow the complete recovery of the animals and a proper assessment of the new metabolic status.

Blood sampling was performed using two different methods. For perioperative sampling, a venous catheter was placed at the level of dorsal auricular vein. For long-term postoperative sampling, Accu-Check Active glucometer rapid test and strips was used. Prior to blood sampling, adequate grooming and disinfection with topical antiseptics (70% sanitary alcohol) of the puncture area was performed.

Six months after the endoscopic procedure, the animals were euthanized by a single intravenous dose of sodium pentobarbital and necropsy was performed.

Statistical analysis

t-Test was used to evaluate the statistical differences between EUGB and control group and pre-operative and post-operative levels in the echo-endoscopic group. A *p*-value of less than 0.05 was considered significant.

Results and discussions

All procedures were successful, with necropsy indicating only mild adhesions between the stomach and the jejunal loop adjacent to the anastomosis, but without any signs of local or disseminated sepsis.

The animals in the EUGB group had a preoperative (T_0) weight of 31.2 ± 1.64kg, and reached an average weight of 71 ± 2.45kg in the 6th month of follow-up. There were no statistical differences between groups at concerning the preoperative weight. Although there was a statistically significant weight increase in both groups, the weight gain in the control group was more abrupt by a mean difference of 20kg (table 1). Statistically significant differences were observed between control and EUGB at every weight determination (T_2 - T_{11}) except for the preoperative determination (T_0).

Moment of sampling	Control group
T_0	32 ± 2.94*
T_2	56 ± 4.76*
T_3	59.5 ± 5.32*
T_4	63.25 ± 4.86*
T_5	67.25 ± 5.5*
T_6	71.25 ± 5.68*
T_7	74.75 ± 5.44*
T_8	79.25 ± 6.4*
T_9	83 ± 6.16*
T_{10}	87 ± 6.88*
T_{11}	91 ± 6.88*

Table 1
EVOLUTION OF WEIGHT IN THE CONTROL GROUP (IN kg), AVERAGE ± STANDARD DEVIATION
*$p<0.05$, DIFFERENCES ARE STATISTICALLY DIFFERENT BETWEEN T_0 (PREOPERATIVE) AND T_2-T_{11} (POSTOPERATIVE TIME FRAME)

Moment of sampling	GJA group
T_0	31.2 ± 1.64*
T_2	47.5 ± 0.55¥#
T_3	49.6 ± 1.94¥#
T_4	51.8 ± 2.59¥#
T_5	54.4 ± 2.7¥#
T_6	57.2 ± 2.59¥#
T_7	60 ± 2.35¥#
T_8	62.8 ± 1.92¥#
T_9	65.4 ± 1.67¥#
T_{10}	68.2 ± 2.39¥#
T_{11}	71 ± 2.45¥#

Table 2
EVOLUTION OF WEIGHT IN THE GJA GROUP (IN Kg), AVERAGE ± STANDARD DEVIATION
*$p<0.05$, STATISTICALLY NON-SIGNIFICANT DIFFERENCE BETWEEN T_0 IN CONTROL GROUP AND T_0 IN GJA GROUP
¥ $p<0.05$, STATISTICALLY SIGNIFICANT DIFFERENCES BETWEEN T_2-T_{11} AND T_0 IN THE CONTROL GROUP,
# $p<0.05$, STATISTICALLY SIGNIFICANT DIFFERENCE BETWEEN T_2-T_{11} IN THE GJA GROUP COMPARED TO T_2-T_{11} IN THE CONTROL GROUP
CONTROL GROUP

Average values of blood glucose reached a peak of 91 ± 5.35mg/dl in the control group at T_3, which was comparable to the preoperative moment, while in the EUGB the mean glycemic levels decreased by an average of 18mg/dL, only achieving a maximum of 74.6 ± 18.69mg/dl in the postoperative setting.

Overall, the evolution of glycaemic levels in the EUGB was characterized by statistically significant differences of postoperative glycaemic levels compared to preoperative values. When compared to the control group, preprandial glycemic levels in the EUGB pigs indicated

Moment of sampling	Control group
T_0	91 ± 13.34*
T_2	90.25 ± 17.67*
T_3	91 ± 5.35*
T_4	89 ± 8.25*
T_5	88.5 ± 4.43*
T_6	88.5 ± 7.68*
T_7	90.5 ± 1*
T_8	89 ± 2.16*
T_9	89.5 ± 5.57*
T_{10}	90 ± 7.07*
T_{11}	90.5 ± 2.65*
T_{12}	90.75 ± 6.18*

Table 3
PREPRANDIAL (A JEUNE) LEVELS OF BLOOD GLYCEMIA IN CONTROL GROUP (IN mg/dL), AVERAGE ± STANDARD DEVIATION
*$p>0.05$, DIFFERENCES NOT STATISTICALLY SIGNIFICANT, BETWEEN T_0 AND T_{12} SAMPLES

Moment of sampling	GJA group
T_0	91.6 ± 3.36*
T_2	73.8 ± 6.22**
T_3	74.2 ± 1.3**
T_4	73.8 ± 3.77**
T_5	73.4 ± 9.37**
T_6	71.2 ± 10.18**
T_7	74.6 ± 18.69**
T_8	74.2 ± 6.76**
T_9	71.2 ± 2.28**
T_{10}	71.2 ± 2.95**
T_{11}	71 ± 2.35**
T_{12}	71.8 ± 3.35**

Table 4
PREPRANDIAL (A JEUNE) LEVELS OF BLOOD GLYCEMIA IN OPERATED GROUP (GJA) (IN mg/dL), AVERAGE ± STANDARD DEVIATION
*$p<0.05$, STATISTICALLY NON-SIGNIFICANT DIFFERENCES BETWEEN T_0 AND CONTROL GROUP
** $p<0.05$, STATISTICALLY SIGNIFICANT DIFFERENCES OF T_2-T_{12} COMPARED TO T_0 AND CONTROL GROUP

significantly lower values at every determination time frame except for T_0 (table 3, 4).

The results of the statistical analysis indicate that EUGB had a determinant role in the metabolic response of the recipient, achieving significant and long-term decreases in body weight and blood glucose levels. These results have multiple repercussions, as gastrojejunostomy, either open or laparoscopic, is a widely used procedure with multiple indications, varying from oncologic to bariatric and metabolic surgery. [3] However, the current tendency is to reduce the surgical trauma, given the frailty of the patients to whom this kind of procedure is addressed to. One potential solution is the use of flexible endoscopy alone or in conjunction with other techniques (i.e. endoscopic ultrasonography) as the main frame for the introduction of new *ultra*-minimally invasive methods in the clinical setting [4-6].

Conducting this experimental study on pigs provides the known advantages of this large-animal model, such as its similarities with humans in terms of metabolic features, physiology of digestion and omnivorous nutritional habits. Moreover, an almost identical surgical technique can be used for different experimental and clinical purposes due to comparable organ sizes and anatomy [7, 8].

In the current study, it was used a non-obese, non-diabetic pig model, because the aim was to assess the typical weight gain and glycemic response pattern after the endoscopic gastric by-pass procedure, without the interference of other preoperative co-morbidities.

For example, contrary to human diabetic patients, several experimental studies noticed that diabetic animal models had a negative energy balance and thus failed to obtain a normal weight curve. Thereafter, EUGB would have provided contradictory results in obese, diabetic pigs than in obese human patients with type 2 diabetes mellitus [9, 10].

There are several limitations to this study. The most important drawback is the low statistical significance caused by the small number of subjects. The second limitation is related to the poor correspondence between the experimental and clinical setting, mainly due the animal model itself. For example, the metabolic role of altered gut microbiota after gastric by-pass procedure in humans are well documented. However, despite the multiple similarities, there is no proof that the same type of changes will occur in pig model, given the differences in gut microbiota. More important, it seems that the control of energy balance in various animal models is different than in humans, leading to a different mechanism of weight loss and glycaemic control after different bariatric techniques [11].

Future studies, both clinical and of adipokines and biomarkers of inflammation will better describe the mechanisms through which this type of intervention generates metabolic changes. There are already some comparative studies regarding the changes in the level of the adipokines in peripheral blood in patients or animals operated with different procedures of bariatric surgery, but the subject is only at the beginning of exploration [12-15].

Conclusions

Endoscopic ultrasound-guided gastric bypass procedure provides a safe and efficient method for weight and glycemic decrease in the controlled experimental framework. However, the early stage of research requires additional data before the introduction of this technique in the clinical setting.

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