Effect of Preoperative oral Carbohydrate on Intestinal Flora in Patients with Multiple Trauma

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Research

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Abstract

Background: In recent years, the advanced and safety of the concept of ERAS (enhanced recovery after surgery) have been certified by many scholars internationally. One of its core concepts, preoperative oral carbohydrate, has been confirmed by many studies to play an important role in improving patients’ comfort and postoperative insulin resistance. In the previous experiment conducted by our team to explore the effect of preoperative oral carbohydrate on postoperative insulin resistance in patients with multiple trauma, we also found that the postoperative intestinal function of patients in the preoperative oral carbohydrate group recovered faster. A few scholars have reached the same conclusion before, but there has been no further discussion on this phenomenon from the perspective of intestinal flora. Therefore, we aim to further explore the role and advantages of preoperative oral carbohydrate through intestinal flora.

Methods: A total of 43 multiple trauma patients scheduled for elective surgery were included in the study. They were randomly divided into Cho group (n = 23) and Normal group (n = 20). The patients in CHO group were given 400ml carbohydrates 3 hours before the operation, and the patients in Normal group were forbidden to drink anything 2 hours before the operation. The subjective comfort, the first anal exhaust time and defecation time after operation of the two groups were scored, the last fecal intake before the operation and the first fecal intake after operation were taken for 16SrRNA gene amplicon sequencing.

Result: Compared with the Normal group, the subjective comfort of CHO group was improved, the first anal exhaust time and defecation time were advanced, and the length of stay was shortened (all p<0.01). By 16SrRNA gene amplicon analysis, preoperative oral carbohydrates regulated the intestinal microflora of patients with multiple trauma. In the first postoperative feces of CHO group, the Megasphaera_elsdenii played a significant role.

Conclusion: Preoperative oral carbohydrates can cause changes of intestinal flora in patients with multiple trauma, to protect intestinal function and accelerate postoperative recovery. Regulating the relative abundance of Megasphaera_elsdenii and Prevotella may be another way to improve intestinal function and reduce insulin resistance in patients with multiple trauma.

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Introduction

ERAS (enhanced recovery after surgery) was developed by Danish doctor Henrit Kehlet (1) proposed for the first time in 1997 and was first introduced into China by academician of Chinese Academy of Engineering- Jieshou Li in 2007. ERAS refers to the implementation of various proven effective methods in the perioperative period to maintain the preoperative organ function, to reduce the stress and complications of patients, reduce the physical and psychological trauma, reduce the mortality and shorten the length of hospital stay, and speed up the recovery of patients. The core measures of ERAS
include preoperative education, preoperative nutritional support, avoidance of long-term food intake during the perioperative period, preoperative oral carbohydrates, corresponding anaesthesia and analgesia programs, and early mobilization (2).

Previously, to ensure that the gastric contents are completely emptied and avoid the occurrence of reflux and aspiration during anaesthesia, patients undergoing elective surgery are often required to fast for 6–8 hours or even longer. Most patients have preoperative discomfort after long-term fasting, including anxiety, thirst and hunger (3). Jonas Nygren et al (4) suggested in a study that preoperative fasting for a long time would reduce the metabolic rate of the body, induce insulin resistance, cause postoperative muscle function damage and nitrogen level disorder. In a prospective cohort study in 2015, De Amorim et al (5) found that preoperative fasting increased inflammatory response and prolonged postoperative recovery time in patients. Fasting before operation causes a stress reaction in the body, resulting in the increase of pro-inflammatory factors and growth factors, activation of endothelium and leukocytes and release of a large number of reactive oxygen species, which further aggravates the damage of endothelial cells and tissues (6). The increase of inflammatory factors can also cause the damage of intestinal mucosal barrier, thus aggravating the disorder of intestinal flora. The above-combined effect, prolonged the postoperative recovery time, increased the length of hospital stay. As the core measure of eras, preoperative carbohydrate loading has been proved to be safe and effective by more and more scholars. For more than a decade, many studies have confirmed that giving carbohydrates 2 hours before surgery does not increase the risk of aspiration during anaesthesia. A large number of international guidelines suggest shortening the time of fasting and drinking before surgery, including 6h of solid fasting and 2h of clear liquid fasting before surgery. In 2009, the American Society of Anesthesiologists issued “the Guidelines on Fasting and Drinking Before Anesthesiology”. It is recommended that carbohydrate should be taken orally 2 hours before surgery. The European Society of Anesthesiology (7) points out that it is safe and reliable for patients undergoing elective surgery to take oral carbohydrate clear night before the operation. According to “the guidelines for preoperative anaesthesia for adults and children” issued by the anesthesiology branch of Chinese Medical Association in 2014, oral carbohydrate 2 hours before the operation can prevent dehydration, improve circulation stability, relieve postoperative nausea and vomiting, and reduce the incidence of postoperative insulin resistance. Compared with standard glucose solution, carbohydrate osmotic pressure is lower, which leads to higher gastric emptiness rate (8) and oral administration is safer than intravenous administration, comfort is higher than intravenous administration, and patients' acceptance rate is higher. A large number of studies have shown that (3, 9, 10) preoperative oral carbohydrate can reduce the postoperative stress response, insulin resistance, hunger, thirst and anxiety of patients, and improve the comfort of patients. It can reduce the production of inflammatory factors and reduce the occurrence of inflammatory reaction and immune reaction (11). In recent years, the reliability and advantages of ERAS concept have been confirmed by more and more studies, supported by more experimental data, and accepted by more and more doctors and patients. In our previous prospective study, it was confirmed that preoperative oral carbohydrate can improve insulin resistance and comfort after multiple trauma surgery, including nausea and vomiting. At the same time, we also found that the first defecation time of patients in the CHO group (preoperative oral carbohydrate
was shorter than that in the Normal group (Preoperative fasting), and the stool characteristics were more soft and rotten. Previous studies have shown that preoperative oral carbohydrate is helpful to the recovery of postoperative intestinal function and shorten the length of hospital stay (12). Intestinal function is closely related to the balance of intestinal flora, but there are few studies on the effect of intestinal flora. Therefore, we designed this prospective experiment to actively explore the influence of preoperative oral carbohydrate on intestinal flora from the perspective of intestinal flora by 16SrRNA gene amplicon sequencing.

Patients And Methods

Patients

From June 2020 to December 2020, patients with multiple trauma were selected from the Department of emergency surgery, Affiliated Hospital of Nantong University. Inclusion criteria of research subjects: 1. The research subject conforms to the diagnostic criteria of multiple trauma. See “Consensus on medical documentation and diagnosis of multiple injury” (13) in 2013 for the specific criteria. 2. The age is 18–80 years old. 3. ISS (Injury Severity Score) < 16. Exclusion criteria: 1. GCS (Glasgow Coma Scale) < 15. 2. Incomplete clinical data. 3. The patients had digestive system diseases in the past, had gastrointestinal surgery, affected the normal digestive system function, and had abdominal pain, abdominal distension, diarrhea and other symptoms of intestinal infection. 4. Severe underlying diseases, history and signs of metabolic diseases, taking metabolic drugs, weight loss > 5% in the past week. 5. Gastric emptying disorder related diseases. 6. Patients with abnormal glucose metabolism. This study is in line with the "Declaration of Helsinki" and the "international ethical guidelines for biomedical research in human body" of CIOMS (The Council for International Organizations of Medical Sciences) and has been approved by the ethics committee of the Affiliated Hospital of Nantong University. All subjects read and understood the study and signed informed consent.

Methods

Carbohydrate intervention

The patients were randomly divided into the CHO group (n = 23) and Normal group (n = 20) by the double-blind method. Patients in the CHO group were given 400ml carbohydrates orally 3 hours before the operation, and the use was completed within 2 hours. Before, during and after the oral administration of carbohydrates, the patients were examined by B-ultrasonography in the lower gastric fundus, gastric body, gastric antrum, and gastric empty rate. Communicate with the anesthesiologist before the operation, record intraoperative fluid supplement and intraoperative bleeding.

Index observation and sample collection

Subjective comfort: VAS (visual analogue assessment) was used to evaluate the subjective comfort of patients (including thirst, hunger, anxiety and nausea). The specific operations were as follows:
communicate with the patients who were conscious on the day of operation 6 h, 4 h, 2 h before the operation, 2 h, 4 h, 6 h after operation, and give them a white note with 10 scales to represent the discomfort degree of thirst, hunger, anxiety and nausea. They are "0" and "10" respectively. "0" means no discomfort; "10" means unbearable. The higher the value is, the more serious the degree of discomfort is, so that patients can point out the most similar value of comfort.

**Fecal collection and analysis**

We guarantee that feces will be collected immediately and stored in a Norgen tube in a refrigerator at −80 °C. The tube contains liquid fecal preservatives to prevent the growth of G⁻ and G⁺ bacteria and fungi, ensure the freshness of feces and the stability of flora, and ensure the accuracy of 16SrRNA gene amplicon sequencing analysis. Using the MiseqPE250 sequencing platform of Illumina company, the MIS sequencing library was obtained by PCR technology, and the products were further analyzed by Illumina Miseq.

**Statistics And Network Analysis**

SPSS 22.0 was used for statistical analysis. Chi-square test was used for frequency data. The normal distribution of measurement data are expressed as mean ± standard error (x ± s), t-test was used for intergroup comparison, M(Q1, Q3) was used for non-normal data, and Mann-Whitney U test was used for intergroup comparison. A P value < 0.05 was considered statistically significant.

**Results**

A total of 43 patients participated in this study. The data of all subjects are complete. The baseline characteristics are shown in Table 1. Except for the length of hospital stay, there was no significant difference in baseline data between the two groups. The length of hospital stay of the CHO group was shorter than that of the Normal group, and the results had a significant difference. (P < 0.01)
|                                     | CHO group | Normal group | P-value |
|-------------------------------------|-----------|--------------|---------|
|                                    | n=23      | n=20         |         |
| Gender (F/M)                        | 11/12     | 9/11         | 0.853   |
| Age (years)                         | 46 (37.63) | 53.5 (37.5, 64.75) | 0.643   |
| BMI (Kg/m²)                         | 22.7 ±3.2 | 23.3 ±4.1    | 0.593   |
| Smoking (yes/no)                    | 13/10     | 8/12         | 0.280   |
| ISS score                           | 10.17 ±2.401 | 10.33 ±2.875 | 0.843   |
| Intraoperative blood loss (ml)      | 12.1 ±7.2 | 11.4 ±4.3    | 0.706   |
| Fluid intake during surgery (ml)    | 489 ±130  | 527 ±230     | 0.501   |
| The operation time (hours)          | 1.2 ±0.3  | 1.15 ±0.7    | 0.757   |
| Length of stay (days)               | 4 ±1.5    | 6 ±1.7       | <0.001  |

**Oral carbohydrate before operation changed the subjective feelings of the two groups**

The scores of anxiety, hunger and thirst in the CHO group were significantly lower than those in the Normal group (P < 0.01), and there was no obvious nausea and reflux of gastric contents. After the operation, there was no significant difference in anxiety between the two groups, but the scores of thirst, hunger and nausea in the Normal group were higher than those in the CHO group at 2 h, 4 h and 6 h after operation, the results had a significant difference. (P < 0.01)(Fig. 1).

**Preoperative oral carbohydrate accelerated the recovery of intestinal function**

Compared with the Normal group, the first anal exhaust time and defecation time in the CHO group were earlier, and the results of the two groups were significantly different. (P < 0.01)(Fig. 2)

**Preoperative oral carbohydrate changes in intestinal flora**

In Fig. 3 and Fig. 4, we found that Prevotella showed a significant influence in the intestinal microenvironment of the patients in the Normal group and the patients in the CHO group before the operation, and the patients in the CHO group showed the increase and significant influence of Megasphaera_elsdenii after the operation.
Discussion

Intestinal microorganisms play an important role in the occurrence and development of human health and diseases. They participate in many aspects of human metabolism, immunity, neuroendocrine and so on. The fermentation products of intestinal microorganism, propionic acid and butyric acid, play an important role in maintaining intestinal mucosal integrity, intestinal epithelial cell stability and constructing good intestinal barrier (14). In this study, we concluded that preoperative oral carbohydrates can reduce preoperative anxiety, postoperative nausea, preoperative and postoperative hunger and thirst. First anal exhaust and defecation time in CHO group were earlier than those in Normal group. In Fig. 3 and Fig. 4, the influence of Megasphaera_elsdenii was significantly increased and the influence of Prevotella was decreased in the postoperative intestinal flora of CHO group, As a kind of rumen cocci, Megasphaera_elsdenii is a strict anaerobic bacteria. It is a colonized bacteria in the intestine of cattle, sheep and other mammals. Because it can use lactate to prevent the accumulation of lactic acid in the intestine, ferment lactic acid into propionic acid, and participate in the metabolism of lactic acid and amino acids, it is widely added to the ruminant feed, but its regulatory effect on human intestinal microenvironment is rarely studied (15). Lactate is the product of bacterial aggregation and degradation in the intestine. When the intestinal motility is insufficient, the intestinal blood supply is reduced, and the intestinal contents are accumulated, which leads to anaerobic digestion, resulting in a large amount of lactate. At the same time, with the intestinal mucosal damage, the intestinal permeability is enhanced, and it enters the blood, causing serious complications. In recent years, many randomized controlled trials have concluded that lactate is an important indicator for the diagnosis of intestinal ischemia. Yingying Liao et al (16) found a strong positive correlation between lactate level and endotoxin level in a randomized controlled trial on intestinal mucosal function in patients with severe acute pancreatitis. VAN et al (17) found that lactate has a high diagnostic value for intestinal obstruction. Another study (18) suggests that although lactate level is not a standard linear relationship with ischemic obstruction of intestinal obstruction, it plays an important role in suggesting early intestinal ischemia. Therefore, we considered that the defecation time and anal exhaust time of CHO group were shortened, and the changes of fecal characteristics were correlated with the increase of the genus of Megasphaera_elsdenii. Carbohydrates were taken orally before surgery, and the increase of carbohydrates in the intestines led to the increase of available substrate of Megasphaera_elsdenii in the intestines of patients in the corresponding CHO group, which resulted in the increase of Megasphaera_elsdenii in the intestines. Megasphaera_elsdenii fully fermented lactate into propionic acid. Propionic acid has anti-inflammatory and anti-oxidation effects through regulating signalling pathways, thus stabilizing intestinal epithelial cells (19), improving intestinal microenvironment, forming a certain protective effect on the intestinal mucosa, and accelerating the recovery of intestinal function. Recently, some studies (19) ave concluded that the increase of propionic acid content can reduce the occurrence of insulin resistance to a certain extent, which is also related to the result of our previous experiment that preoperative oral carbohydrate can improve the occurrence of postoperative insulin resistance. In this experiment, we also found that the
concentration of Prevotella was higher in the Normal group after surgery and CHO group before surgery, and the expression of Prevotella was significantly lower in CHO group after surgery. Prevotella is a strict anaerobic bacteria, which widely exists in the oral and intestinal tract (20), Aida et al (21) concluded from the study on the correlation between Prevotella and colitis in mice that the long-term colonization of Prevotella in the intestine leads to the decrease of acetic acid, which in turn leads to the decrease of IL-8 level, thus aggravating the intestinal inflammatory reaction and further leading to the destruction of the intestinal mucosa. Moreover, the increase of Prevotella was positively correlated with insulin resistance (22). Before this, preoperative oral carbohydrate intake increased glycogen reserves in the body on the one hand and co-regulated blood glucose levels through the entero-brain axis on the other. Preoperative oral carbohydrates lead to the increase of portal blood glucose, which is transmitted to the hypothalamus through the portal blood glucose receptor, thus enhancing insulin sensitivity, reducing blood glucose and alleviating postoperative insulin resistance. At the same time, the hypothalamus can regulate blood glucose by regulating the balance between sympathetic nerve and parasympathetic nerve, and excite parasympathetic nerve to reduce blood glucose (23, 24). Other studies have confirmed that preoperative oral carbohydrate can also reduce the incidence of postoperative insulin resistance by regulating PI3K/AKT/ mTOR pathway (25). We conclude that preoperative oral carbohydrate can improve the intestinal microenvironment, to accelerate the recovery of intestinal function and shorten the length of hospital stay in patients with multiple trauma. At the same time, the change of intestinal flora will also have a certain impact on postoperative insulin resistance. Regulating the relative abundance of Megasphaera_elsdenii and Prevotella may be another way to improve intestinal function and reduce insulin resistance in patients with multiple trauma.

**Conclusion**

Through the randomized controlled trial of preoperative oral carbohydrate, we concluded that preoperative oral carbohydrate can reduce postoperative insulin resistance, accelerate the recovery of postoperative intestinal function and shorten the length of hospital stay by improving intestinal microenvironment and regulating intestinal bacterial balance.

**Declarations**

**Ethics approval and consent to participate:** This study has been performed under a project license(No: 2018-L062) granted by the Ethics Committee of the Affiliated Hospital of Nantong University.

**Consent for publication:** We agree that once our paper is accepted, the statement I provided will be published as additional information for readers.

**Availability of data and materials:** All data generated or analysed during this study are included in this published article.

**Competing interests:** The authors have no conflicts of interest to declare.
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**Authors' contributions:** (I) Conception and design: ZH and HJ; (II) Administrative support: ZH and YL; (III) Provision of study materials or patients: RW and YR; (IV) Collection and assembly of data: RW and YW; (V) Data analysis and interpretation: GM and LQ; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors

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Figures
Figure 1

A total of 43 patients participated in this study. The data of all subjects are complete. The baseline characteristics
Figure 2

(a.) The VAS score of CHO group 2 hours before operation (1 hour after taking carbohydrates) was lower than that of conventional fasting group. The results showed a significant difference, but there was no significant difference after the operation. (b.) The degree of postoperative nausea in the Normal group was more obvious than that in the CHO group, and the VAS scores of the two groups were significantly different. (c.d) After taking carbohydrates orally, the hunger and thirst of the CHO group decreased and maintained at a low level after the operation. *p<0.01 VAS: Visual Analogue Score -6,-4,-2, 2,4,6 hours preoperative, 4 hours preoperative, 2 hours preoperative, 2 hours postoperatively, 2 hours postoperatively
There were significant differences in the first postoperative anal exhaust time and defecation time between CHO group and the Normal group. *p<0.01
Figure 4
(a.) The evolutionary branching chart from the inside out (from the phylum to the species level), shows the intestinal flora that plays an important role after surgery in the CHO group and the Normal group, and the diameter of the circle is proportional to the relative abundance of the flora. (b.) Among the intestinal flora with statistically significant differences, we chose the flora with significant influence on the intestinal microenvironment as shown in (b).
Figure 5

(a.) the difference of intestinal microflora before and after the operation in the CHO group, and the diameter of the circle was directly proportional to the relative abundance of microflora. (b.) Among the intestinal microflora with the statistical difference between preoperation and postoperation in CHO group, we selected the microflora with significant influence on the intestinal microenvironment, as shown in (b).