Reverse Transposition Of The Ileum Is A New Surgical Model For The Study Of The «Hindgut" Hypothesis In Diabetes Mellitus

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Research article

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Abstract

**Background.** The study reports on a newly developed experimental surgical technique for studying the role of the ileum in diabetes mellitus, tested in healthy outbred white rats without obesity.

Despite the generally accepted positive effect of metabolic operations in type 2 diabetes mellitus, there are several theories, based on the opposite hormonal mechanisms, explaining these positive changes. The combined actions of these hormones, as well as their balance resulting in normal glucose metabolism, are under discussion.

**Aim.** To develop and study the possibility of reverse transposition of the small intestine for investigating the role of the ileum in diabetes mellitus.

**Materials and methods.** The idea behind the reverse transposition model proposed by us consists in the incomplete rotation of the small intestine after transection and a new reversible (antiperistaltic) arrangement of the entire intestinal tube.

To create a model, outbred white rats, identical in all parameters, were randomly selected. The animals were divided into two groups: Group I – the rats that underwent reverse transposition of the small intestine; Group II – the rats, in which pseudo-operations were performed. Both groups were monitored for body weight, fasting plasma glucose levels and blood glucose level after oral glucose tolerance test (OGTT).

**Results.** We did not obtain significant differences in the surgery duration, body weight of the animals and fasting blood glucose levels, as well as after OGTT in the studied groups.

**Conclusion.** The reverse transposition technique proposed by us is feasible. The anatomical changes obtained during the operation suppose early contact of the chyme with the terminal portion of the small intestine, and the reverse position of the intestine causes a longer passage of a bolus in a distant direction, which presumably may cause changes in nutrient absorption, as well as in the release of ileal hormones (incretins) affecting glucose metabolism.

1. **Background.**

Metabolic surgery is an effective treatment for patients with diabetes mellitus. The effectiveness of metabolic operations in diabetes is due to changes in the anatomy of the gastrointestinal tract and, as a result, a complicated complex of molecular pathophysiological mechanisms which contribute to the normalization of glucose levels and are still the subject of discussion [1, 2].

Based on the anatomical changes that occur during the most efficient antidiabetic procedures Roux-en-Y Gastric Bypass and biliopancreatic diversion (RYGB and BPD), the Hindgut hypothesis arose. This hypothesis postulates that the accelerated release of undigested nutrients into the terminal portion of the small intestine and their contact with L-cells promotes enhanced production of glucagon-like peptide-1
and peptide tyrosine tyrosine (GLP-1 and PYY) incretins, which in turn contribute to enhanced production of insulin and increased glucose tolerance [3, 4].

To test the hypothesis in the experiment, different techniques were proposed to achieve accelerated transport of chyme into the lumen of the ileum, and the most common of them is ileal transposition (interposition), which, since its presentation, has undergone many modifications regarding the procedure, length and localization of the transposed portion of the ileum: interposition of the distal part of the jejunum, 5.0 cm long, proximad at a distance of 5.0 cm from the ligament of Treitz [5], creation of a jeuno-ileal anastomosis at different distances from the ligament of Treitz [6], a combination of the ileal interposition and RYGB [7], 10 cm interposition of the ileum at the level of the duodenum [8], transposition of the ileum between the stomach and the duodenum [10].

Along with that, some authors express doubts about the pathophysiological importance of the Hindgut hypothesis, which is explained by the lack of an effect equivalent to metabolic operations in response to antidiabetic drugs that stimulate the secretion of GLP-1 [9]. Moreover, in contrast to the positive effects of the incretin mechanism and the Hindgut hypothesis, hormonal pathways with adverse effects on glycaemic homeostasis are assumed. It is a so-called anti-incretin mechanism, which is triggered in response to the passage of nutrients through the proximal parts of the small intestine and the presence of which explains the contrary hypothesis “Foregut” or “high small intestinal theory”. The anti-incretin effect consists in the inhibition of the incretin effect – it causes a decrease in insulin release, a decrease in the proliferation of beta cells and the inhibition of the insulin action to prevent hypoglycemia [10]. In this regard, the development of innovative experimental surgical techniques for studying the effects of the ileum remains a promising direction.

This article reports on the development of a new experimental surgical model to investigate the specific role of the ileum in glucose metabolism. This model cannot be replicated in humans as a metabolic / bariatric procedure and as a biological model we used healthy non-obese non-pedigree rats. We called it the reverse transposition (RT) of the small intestine, since almost the entire intestinal tube after resection, incomplete rotation and anastomoses, is placed in the opposite direction (antiperistaltic). The underlying idea is to invert the ileal part of the small intestine into the proximal intestinal segment after the duodenum, to reduce the number of anastomoses during transposition, preserving the integrity of the intestinal tube, and to create conditions for a longer passage of the chyme in the distal direction due to antiperistalsis, as well as to determine the effects of the ileum in these conditions of the configuration change of the gastrointestinal tract. Many authors have proposed various models for changing the configuration of the gastrointestinal tract to study the role of the ileum in glucose metabolism. Most of them are represented by various versions of ileal interposition (transposition), but we propose a new method that has never been published before.

Thus, we open up a wide range of new possibilities for studying the role of the entire small intestine, and in particular, the ileum, in changing the parameters of glucose metabolism after a new reproducible experimental surgical technique – reverse transposition.
2. Methods.

The study was carried out in the animal research facility and the shared research laboratory in. The study was conducted on the adult sexually mature white short-haired non-pedigree male rats in accordance with the order of the Minister of .... dated April 2, 2018 N 142 "On Approval of the Rules for Conducting Biomedical Experiments, Preclinical (Nonclinical) and Clinical Studies, as well as Requirements for Preclinical and Clinical Sites” and compliance with the international principles of the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes, after obtaining the approval of the University Ethics Committee.

2.1 Animals.

Sexually mature male rats comparable in weight and age, weighing 300–320 g, without obesity – 30 animals – before the experiment were kept for 7 days in identical conditions (temperature and humidity, diet): with a 12-hour cycle day / night with natural light during the day, with constant access to food and water. After that, the animals were randomly distributed into groups: an intervention group (reverse transposition, Group I) and a control group (pseudo-operations – Sham, Group II). Weighing and labeling, as well as identification of the sex of the animals were carried out. Female rats were not used to avoid cyclic changes in gonadotropins and their effect on glucose metabolism. The animals were sacrificed in a 28 days after surgery by cardiac puncture and blood sampling under anesthesia [11].

2.2 Weight control, basal glycemia and OGTT.

Before surgery and once a week during the entire follow-up period, the weight of the animals was monitored. After an overnight fast (12 hours) on the day of surgery, blood was taken under general anesthesia by puncture of the lateral tail vein to determine the basal glucose level (Satellite Express glucometer, Russia). Then orally, through an orogastric tube (32.50.13.110–00005211 subclavian catheter, Russia), a 40% glucose solution (2 g/kg body weight) was injected with repeated blood sampling after 30 minutes. Further, surgical interventions in groups were performed. Repeated measurements were taken on postoperative days 7, 14, 21, and 28.

2.3 Technique of surgical procedures.

Technically, the experimental surgical technique of reverse transposition of the ileum was performed as follows: after anesthesia (intramuscular Ketamine injection 50 mg/ml at the rate of 80 mg/kg body weight), placing the animal on an operating plate and fixing the limbs, hair was removed from the anterior abdominal wall with using a shaver. The surgical field was treated with chlorhexidine twice, after which a 2.5 cm mid-median incision was made. The surgical field was covered with sterile dressings. Using anatomical forceps, the cecum and the terminal ileum, as well as the small intestine proximal part, were delivered into the wound. Having identified the ileocecal angle, as well as the ligament of Treitz, a complete intersection of the small intestine was made in a distance of 5 cm from the indicated anatomical structures on both sides. Further, the free ends of the intersected small intestine were placed in the opposite direction - the terminal ileum was brought to the duodenum and the jejunum was placed
in the direction of the ileocecal angle. The segments of the intestine in the intersection area were washed by a syringe and a blunt needle with isotonic sodium chloride solution. A microvascular clamp was applied to the anastomosed ends of the intestinal tube, followed by an end-to-end intestinal anastomosis, reversibly (anti-peristaltic) with interrupted 5–0 Vicryl sutures using microsurgical techniques at 3.5x magnification (Fig. 1. Schematic representation of the operation).

Thus, the distal ileum moves in the proximal direction for faster contact with the undigested chyme, and the reverse position of the intestine causes a longer passage of the food bolus in the distal direction.

After applying anastomoses and washing with warm isotonic sodium chloride solution, the intestine was placed into the abdominal cavity. The anterior abdominal wall was sutured in layers with a 2–0 Vicryl. The postoperative wound was retreated with chlorhexidine followed by aseptic bandaging.

The surgical technique of reverse transposition, like a sham surgery, was identical in all cases and was performed by the same operating surgeon. Technically, a sham surgery differed only in the volume of the intervention performed - after transection in the proximal and distal sections; small intestinal anastomosis was performed in the same places, without moving. The duration of the intervention was specially prolonged until the time of the reverse transposition.

In the postoperative period, all the animals were housed separately. Ibuprofen D 100mg/5 ml (120 ml) Suspension at a dose of 20 mg/kg was used for analgesia. During the first two postoperative days, a liquid diet in the form of a 5% glucose solution was assumed, after which the typical feeding schedule was established.

2.4 Statistical methods.

Statistical analysis was performed using statistical software SPSS, version 24.0. For all quantitative variables, the mean (M), mean error (m), standard deviation (SD), limits of 95% confidence interval (± CI); check for normality of distribution were determined. The significance of differences in quantitative variables was determined using the Mann Whitney U test.

3. Results.

Surgical duration in the groups was comparable and was 46.9 (± 3.9) minutes for group I and 46.6 (± 4.0) minutes for the comparison group.

The overall mortality rate was 16.6%. Animal mortality in group I was 20% (3 individuals); it was 13.3% in group II (2 individuals). None of the cases occurred during surgery. Animals died within 48 to 96 hours (days 2–4) due to anastomotic leak. Signs of impaired blood circulation of the mesenteric vessels, as well as obstruction due to the reverse position of the intestinal tube in the dead animals, were not observed (Fig. 2B). The rest of the animals were sacrificed on postoperative day 28.
The average body weight in the groups before surgery was: 297.6 g (± 16.5) in group I (RI), and 302.2 g (± 14.3) in group II (Sham). There was a decrease in the body weight of the animals in both groups for two weeks after surgery. A slight increase in body weight in both groups was noted on day 21. It was more pronounced in the Sham surgery group. There were no significant differences at all observation stages (Fig. 3).

By the end of the observation period, the average body weight in group I was 277.1 g (± 20.5), and 292.2 g (± 12.5) in group II. Weight recovery to baseline values in the groups was also not noted during the observation period. Appearance of the stool was noted on postoperative day 1 or 2.

Basal glucose level after the 12-hour fast (Fig. 4), as well as blood glucose level in 30 minutes after OGTT (Fig. 5), measured in mmol/L, did not show any significant changes in both groups. When comparing blood glucose levels in both groups, no significant differences were found, both before the surgery and at all studied time intervals after it.

4. Discussion.

We present a novel experimental surgical procedure to investigate the role of the ileum in diabetes mellitus and the Hindgut hypothesis. As in most similar previous studies, surgical technique proposed by us is based on the movement of the ileum in the proximal direction and its location after the ligament of Treitz and duodenum, but there is not only interposition of the terminal segment of the ileum [12,13,14], but also reverse transposition - antiperistaltic location of almost the entire small intestine.

The surgery proposed is performed using a microsurgical technique under magnification. Unlike the well-known ileal interposition operation in its most common version [15], the reverse transposition technique decreases the number of bowel intersections and anastomoses, thereby it reduces invasiveness.

The number of postoperative complications and animal mortality are due to technical errors at the stage of developing and mastering the surgical technique. The cause of death of the animals was anastomotic leak, as a consequence, the development of peritonitis. It is difficult to say whether a leaking intestinal anastomosis was a consequence of the antiperistaltic location of the intestinal tube and dynamic intestinal obstruction. It was not confirmed by macroscopic examination of sacrificed animals. In our study, animal mortality was 16.6%, the mortality in the study group was 20%, and in comparison with similar studies it was comparable.

Conclusions.

Most of the studies aimed at studying metabolic effects of the ileum after ileal interposition are carried out in animals with various pathological conditions [16, 17]. At this stage, our study demonstrates technical feasibility of performing reverse transposition of the small intestine, as well as indicators of weight, fasting blood glucose level and blood glucose level after OGTT during the period of survival and...
adaptation of the experimental model to the new anatomical conditions of the intestinal tract. For further studies of the deeper glycometabolism regulating mechanisms is planned to use obesity-induced diabetic models.

**Abbreviations.**

OGTT - Oral glucose tolerance test.

RYGB - Roux-en-Y gastric bypass.

BPD - biliopancreatic diversion.

RT - reverse transposition.

GLP-1 - glucagon-like peptide-1.

PYY - peptide tyrosine tyrrosine.

**Declarations.**

**Ethics approval and consent to participate.** This experimental study was conducted after approval by the ethical committee of the Medical University of Karaganda in accordance with the legislation of the Republic of Kazakhstan "On Approval of the Rules for Conducting Biomedical Experiments, Preclinical (Nonclinical) and Clinical Studies, as well as as Requirements for Preclinical and Clinical Sites" and compliance with the international principles of the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes.

**Consent for publication.** Not applicable.

**Availability of data and materials.** The data sets used and analyzed in the current study are available from the corresponding author on reasonable request.

**Competing interests.** The authors state that they have no competing interests.

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**Authors' contributions.** All of the authors have made significant and equal contributions to the current study. Ye. M. Turgunov - study planning and coordination, J-P. Faure - study planning, coordination of metabolic/bariatric surgery. L.V. Sevastyanov and D.V. Shestakov - experimental study and data analysis.

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Figure 1

Schematic representation of the operation

Figure 2

B. Type of anastomoses on postoperative day 28
Figure 3

Dynamics of body weight in groups RI and Sham surgery
Figure 4
Basal glycemia in both groups before and after surgery
Figure 5

Blood glucose (BG) 30 minutes after OGTT at different periods of RT and Sham

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