Digestive Surgery

Research Article

Dig Surg 2020;37:368–375
DOI: 10.1159/000505515

Received: February 12, 2019
Accepted: December 17, 2019
Published online: March 10, 2020

Preoperative Oral Carbohydrate Reduces Postoperative Insulin Resistance by Activating AMP-Activated Protein Kinase after Colorectal Surgery

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Keywords
Colorectal surgery · Insulin resistance · Preoperative oral carbohydrate · AMP-activated protein kinase

Abstract
Background: Postoperative insulin resistance (PIR) is a common response after colorectal surgery and an independent risk factor for recovery. Preoperative oral carbohydrate (POC) has been known to reduce PIR. Herein, we investigated whether its mechanism of action involves AMP-activated protein kinase (AMPK) and mTOR/S6K1/insulin receptor substrate-1 (IRS-1) pathways. ***Methods:*** Patients undergoing colorectal cancer resection were randomly assigned to a POC, fasting, or placebo group. The exclusion criteria were association with diseases or intake of medication affecting insulin sensitivity. Pre- and postoperative insulin resistance, and protein phosphorylation of AMPK, mTOR, and IRS-1 in the rectus abdominis muscle were evaluated. ***Results:*** From January 2017 to December 2017, 70 patients were randomized and 63 were evaluated. No difference was found in the clinical and operative characteristics among the 3 groups. In the POC group, the levels of blood glucose, blood insulin, and homeostasis model assessment of insulin resistance were significantly lower in the POC group than the fasting and placebo groups, and the insulin sensitivity index was significantly higher. The phosphorylation of AMPK in the POC group was significantly higher than that in the other 2 groups, whereas the phosphorylation of mTOR and IRS-1 was significantly lower. ***Conclusion:*** PIR involves AMPK and mTOR/S6K1/IRS-1 pathways. POC reduces PIR by the stimulation of AMPK, which suppresses the phosphorylation of mTOR/IRS-1 and attenuates PIR after colorectal resection.

Background

Colorectal cancer is one of the common malignant tumors of the digestive system globally [1], and surgical treatment through radical cancer resection has been the most effective but invasive treatment for this cancer type [2]. Postoperative insulin resistance (PIR) is the most common metabolic disorder after this surgery, which is characterized by increased blood glucose levels and decreased insulin sensitivity after operation [3]. Numerous studies have revealed that PIR increases the risk of infection and cardiovascular and cerebrovascular dysfunction, as well as dysfunction of other organs, which increases postoperative morbidity and mortality [4]. The degree of

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PIR has been considered an independent factor for the risks of postoperative complications, which lead to prolonged stay in the hospital and increased costs [5, 6].

Several perioperative managements aiming at maintaining euglycemia, such as intensive insulin therapy [7], shortened preoperative fasting [8], and patient-controlled epidural analgesia [9], have been reported to markedly reduce postoperative complications and improve the outcome of the patients after surgery. Moreover, preoperative oral carbohydrate (POC) therapy has been confirmed to be a safe and effective treatment to reduce PIR [10, 11]. A recent review reported enhanced gastrointestinal recovery and shorter hospital stay after the use of POC, which does not influence postoperative complication rates [12]. Multiple randomized controlled trials have demonstrated improved postoperative metabolic response after POC administration, including reduced insulin resistance, protein sparing, improved muscle function, and preserved immune response [13–16]. However, the mechanism by which POC treatment reduces PIR remains unclear.

Insulin receptor substrate-1 (IRS-1) binds the insulin receptor to phosphoinositide-3-kinase (PI3K), which regulates the translocation of glucose transporter 4 from intracellular pools to the plasma membrane, and stimulates the glucose uptake in muscles and adipocytes [17]. The phosphorylation of IRS-1 by upstream signals leads to disassociation of the bonding of the IRS-1 signal transduction to PI3K, resulting in insulin resistance [18]. mTOR/S6K1 is the most important signaling pathway for IRS-1 phosphorylation [19], and the AMP-activated protein kinase (AMPK) is the molecular factor that suppresses mTOR activation [20]. The purpose of the present study was to determine whether the AMPK/mTOR signal pathway contributes to the development of PIR and to further elucidate the mechanism of PIR attenuation by POC treatment.

Method

The study was carried out in accordance with the Helsinki Declaration and the guidelines published by the CONSORT group. Prior approval of the research project was granted by the Ethics Committees of the Second Military Medical University and Changzheng Hospital, Shanghai, China. Before participation, the patients received information about the study, including the details of the treatment procedure, after which they gave their written consent. Patients undergoing elective open colorectal cancer resection were eligible for inclusion and were randomly assigned to one of the following 3 groups: the POC group, the overnight fasting group, or the placebo group. The following exclusion criteria were applied: age younger than 20 or older than 75 years, diabetes mellitus or impaired glucose tolerance, intake of metformin and other medication affecting insulin sensitivity, symptoms of obstruction, renal insufficiency, hepatic insufficiency (Child-Pugh grade B or above), coexisting diseases that could affect the reliability of clinical assessments, and pregnancy, having received preoperative chemoradiotherapy. All the patients had regular food until 8 p.m. on the day before surgery, at which time they received oral bowel preparation with polyethylene glycol electrolyte solutions. The patients in the POC group consumed 400 mL Nutricia preOp solution (12.5% carbohydrate, 0.5 kcal/mL, 10 g polysaccharides, 2.1 g sugars, and <0.025 g lactose per 100 mL, 240 mOsm/kg, pH 4.9; lemon flavored; Nutricia, Zoetermeer, The Netherlands) 2 h before induction of anesthesia [12]. Patients in the placebo group received the same amount of noncarbonated, lemon-flavored water (pH 4.9), consumed as in the POC group. Patients in the fasting group were fasted overnight. No infusion was administered before the operations in all the 3 groups. All patients received general anesthesia. No patient received intraoperative allogeneic blood transfusion, and no glucose, amino acids, corticosteroids, exogenous insulin, or other medicine that could affect insulin sensitivity was infused during surgery.

Blood samples were taken 3 h before and at the end of the operation. Blood glucose and insulin levels were measured by using an automatic biochemistry analyzer (Hitachi 7600; Hitachi, Tokyo, Japan) and an automatic chemiluminescence immunoassay analyzer (IMMULITEBB1000; Siemens, Deerfield, IL, USA). Muscle samples were taken from the rectus abdominis muscle through the abdominal surgical wound just before the opening of the peritoneum and at the end of the operation. The samples were frozen in liquid nitrogen immediately for evaluation of the phosphorylation of AMPK, mTOR, S6K1, and IRS-1.

Insulin Resistance Assessment

Insulin resistance was calculated using homeostasis model assessment of insulin resistance (HOMA-IR) and by using the formula: HOMA-IR = (blood glucose [mmol/L] × blood insulin [μunits/mL])/22.5. A HOMA index value of 2.4 or more indicated insulin resistance. The index of insulin secretion was calculated as HOMA-β = {20 × (blood insulin [μunits/mL]/blood glucose [mmol/L]) − 3.5} and the insulin sensitivity index (ISI) as ISI = 1/{lg(blood glucose [mmol/L]) + lg(blood insulin [μunits/mL])} [21, 22].

Western Blot Analysis

Protein homogenates were run on an SDS polyacrylamide gel (4–15% gradient; Bio-Rad) and transferred onto a polyvinylidene fluoride membrane (Bio-Rad). The membranes were then stained with Ponceau S (1% in 5% acetic acid) to ensure even transfer and blocked in Tris-buffered saline (pH 7.5) containing 0.05% Tween-20 and 5% milk for 1 h at room temperature. Further, they were co-incubated overnight with primary antibodies against total and phosphorylated AMPK Thr172 (Cell Signaling Technology, Danvers, MA, USA), mTOR Ser2448 (Cell Signaling Technology, Danvers, MA, USA), S6K1 Thr389 (Cell Signaling Technology, Danvers, MA, USA), and IRS-1Ser636/639 (Cell Signaling Technology, Danvers, MA, USA). Next, the samples were incubated with a secondary antibody conjugated to horseradish peroxidase (GE Healthcare, Little Chalfont, UK) diluted 1:5,000 and transferred into an enhanced chemiluminescence solution. Finally, the

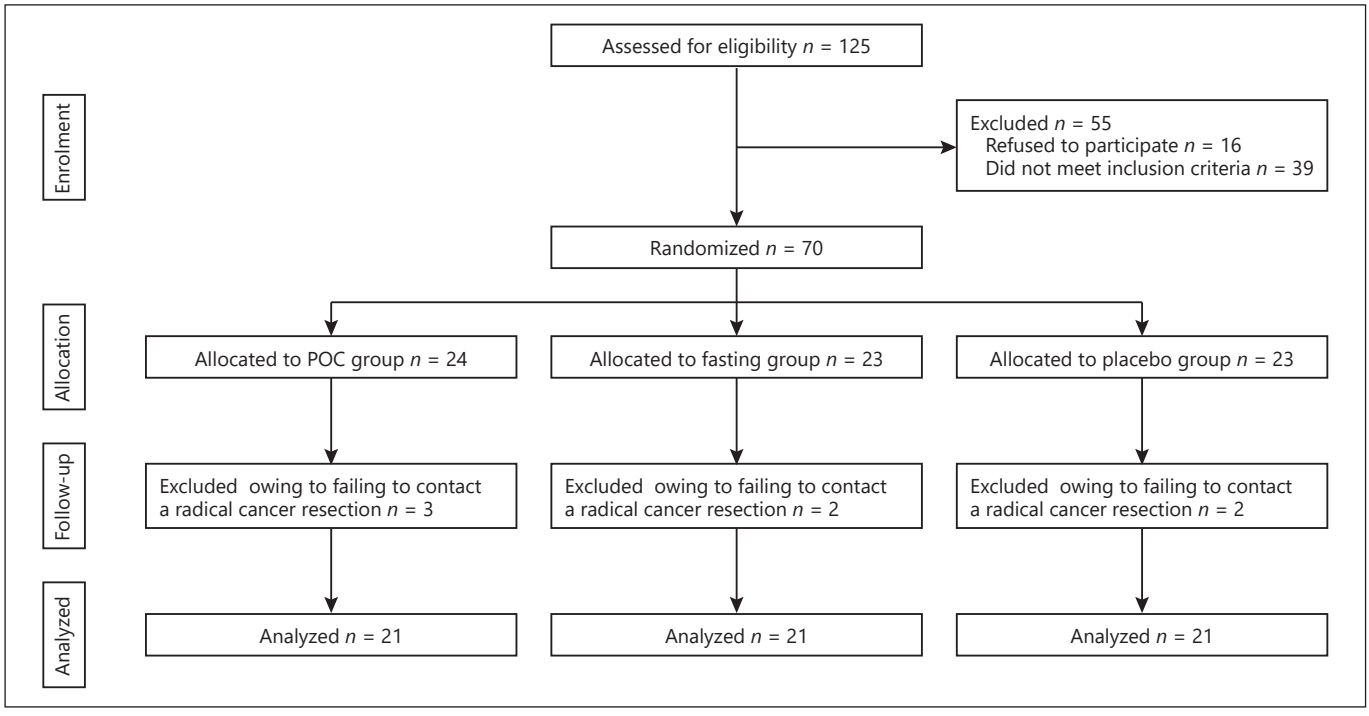

Fig. 1. CONSORT diagram for the trial. POC, preoperative oral carbohydrate.

signal intensities of the phosphorylated and total proteins were quantified and analyzed using Kodak image station 1000 and its accompanying software packages (Kodak, New Haven, CT, USA).

Statistical Analysis

To estimate the sample size, we calculated that a minimum of 16 patients would be required in each group to detect a significant difference in PIR ($\alpha = 0.05$, power = 80%), based on the findings of Soop et al. [14], which were similar to our research. Quantitative data are expressed as median (range), unless otherwise indicated. Comparisons between the 3 groups were performed by the Kruskal-Wallis test. The χ^2 test was employed for comparison of categorical variables. Comparisons within groups were made using the Wilcoxon signed-rank and Mann-Whitney *U* tests. $p < 0.05$ was considered statistically significant. Statistical analysis was performed by SPSS version 11.5 for Windows (SPSS Inc., Chicago, IL, USA).

Results

A total of 125 patients undergoing open colorectal surgery for colorectal cancer from January 2017 to December 2017 were eligible for inclusion in the study, and 70 patients were included for randomization. Of these 70 patients, 7 did not complete the study because distal metastasis was discovered during the operation, and thus, a radical resection for colorectal cancer could not be conducted. A total of 21 patients in each group remained for analysis (Fig. 1). The clinical features of these 63 patients included in the 3 groups are listed in Table 1. No significant differences in sex, age, body mass index, American Society of Anesthesiologists scores, operation procedure, duration of operation, intraoperative blood loss, postoperative complications, and pathological tumor node metastasis stage were found among the 3 groups. The preoperative carbohydrates were well tolerated, and no apparent or suspected pulmonary aspiration or other complications related to other oral intake were observed during or after the operation.

As can be seen in Figure 2, at the end of the operation, the levels of blood glucose, blood insulin, and HOMA-IR significantly increased, whereas ISI decreased in all the 3 groups in comparison with their initial values 2 h before the operation ($p < 0.05$). We established no significant difference in the levels of blood glucose, blood insulin, HOMA-IR, ISI, and HOMA-β among the 3 groups 2 h before the operation ($p > 0.05$). At the end of the operation, the levels of blood glucose, blood insulin, and HOMA-IR were significantly lower in the POC group than the fasting and placebo groups, and ISI was significantly higher in the POC group than the fasting and pla-

Table 1. Demographic and clinical data

Factor	POC group (n = 21)	Fasting group (n = 21)	Placebo group (n = 30) (n = 21)
Sex			
Male	11	11	11
Female	10	10	10
Age, years	55 (36–74)	56 (40–72)	55 (42–72)
Body mass index, kg/m^2	23 (20–25)	22 (19–24)	23 (18–26)
American Society of Anesthesiologists score			
I	3	4	3
II	18	17	18
III	0	0	0
Operation procedure			
Right hemicolectomy	7	8	8
Left hemicolectomy	2	1	2
Sigmoid colectomy	3	2	2
Anterior resection	9	10	9
Duration of operation, min	95 (65–150)	100 (75–160)	98 (70–155)
Intraoperative blood loss, mL	110 (100–150)	150 (100–200)	125 (110–180)
Pathological tumor node metastasis stage			
I + II	4	3	4
III	17	18	17
IV	0	0	0

Values are expressed as median (range) or a number. There was no statistical difference among the 3 groups. POC, preoperative oral carbohydrate.

cebo groups ($p < 0.05$). There were no differences in HOMA-β among the 3 groups before and after the operation.

As illustrated in Figure 3, the phosphorylation of mTOR Ser244, S6K1 Thr389, and IRS-1Ser636/639 increased significantly, whereas the phosphorylation of AMPK significantly decreased after the operation in all 3 groups. There were no significant preoperative differences in the phosphorylated mTOR, S6K1, IRS-1, and AMPK among the 3 groups. After the operation, the levels of the phosphorylated mTOR, S6K1, and IRS-1 were significantly lower in the POC group than the fasting and placebo groups; the level of phosphorylated AMPK was significantly higher in the POC group than the other 2 groups.

Discussion

The findings of the present study showed that immediately at the end of colorectal resection, PIR and the mTOR/S6K1/IRS-1 pathway were activated, but the phosphorylation of AMPK was decreased. In the POC group, the phosphorylation of AMPK was elevated, whereas the phosphorylation of mTOR/S6K1/IRS-1 was reduced. These data showed that POC activated AMPK, which suppressed the mTOR/S6K1/IRS-1 pathway and attenuated PIR after colorectal resection.

PIR is a compensatory response of the body to traumatic stress and the central link of the metabolic reaction induced by surgical trauma. It can guarantee the stability of circulation, enabling the brain cells and the red blood cells to obtain sufficient glucose for their metabolism. However, hyperglycemia induced by insulin resistance damages important organs, such as the brain, heart, kidney, liver, and pancreas, increasing the incidence of postoperative complications (infection, thrombosis, etc.). Insulin resistance has been shown to be an independent predictor of the length of hospital stay after elective surgery [6, 7]. Avoiding preoperative fasting by using POCs has been suggested as a measure to prevent and reduce the extent to which such derangements occur [12]. In 2 clinical trials, POC application reduced insulin resistance by 47 and 57% in patients undergoing colorectal [13] and hip replacement surgery [14], respectively. Although the homoeostatic model (HOMA-IR) is not the "gold standard" for quantifying insulin resistance as compared to clamp [23], we did confirm a significantly reduced PIR in

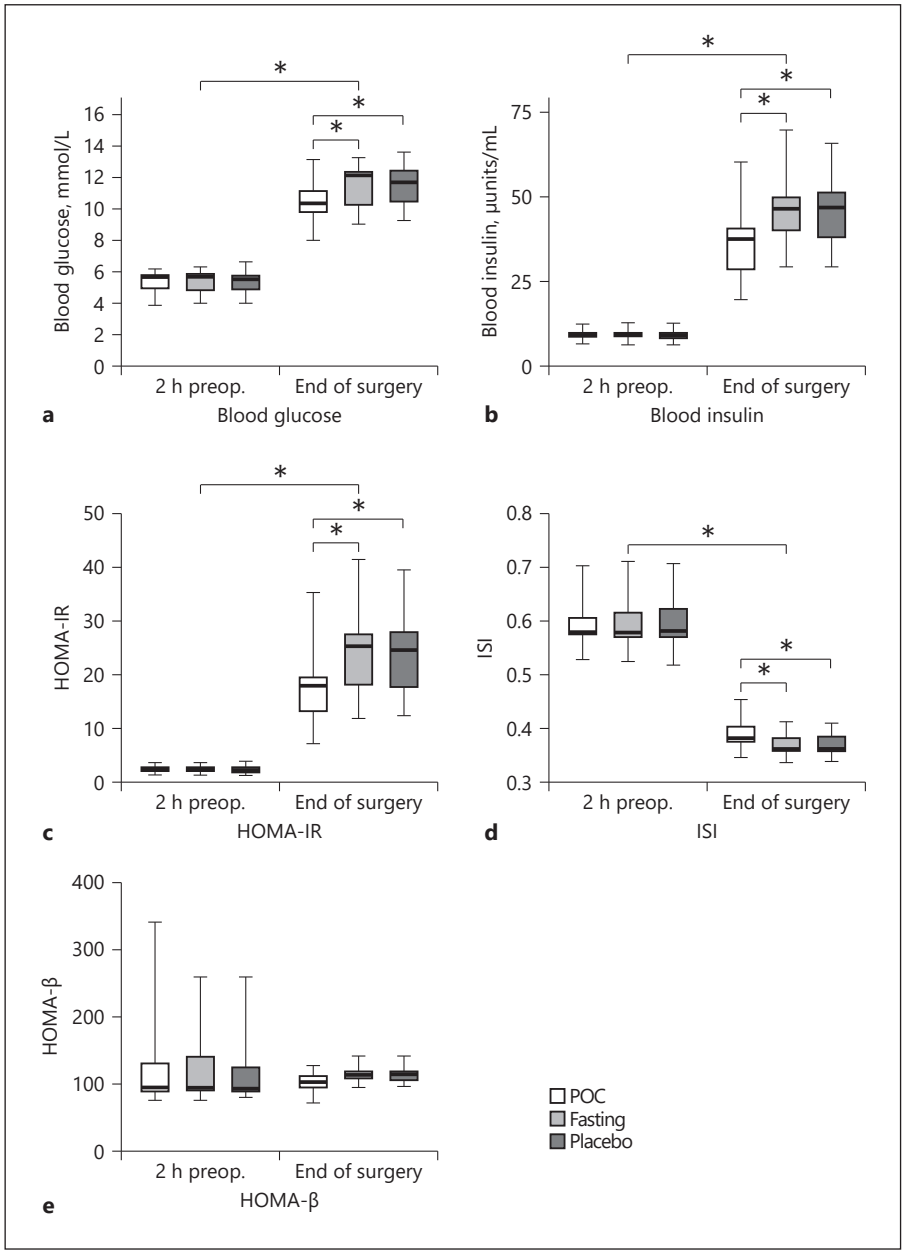

Fig. 2. PIR 4 h before and immediately after colorectal cancer resection. **a** Blood glucose. **b** Blood insulin. **c** HOMA-IR. **d** ISI. **e** HOMA-β. POC, preoperative oral carbohydrate. Values are median, interquartile interval, and range. *$p < 0.05$.

the POC group compared with that in the fasting group. Therefore, POC treatment effectively and safely reduces PIR in colorectal resection for cancer.

The precise mechanism by which POC attenuates PIR still remains unclear. Surgical stress is known to activate inflammation pathways in the skeletal muscle and adipose tissue [24] and attenuate insulin-stimulated glycogen synthase activity [25]. IRS-1 is a signaling protein in the insulin signaling pathway, which binds the insulin receptor to PI3K. After binding with insulin, the insulin receptor recruits IRS-1, and the latter induces autologous phosphorylation of tyrosine, which in turn phosphorylates PI3K [26]. However, the serine phosphorylation of IRS-1 impairs the autologous phosphorylation of tyrosine, which disassociates IRS-1 signal transduction to PI3K and impairs insulin signal transduction. It has been observed that skeletal muscle and liver cells had elevated IRS-1 serine phosphorylation and impaired insulin signaling through the PI3K pathway in obese rats [27]. Increased serine phosphorylation of IRS-1 was observed in several types of

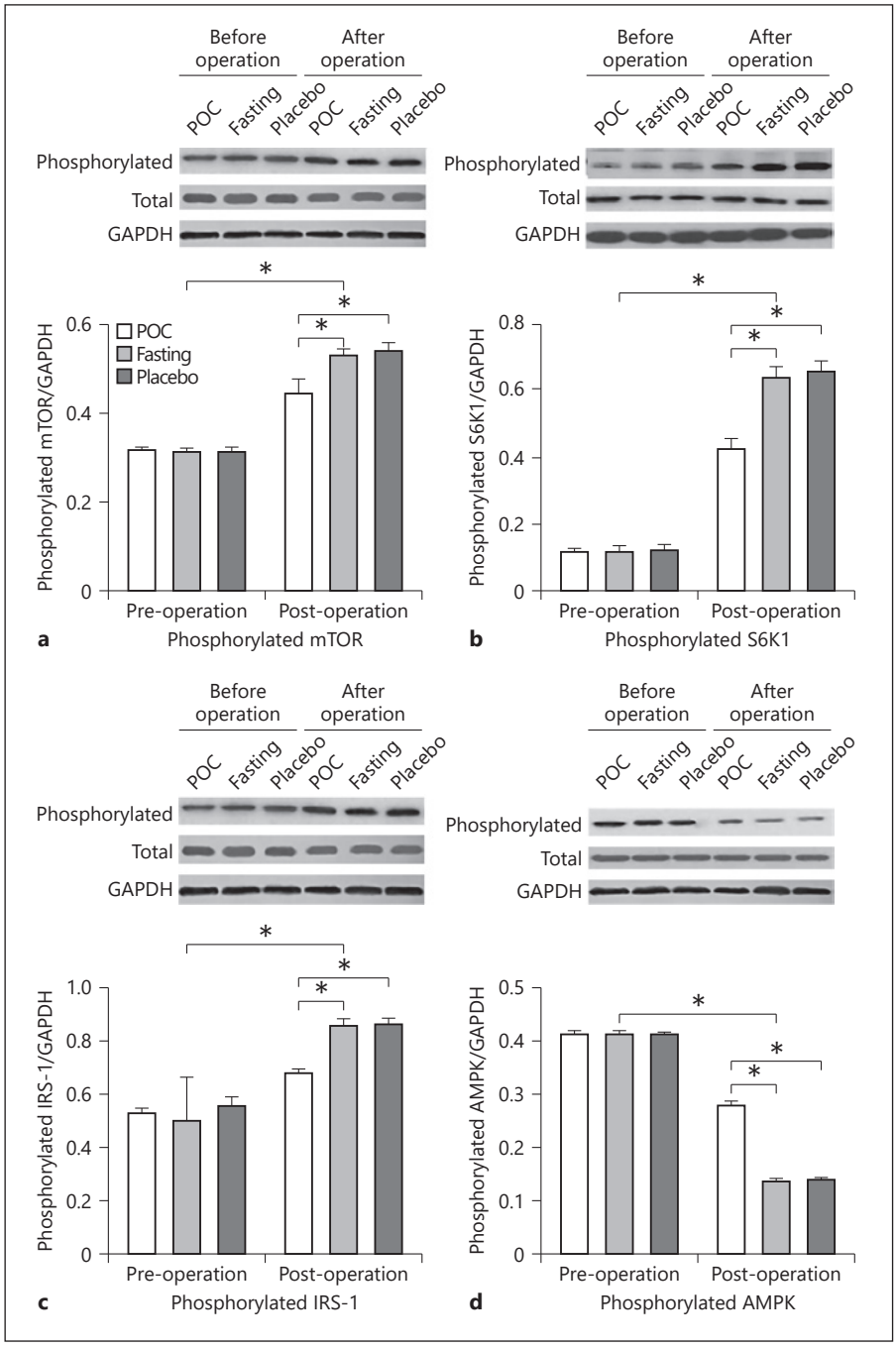

Fig. 3. Western blot for mTOR (**a**), S6K1 (**b**), IRS-1Ser (**c**), and AMPK (**d**) immediately before and after colorectal cancer resection. POC, preoperative oral carbohydrate. Values are median, interquartile interval, and range. *$p < 0.05$.

insulin resistance [18]. Here, we discovered that the increased serine phosphorylation of IRS-1 is accompanied by hyperglycemia, high concentration of insulin, and increased insulin resistance index after colorectal resection. These findings demonstrate that serine phosphorylation of IRS-1 attenuates the signal transduction from the insulin receptor to PI3K, which resulted in PIR.

An increase in the serine phosphorylation of IRS-1 can be observed in some metabolites, including free fatty acids, diacylglycerols, and fatty acyl-CoAs [28]. mTOR/S6K1 is the most important upstream signal system of IRS-1. The mTOR/S6K1 pathway regulates the serine phosphorylation of IRS-1, and previous studies have showed that the increased phosphorylation of mTOR and

S6K1 caused subcellular redistribution of IRS-1 and inactivation of IRS-1 by increasing its serine phosphorylation, leading to insulin resistance [27, 29, 30]. It has been reported by Bae et al. [31] that exercise and dietary change stimulate the mTOR signaling pathway and ameliorate insulin resistance. Here, we found an increased phosphorylation of the mTOR/S6K1 pathway in the muscle cells after colorectal resection, while POC suppressed the activation of the mTOR/S6K1 pathway, and PIR was reduced.

AMPK belongs to a highly conserved eukaryotic protein family and is involved in cellular energy homeostasis. AMPK activity is regulated by the AMP:ATP ratio, intracellular calcium level [32], exercise (muscle contraction) [33], metformin [34], and inflammation factors such as TNF-α [35]. The phosphorylation of AMPK inhibits mTOR activation by phosphorylating TSC2 and raptor and suppressing the mTOR/S6K1 pathway. Our Western blot analysis results showed that the AMPK phosphorylation was decreased after surgery. Compared with the fasting and placebo groups, the phosphorylation of AMPK was significantly higher in the POC group. These findings show that POC reduced PIR by activating AMPK. The latter suppressed the mTOR/S6K1 pathway, which in turn reduced the serine phosphorylation of IRS-1 and promoted the insulin signal transduction from the insulin receptor to PI3K.

In summary, the present study shows that PIR after colorectal resection involves the mTOR/S6K1/IRS-1 pathway. POC intake reduces the PIR by the stimulation of AMPK, which suppresses mTOR/S6K1/IRS-1 and facilitates insulin signal transduction from the insulin receptor to PI3K. Further studies are needed to investigate the regulation mechanism of the AMPK/mTOR pathway during PIR. More clinical treatment could focus on the AMPK/mTOR pathway in order to reduce PIR after colorectal surgery.

Statement of Ethics

The patients gave their informed consent, but our university is a military medical university, and some of the patients included in our clinical research were soldiers and veteran. For this reason, our study was not registered.

Disclosure Statement

The authors declare no conflict of interests related to this work.

Funding Sources

This research was funded by the National Natural Science Foundation of China (No. 81100629).

Author Contributions

Zhenxin Zhu and Qingping Cai conceived and designed the study. Mengyao Shi and Zunqi Hu performed the experiments. Mengyao Shi and Zunqi Hu wrote the paper. Mengyao Shi, Zunqi Hu, Dejun Yang, and Zhenxin Zhu reviewed and edited the manuscript. All authors read and approved the manuscript.

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