CHAPTER NINE

Preservation of the gut by preoperative carbohydrate loading improves postoperative food intake

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ABSTRACT

Background A carbohydrate (CHO) drink given preoperatively changes the fasted state into a fed state. The ESPEN guidelines for perioperative care include preoperative CHO loading and re-establishment of oral feeding as early as possible after surgery. An intestinal ischaemia reperfusion (IR) animal model was used to investigate whether preoperative CHO loading increases spontaneous postoperative food intake, intestinal barrier function and the catabolic response.

Methods Male Wistar rats (n = 65) were subjected to 16 hours fasting with ad libitum water and: A) sham laparotomy (Sham fasted, n = 24); B) intestinal ischaemia (IR fasted, n = 27); and C) intestinal ischaemia with preoperatively access to a CHO drink (IR CHO, n = 14). Spontaneous food intake, intestinal barrier function, insulin sensitivity, intestinal motility and plasma amino acids were measured after surgery.

Results The IR CHO animals started eating significantly earlier and also ate significantly more than the IR fasted animals. Furthermore, preoperative CHO loading improved the intestinal barrier function, functional enterocyte metabolic mass measured by citrulline and reduced muscle protein catabolism, as indicated by normalization of the biomarker 3-methylhistidine.

Conclusions Preoperative CHO loading improves food intake, preserves the GI function and reduces the catabolic response in an IR animal model. These findings suggest that preoperative CHO loading preserves the intestinal function in order to accelerate recovery and food intake. If this effect is caused by overcoming the fasted state or CHO loading remains unclear.

INTRODUCTION

Fasting before surgery has serious consequences for organs such as the gastrointestinal (GI) tract, liver, kidney, heart, and lungs, probably because it exhausts the energy reserves of the body (1). Fasting further increases the effects of surgical stress, it results in depletion of glycogen stores, dehydration, muscle wasting, a weakened immune response and production of inflammatory mediators (2). Furthermore, overnight fasting has been reported to induce postoperative insulin resistance, resulting in decreased cellular uptake of glucose, despite high levels of glucose and adequate levels of insulin in the blood. Insulin resistance is an unwanted phenomenon in modern surgical practice because it may lead to an increased infectious complication rate and prolonged length of hospital stay (3).

A carbohydrate (CHO) drink containing at least 48 g of CHO's given preoperatively changes the fasted state into a fed state and counteracts the disadvantageous effects of fasting on patients' well-being. In addition, it is safe and empties rapidly from the stomach to decrease the risk of gastric aspiration (4). CHO loading reduces postoperative insulin resistance, improves muscle strength, has a positive effect on well-being, and shortens length of hospital stay (5-8). Therefore the use of preoperative CHO loading has been incorporated in the ESPEN guidelines (European Society for Parenteral and Enteral Nutrition) (9). These guidelines for perioperative care include avoidance of long periods of preoperative fasting and re-establishment of oral feeding as early as possible after surgery.

Previously, we have shown in an intestinal ischaemia reperfusion (IR) rat model, that preoperative CHO loading preserves the intestinal barrier function and reduces bacterial translocation (10). In this model, the animals were allowed to recover for three hours after surgery under complete narcosis. The results suggest a preservation of the function of the GI tract. Based on this, we hypothesise that preoperative CHO loading may lead to earlier postoperative food intake. To test this hypothesis, ad libitum food intake was measured in an IR rat model, comparing preoperative CHO loading versus fasting. GI function, intestinal barrier function and the catabolic response were also measured 24 hours after intestinal ischaemia. We used a well established experimental setup in which we extended the recovery time to at least 24 hours.

METHODS

Animals

All experimental procedures were approved by an independent animal experiments committee (DEC Consult, Bilthoven, The Netherlands) and complied with the principles of laboratory animal care. Male Wistar rats (Harlan Laboratories, Horst, The Netherlands; 225-250 g on arrival) were pair-housed under a light-dark schedule of 12:12 (lights on at 11 PM) in a temperature and humidity controlled room (21 ± 2°C and 50 ± 5%, respectively). All animals were allowed to acclimate for 2 weeks and had free access to standard rodent diet and tap water unless stated otherwise.

Canulation of the jugular vein

All animals were equipped with a jugular vein catheter for stress-free blood sampling according

to the Steffens method.(11) The animals were allowed to recover, until they all had regained the weight they had prior to this procedure.

Study design

Animals (n = 65) were divided into 3 groups. A fasted sham-operated group (Sham fasted: n = 24) was subjected to 16 hours of fasting and laparotomy. The second group (IR fasted: n = 27) was also fasted for 16 hours followed by laparotomy and intestinal ischaemia by clamping the superior mesenteric artery for 70 minutes. The remaining group was subjected to laparotomy and intestinal ischaemia, and had ad libitum access to a clear CHO drink (IR CHO: n = 14; 12.6% carbohydrates, Nutricia preOp, Nutricia N.V., Zoetermeer, The Netherlands). Water remained ad libitum for all animals and all animals in the IR CHO group voluntarily ingested 35ml of the provided CHO drink prior to surgery. Surgery was performed under O_2/N_2O/ isoflurane anesthesia (Forene Abbott, Hoofddorp, The Netherlands) and body temperature of the animals was maintained at 37°C. Peritoneal fluid resuscitation was given during the intestinal ischaemia. Buprenorfine was injected subcutaneously once (3 mg/ml, 1 mg/kg) immediately after surgery. Animals were housed individually in food registration cages with free access to food and water. Spontaneous food intake was automatically recorded every 5 minutes (Food Intake Monitor for Rat, MED Associates Inc., Georgia, Vermont, USA). Animals were dissected 24 or 48 hours after surgery. Blood was drawn via heart puncture. The distal ileum was removed for determination of *ex vivo* intestinal barrier function and the jejunum was removed for ***ex vivo*** measurements of the motility.

Ex vivo analysis of intestinal barrier function

After removal of the distal ileum, the epithelium was stripped from the external muscle layer and mounted in ussing chambers. This procedure was repeated five more times per animal. Horseradish peroxidase, a 40 kD protein (HRP, UKO protein, 15µL, $1*10^{-3}$ M) was added to the mucosal compartment, and the mucosal to serosal flux of HRP was determined after 150 minutes. The serosal concentration of enzymatically active HRP was measured using a method based on Gallati and Pracht (12). The transepithelial electrical resistance (TER) was determined after 150 minutes, using an epithelial voltohmmeter (WPI, Sarasota, Florida, USA).(13) TER mainly represents paracellular barrier function, whereas the barrier function for HRP is mainly related to endocytosis and represents transcellular barrier function. Both HRP-flux and TER are well established methods for analysis of ***ex vivo*** measurement of intestinal barrier function (10).

This model, in which the superior mesenteric artery is clamped for 70 minutes, is considered to have a great impact on the intestine. To validate this intestinal IR model we wanted to find out whether the damage to the intestine was transient and reversible; 15 animals (Sham fasted: n = 7, IR fasted: n = 8) were allowed to recover for 48 hours after which HRP-flux and TER was measured.

Determination of intestinal motility

Per animal, four approximately one centimeter sections of the jejunum were placed into

organbaths of a myograph (Schuler organbath, Hugo Sachs Elektronik, March, Germany), which measured the contractions of the jejunum. In this experiment we used 8 section of Sham fasted, 8 sections of IR fasted and 4 section of IR CHO jejunum. The sections were incubated with Carbachol (CCh, Sigma, Steinheim, Germany) in increasing concentrations in a range of 0.01, 0.05, 0.1, 0.5, 1, 5, 10 μM for approximately 5 minutes per concentration. Contractions of the jejunum were recorded and the strength of the contraction was calculated by comparing the tension caused by CCh incubation to the preload tension (gr).

Plasma analyses

Plasma glucose was determined colorimetrically (GOD-PAP, Roche Diagnostics, Mannheim, Germany). Plasma insulin was analyzed in triplicate using serially diluted samples with a specific rat ELISA kit (DRG Diagnostics, Diagnostic System Laboratories, Benelux) with a detection limit of 22.6 pmol/L. The insulin resistance index was assessed by homeostasis model assessment (HOMA-IR = 0.403 * [Glucose $_{t=0}$ (mmol/L) * Insulin $_{t=0}$ (pmol/L) / 405]).(14, 15) Plasma citrulline, glutamine and 3-methylhistidine (3-MeHis) were determined by high-pressure liquid chromatography (HPLC) as previously described by Hoorn *et al* (16).

Statistics

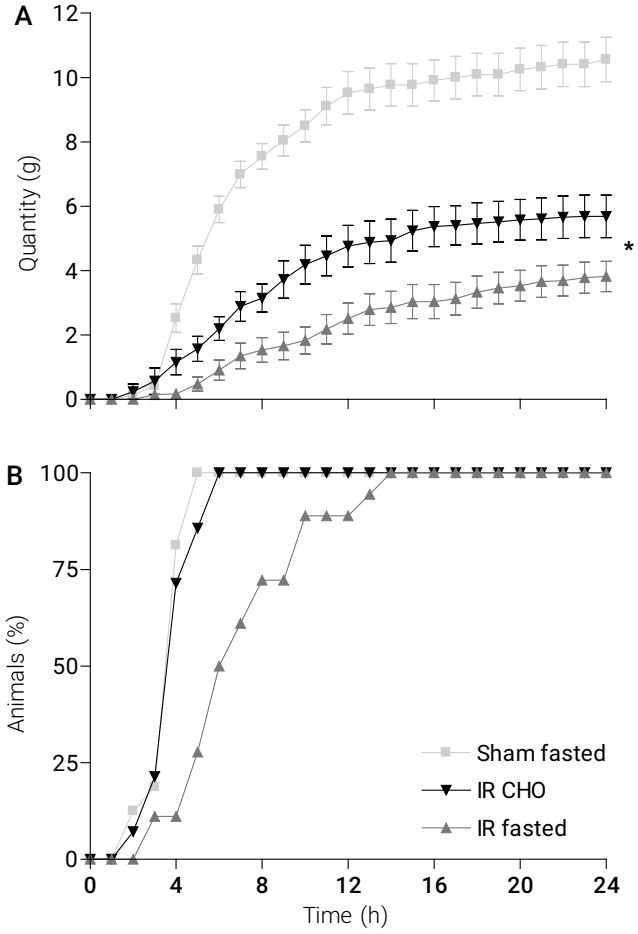
Statistical analyses were performed using SPSS 15.0.1 software (SPSS Benelux, Gorinchem, The Netherlands). Variables were checked for Gaussian distribution with the Shapiro-Wilkes and Kolmogorov-Smirnov test. Levene's test for equality of variance was used to assess the probability of different variances among treatment groups. ANOVA was used for statistical analysis of the data. Differences were considered significant at $P < 0.05$. Correlations between the different parameters were calculated using the Pearson correlation coefficient.

RESULTS

Food intake

Spontaneous postoperative food intake was measured every hour to determine if CHO loading had an effect on the amount that was eaten and the time the animals started eating. After 24 hours, IR CHO animals showed a significant higher overall food intake (5.69 ± 0.66 g, mean ± SEM) compared to IR fasted animals (3.82 ± 0.47 g, mean ± SEM; $P < 0.05$) (Figure 1A). Moreover, the IR CHO animals started eating significantly earlier (4.14 ± 0.29 h, mean ± SEM) than the IR fasted animals (7.28 ± 0.69 h, mean ± SEM; $P < 0.05$) (Figure 1B). The time point at which the IR CHO animals started eating (4.14 ± 0.29 h, mean ± SEM) was the same as in the Sham fasted control animals (3.88 ± 0.22 h, mean ± SEM; $P = 0.446$).

FIGURE 1. *Food intake. (A) CHO loading improved postoperative food intake. IR CHO animals ate significantly more than IR fasted animals. Data presented as mean ± SEM. * P < 0.05. (B) IR CHO animals started eating significantly earlier than IR fasted animals. The time point at which the IR CHO animals started eating was equal to the Sham fasted animals. Data presented as percentages.*

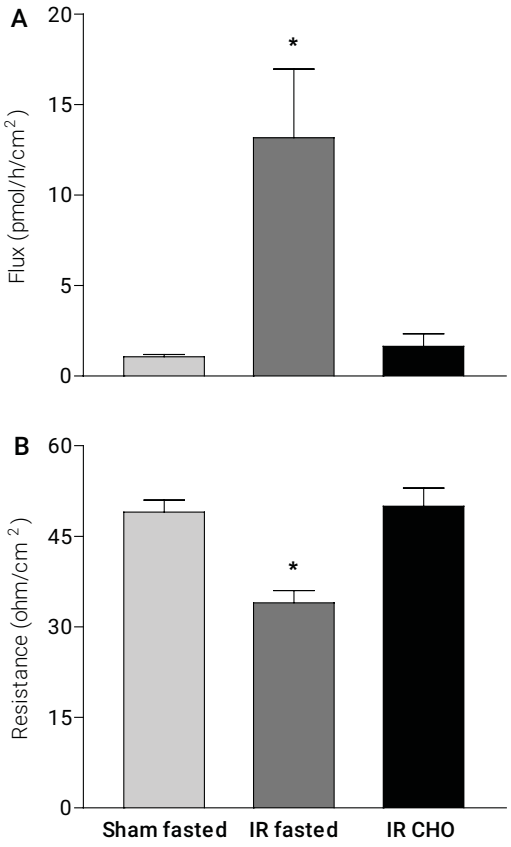


Intestinal barrier function

To study the effects of IR on the intestinal barrier function of the ileum, the HRP flux from the mucosal to serosal side was measured across stripped epithelium mounted in ussing chambers. Intestinal ischaemia in the IR fasted animals caused a significantly higher HRP flux from the mucosal to the serosal side than in Sham fasted animals ($P < 0.05$). Animals that had preoperative access to a CHO drink showed a significantly lower flux of HRP across the epithelium of the ileum when compared to IR fasted animals ($P < 0.05$) (Figure 2A). Transepithelial resistance (TER) measurements supported the HRP-flux findings. IR CHO animals showed a significantly higher TER across the epithelium of the ileum when compared to IR fasted animals ($P < 0.05$) (Figure 2B).

The HRP-flux and TER were already normalized in the IR CHO group after 24 h of recovery. To demonstrate that the impact on the intestine of the IR model was not too severe we performed a pilot study, in which IR fasted animals were allowed to recover for 48 hours. After 48 hours of recovery, HRP-flux was normalized in the IR fasted group (1.34 ± 0.46 pmol/h/cm^2; mean ± SEM), showing comparable results to the Sham fasted animals (1.59 ± 0.46 pmol/h/cm^2; mean ± SEM). Also, the TER was normalized in the IR fasted group (58 ± 4 ohm/cm^2; mean ± SEM) after 48 hours of recovery, showing comparable results to the Sham fasted animals (57 ± 2 ohm/cm^2; mean ± SEM). This indicates that the intestinal damage, to which the animals were subjected in this model, was not too severe.

FIGURE 2. *Intestinal barrier function. (A) Mucosal to serosal flux of horseradish peroxidase (HRP) through segments of the ileal wall. The data of the paracellular flux of the macromolecule HRP is presented here. IR CHO animals showed a significantly lower flux of HRP compared to IR fasted animals. IR fasted animals showed a significant higher HRP flux than in Sham fasted animals. (B) Transepithelial resistance of ileal epithelial. IR CHO animals showed a significantly higher resistance compared to IR fasted animals. IR fasted animals showed a significant lower resistance than the Sham fasted animals. Data presented as mean ± SEM. * P < 0.05.*



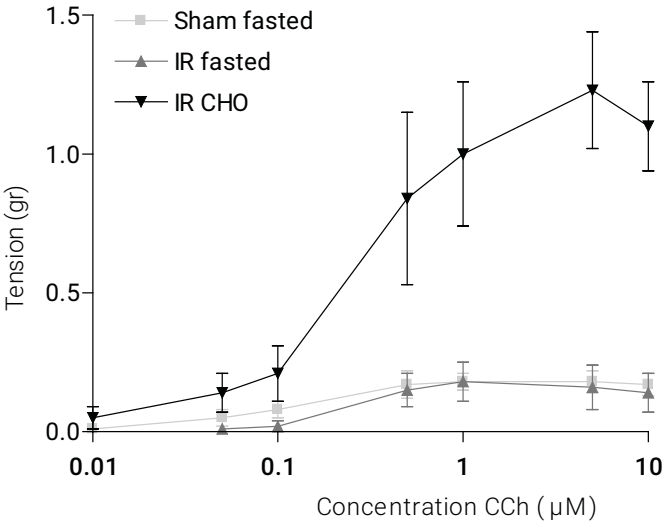
Insulin resistance

Previous human studies show less reduction in postoperative insulin sensitivity after CHO loading, related to a better maintenance of whole body glucose disposal. In the current study, we observe significantly higher postoperative basal insulin levels in the IR fasted animals (181.46 ± 22.79 pmol/L, mean ± SEM) compared to the Sham fasted (95.18 ± 15.89 pmol/L, mean ± SEM; $P < 0.05$). The basal insulin levels of the IR CHO animals (134.82 ± 23.76 pmol/L, mean ± SEM) were not significantly increased. We also see significantly higher postoperative basal glucose levels in the IR fasted animals (7.73 ± 0.54 mmol/L, mean ± SEM) compared to the Sham fasted (5.82 ± 0.45 mmol/L, mean ± SEM; $P < 0.05$). The basal glucose levels of the IR CHO animals were not significantly increased (6.37 ± 0.82 mmol/L, mean ± SEM). The insulin resistance assessed by HOMA-IR is significantly higher in the IR fasted animals (1.17 ± 0.19, mean ± SEM) compared to the Sham fasted animals (0.47 ± 0.09, mean ± SEM) ($P < 0.05$). The IR CHO animals showed a trend towards improvement (0.81 ± 0.15, mean ± SEM).

Intestinal motility

The strength of the motility of the sections of jejunum was measured in a myograph. The effect of CCh on the contraction of the jejunum is presented in Figure 3. From CCh concentrations of 0.5μM and higher, the tension of the sections of jejunum was stronger after preoperative CHO loading compared to the fasted state. The fasted animals (Sham fasted and the IR fasted) showed a minimal response to CCh stimulation (Figure 3).

FIGURE 3. *Intestinal motility. From CCh concentrations of 0.5μM and higher, the tension of the sections of jejunum was higher after preoperative CHO loading compared to the fasted state. Data presented as mean ± SEM.*



Enterocyte metabolic mass

Three hours postoperative the plasma citrulline levels were reduced ($P < 0.05$) in all animals. At 24 hours after surgery, however, a significant increase ($P < 0.05$) in citrulline levels was seen in the IR CHO animals compared to the IR fasted animals (Table 1). Since citrulline is dependent on the glutamine load in time, we also present the plasma glutamine data. The plasma glutamine levels were higher in the IR fasted rats after 24 hours of recovery ($P < 0.05$) (Table 1). No significant correlation was found comparing plasma citrulline and glutamine levels.

Muscle function

In all animals, we found a significant increase in 3-MeHis. However, the IR CHO animals had significantly lower plasma 3-MeHis values compared to the IR fasted animals ($P < 0.05$) (Table 1). Moreover, the IR CHO animals had equal values as the Sham fasted animals ($P = 0.988$).

TABLE 1. *Plasma citrulline, glutamine and 3-methylhistidine levels after surgery.*

Amino acid	Hours	Sham fasted n = 19	IR fasted n = 23	IR CHO n = 14
Citrulline (μM/L)	0	73 ± 2	72 ± 2	68 ± 4
	3	65 ± 2	60 ± 4	57 ± 4
	24	60 ± 2	40 ± 2[A]	47 ± 2[A,B]
Glutamine (μM/L)	0	694 ± 25	657 ± 14	601 ± 22
	3	628 ± 15	541 ± 22	532 ± 18
	24	583 ± 19	721 ± 25[A]	627 ± 18[B]
3-Methylhistidine (μM/L)	0	3.9 ± 0.4	3.7 ± 0.2	2.8 ± 0.1
	3	4.5 ± 0.5	5.6 ± 0.5	3.2 ± 0.2
	24	6.6 ± 0.5	11 ± 0.9[A]	6.5 ± 0.5[B]

[A] Significantly different from Sham fasted, $P < 0.05$. [B] Significantly different from IR fasted, $P < 0.05$. Data presented as mean ± SEM. CHO, carbohydrate; IR, ischaemia reperfusion.

DISCUSSION

We investigated whether preoperative CHO loading compared to fasting could improve postoperative food intake using an intestinal IR animal model. In this model, preoperative CHO loading improved food intake, and intestinal barrier function. Moreover, the biomarkers citrulline and 3-MeHis returned to normal levels. These are distinct parameters reflecting different aspects of intestinal function and well-being.

Our main objective was to evaluate the effect of CHO loading on postoperative food intake. Dag *et al.* recently demonstrated in a randomized controlled trial that early oral feeding after colorectal surgery was not only well tolerated but also affected the postoperative outcomes positively, leading to a shorter hospital stay (17). Early enteral feeding is recommended by the ESPEN guidelines, but it can only be achieved when patients' conditions allow it. In the current study animals started eating earlier voluntarily, which indicates an improved state of well-being. CHO loading diminishes the effect of a major surgery, resulting in comparable outcomes to the Sham fasted animals. From this we can conclude that CHO loading makes early enteral feeding possible.

Previously, we reported that the effect of preoperative CHO loading preserves both the intestinal barrier function and prevents translocation of bacteria to distant organs (10). In this animal model, postoperative ileal HRP flux and transepithelial resistance were measured as indicators of intestinal barrier integrity (13, 18). IR combined with fasting, resulted in a markedly decreased intestinal barrier function. Addition of a CHO drink significantly reduced this effect. CHO loading preserves the intestinal barrier function resulting in a lower HRP-flux and higher resistance.

Clinical studies have shown that fasting before surgery increases insulin resistance when compared with feeding the patient. A CHO drink before surgery is safe and it switches the fasted state to a fed state. Preoperative oral CHO loading reduces the development of insulin resistance by approximately 50% on the day after surgery (19). These findings are supported by the current study in which IR fasted animals had a significant higher insulin resistance index than the Sham fasted animals. The insulin resistance in the IR CHO animals was not significantly different to the Sham fasted animals.

Jejunal motility was measured; because it was hypothesized that intestinal ischaemia would induce an impaired motility. Jejunal motility of the IR fasted animals was equal to the Sham fasted animals. Surprisingly, jejunal motility appeared to be stronger after preoperative CHO loading, suggesting an effect of fasting instead of the intestinal ischaemia on motility. This supports the hypothesis that fasting reduces glucose reserves necessary for intestinal motility. To confirm these findings larger studies are required.

Earlier studies have shown that fasting plasma citrulline correlates with residual bowel length in patients with small bowel syndrome (20, 21). This can be explained by the fact that enterocytes of the small intestine are unique in being able to synthesize citrulline from glutamine. In this study the plasma glutamine levels were not higher in the IR CHO group. In contrary, the glutamine levels were higher in the fasted animals after 24 h. Still the citrulline levels are significantly higher in the IR CHO group. Based on this, we conclude that the elevated levels of citrulline are due to a preservation of the functional enterocyte metabolic mass. This preservation of the intestine is also strengthened by the permeability results. However, the IR CHO animals, could tolerate earlier food intake and ate more, which might explain the higher levels, because enteral glutamine is rather a substrate for citrulline than circulating glutamine (22). Nevertheless, the final measurement of citrulline is done after 24 h, after a light period of 12 h in which the rats are inactive and do not eat, comparable to a fasted state. To substantiate that the increases in plasma citrulline concentrations in IR CHO animals reflect an intestinotrophic effect of CHO loading, a citrulline generation test with a dipeptide alanine-glutamine drink should be performed in humans (23).

3-MeHis is an amino acid which can be used as a biomarker for measuring the rate of muscle protein degradation of skeletal and smooth muscle cells (e.g. from the intestine) (24, 25). 3-MeHis, which is post-translationally formed by methylation of histidine, cannot be reutilized for protein synthesis and is therefore a suitable amino acid for monitoring degradation rate of 3-MeHis-containing proteins (26). 3-MeHis was significantly lower in the IR CHO animals than in the IR fasted animals, indicating that myofibrillar protein degradation was suppressed by CHO loading. This supports the hypothesis that in a fasted state the body will utilize the

proteins within the muscle tissue as a fuel source. According to the results of the current study, the muscle protein catabolism may be reduced with preoperative CHO loading.

These combined effects suggest a multifactorial role for preoperative CHO loading. We are dealing with different mechanisms, on which we would like to elaborate. Preoperative CHO loading leads to earlier spontaneous food intake. The preserved intestinal integrity may be a mechanism, which results in a decreased inflammation and reduced bacterial translocation. Another hypothesis might be that CHO loading preserves the energy status of the liver (27). Consequently, there is a higher capacity to mobilize the fuel for the production of energy necessary for recovery. Accordingly, energy levels in the smooth muscle cells may also be compromised, resulting in decreased jejunal motility of animals in the fasted state. Another factor might be a reduction in insulin resistance influencing energy levels in the liver (28). The probable main factor why catabolism is preserved by CHO loading is the reduction of insulin resistance on the level of skeletal muscle. This is reflected by a reduction of 3-MeHis, from this we may conclude that CHO loading may reduce the length of the catabolic state.

In conclusion, this study shows encouraging results for the future of the patient. A simple CHO drink might be the way to preserve the intestinal function in order to accelerate recovery and food intake. If this effect is caused by overcoming the fasted state or CHO loading remains unclear.

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