MEDICAL SCIENCE MONITOR

ANIMAL STUDY

e-ISSN 1643-3750
© Med Sci Monit, 2015; 21: 9-17
DOI: 10.12659/MSM.891063

Received: 2014.05.19
Accepted: 2014.07.04
Published: 2015.01.01

Preoperative Oral Carbohydrate Improved Postoperative Insulin Resistance in Rats through the PI3K/AKT/mTOR Pathway

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

ABCDEFG 1 Zhiguo Wang
ABCDEFG 2 Yiqing Liu
ABCDEF 1 Qi Li
ABCDEF 1 Canping Ruan
ABCDEG 3 Bin Wu
ABCEFG 4 Qiang Wang
ABCEFG 1 Zhiqian Hu
ABCDFG 5 Huanlong Qin

1 Department of General Surgery, Shanghai Chang Zheng Hospital, Second Military Medical University, Shanghai, China
2 Library of Second Military Medical University, Shanghai, China
3 Department of Thoracic Surgery, Shanghai Chang Zheng Hospital, Second Military Medical University, Shanghai, China
4 Department of General Surgery, Shanghai Zhabei Central Hospital, Shanghai, China
5 Department of General Surgery, Tenth People's Hospital of Tongji University, Shanghai, China

Corresponding Authors: Zhiqian Hu, e-mail: huzq62@163.com; Qiang Wang, e-mail: wang2929@hotmail.com
Source of support: The study was funded by the National Natural Science Foundation of China (Grant number. 81000845)

Background: Preoperative oral carbohydrate (OCH) improves postoperative insulin resistance (PIR) and insulin sensitivity. However, the exact mechanisms involved in the improvement of PIR with respect to preoperative OCH are still not clear. The aim of this study was to investigate the involvement of preoperative OCH and PI3K/AKT/mTOR pathway in reducing PIR in rats.

Material/Methods: Forty male Sprague-Dawley rats were randomly assigned to PreOp, glucose, saline, and fasting groups. Rats in the PreOp, glucose, and saline groups received OCH, 5% glucose solution, and saline, respectively. Rats in the fasting group did not receive anything but were fasted 3 h before surgery. Blood glucose, insulin and leucine levels, and insulin resistance, secretion, and sensitivity indexes were measured before and after surgery. mRNA and protein (total and phosphorylated) levels of mTOR, IRS-1, PI3K, PKB/AKT, and GlUT4 were measured using real-time polymerase chain reaction and Western blot in skeletal muscles.

Results: In the PIR experiment, blood glucose, serum insulin, insulin resistance, and serum leucine levels were all significantly lower in the PreOp group than in the other 3 groups ($P<0.05$) after surgery. HOMA-ISI were higher in the PreOp group vs the other 3 groups after surgery ($P<0.05$), and HOMA-β in the PreOp group was higher than that in the other 3 groups at 30 and 120 min after surgery. Additionally, post-operative phosphorylated IRS-1, PI3K, and AKT protein levels were significantly higher in the PreOp group than in the other 3 groups ($P<0.05$), but no significant differences were observed in their respective protein levels ($P>0.05$).

Conclusions: OCH decreases postoperative insulin resistance and improves postoperative insulin sensitivity in skeletal muscles through the PI3K/AKT/mTOR pathway.

MeSH Keywords: Leucine • Insulin Resistance • Perioperative Care • Phosphoinositide Phospholipase C • TOR Serine-Threonine Kinases

Full-text PDF: http://www.medscimonit.com/abstract/index/idArt/891063

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

Background

Postoperative insulin resistance (PIR) reduces insulin-stimulated glucose uptake in skeletal muscles and adipose tissue, reducing glucose uptake and leading to hyperglycemia [1,2]. Preoperative oral carbohydrate (OCH) can improve post-surgical insulin sensitivity [2,3], as well as post-operative glycemic control, attenuate early post-operative metabolic response, improve insulin resistance (IR), and reduce recovery time [4]. Preoperative OCH intake was found to be safe in patients with colorectal cancer receiving elective open resection [5]. Intake of 400 ml (50 g) of carbohydrates stimulates a release of insulin similar to that seen after a mixed meal [6]. Treatment with this carbohydrate-rich beverage preoperatively attenuates PIR development by 50% measured on the first post-operative day after major abdominal surgery [7].

Peripheral tissues, mostly skeletal muscles, are the major PIR sites, and impaired insulin signaling is the major mechanism for PIR [8]. The insulin/insulin-like growth factor pathway regulates energy homeostasis in animals [9]. The PI3K/AKT signaling pathway plays an important role in the development of PIR [10]. The insulin signaling pathway mainly activates the phosphorylation of kinases of the PI3K/Akt/mTOR pathway in order to improve glucose metabolism [11–13]. However, the exact mechanisms involved in the improvement of PIR with respect to preoperative OCH are still unclear.

Leucine is the most likely activator of mTOR [14]. In skeletal muscles, leucine can stimulate mTOR signaling and increase phosphorylation of eukaryotic initiation factor 4E-binding protein 1 and 70-kDa ribosomal protein S6 kinase 1 [15]. In addition, circulating leucine and isoleucine are both post-operatively decreased when intravenous glucose was administered during upper abdominal surgery. This could stimulate insulin secretion and result in accumulation of substrates such as pyruvate and alanine [16]. However, the effect of circulating leucine on glucose metabolism is unclear.

Therefore, the present study hypothesized that preoperative OCH could decrease circulating leucine and improve PIR by the PI3K/AKT/mTOR pathway. The aim of the present study was to investigate the involvement of preoperative OCH and PI3K/AKT/mTOR pathway in reducing PIR in a rat model of small bowel resection (SBR). The study results may provide further understanding of the molecular mechanisms involved in the improvement of PIR by preoperative OCH.

Material and Methods

Animals and ethics

Forty male Sprague-Dawley rats weighing 150–200 g were purchased from Shanghai SIPPR/BK Laboratory Animals Ltd., Shanghai, China [Certificate number: 2008001618428. Production license: SCXK (Shanghai) 2008-0016]. The rats were acclimatized for a week prior to the experiments, with free access to water and food. The animals were kept under a physiological day/night cycle with an ambient temperature of 23±1°C, humidity of 50–70%, and noise restriction of <50 dB. The experimental protocol was approved by the Animal Ethics Committee of the Shanghai Chang Zheng Hospital.

Preoperative treatment

Forty rats were fasted for 14 h before surgery (from 7: 00 pm the previous night to 9: 00 am on the day of surgery). Rats were randomly assigned to 4 groups (n=10/group): rats in the PreOp, glucose, and saline groups received OCH (PreOp solution, Nutricia, Holland), 5% glucose solution (Baxter International Inc., Shanghai, China), and saline (Baxter International Inc., Shanghai, China), respectively. Rats in the fasting group did not receive anything, but were fasted 3 h before surgery. The PreOp solution contained 0.2 g/mL glucose, 1.3 g/mL sucrose, 0.7 g/mL maltose, 10 g/mL glycan, 0.122 g/mL potassium, 0.006 g/mL calcium, 0.006 g/mL chloride, 0.001 g/mL phosphorus, and 0.001 g/mL magnesium.

PIR surgery model in rats

Three hours after the above treatments, rats underwent anesthesia using 10% chloral hydrate (Shanghai Reagent Co., Ltd, China) 500 µl/100 g by intraperitoneal injection. Sedated rats were fixed in the dorsal position, abdominal fur was removed, and the abdominal area was disinfected [1]. A 5-cm midline abdominal incision was made, the small intestine was exposed, and 2–3 mesenteric vessels were disconnected at 5 cm from the ligament of Treitz. A 5-cm length of small intestine was removed. Small intestine was anastomosed, followed by abdomen closure using 6/0 Prolene sutures. Average surgery time was 20 min. After surgery, rats were kept in individual cages and under a heat lamp to maintain body temperature.

Blood and tissue collection

In the PIR experiment, blood samples (300 µL) were collected 4 h before the surgery, and 30, 60, 90, and 120 min after surgery. Blood samples were centrifuged at 4°C at 2000 rpm for 10 min, and serum was collected and stored at –20°C. Rats were killed 120 min after the last blood sampling.

Abdominal muscle tissue was removed after the experiment and quickly put into cold saline. Gastrocnemius tissue was washed 3 times in cold saline to remove the blood, and was stored in liquid nitrogen for measurements of mTOR, PI3K, AKT, and/or GLUT4 mRNA and protein levels.

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

Blood glucose and insulin assay

Blood glucose levels were determined using the Wako LabAssay™ Glucose kit (Wako Pure Chemical Industries, Ltd., Japan), according to the manufacturer's protocol. Serum insulin levels were measured using the BlueGene Rat Insulin enzyme-linked immunosorbent assay kit (Shanghai BlueGene Biotech CO., Ltd., China), according to the manufacturer's protocol.

Homeostatic model assessment (HOMA) of PIR

The following formulae were used to evaluate PIR in each animal through HOMA [17–19]:

1. HOMA-IR (insulin-resistant) = (blood glucose level × serum insulin level)/22.5
2. HOMA-β = (serum insulin level ×20)/(blood glucose level – 3.5)
3. HOMA-ISI (insulin sensitivity index) = 1/[log(blood glucose level) + log(serum insulin level)]

Serum leucine evaluation by high-performance liquid chromatography (HPLC)

Serum leucine levels were measured using HPLC (Perkin Elmer, USA). To prepare the sample solution, 200 µL of amino acid standards and serum samples were added into individual 1.5-mL microcentrifuge tubes. Fifty µL of norleucine (1 g/L diluted in 10 mol/L; Sangon Biotech, China) was added to each tube as internal standard. Then, 100 µL of acetonitrile triethylamine solution (triethylamine: acetonitrile (v/v)=500: 8.6, Sangon Biotech, China) and 100 µL of phenyl isothiocyanate acetonitrile solution (isothiocyanate: acetonitrile (v/v)=250: 1, Sangon Biotech, China) were added to each tube, mixed well, and kept aside for 1 h at room temperature. Following that, 400 µL of hexane was added into each tube, mixed well, and kept aside for 10 min at room temperature to form 2 phases. The lower phase, which contained amino acids, was filtered and transferred into a fresh tube using a 0.45-µm syringe filter. Then, 200 µL of phenylthiocarbamoyl amino acid was diluted in 800 µL of sterile water as sample solution. A Venusil AA chromatographic column was used (4.6×250 mm and 5 µm; Agela Technologies, China), and column temperature was kept at 40°C. Ten µL of each sample solution was used for the measurement of amino acid concentration, and the wavelength was 254 nm.

RNA isolation and real-time polymerase chain reaction (PCR)

Frozen abdominal muscle (100 µm) was sectioned on a cryostat. RNA was extracted from muscle sections using Trizol (Life Technologies, USA), according to the manufacturer's instructions. cDNA was synthesized from 1 µg of total RNA using the Tiangen Quantscript Reverse Transcriptase kit (KR103, Tiangen Biotech, China). Quantitative real-time PCR was performed on a Real-Time PCR System (Bio-Rad, USA) using TIANGEN SuperReal PreMix (SYBR Green, FP204, Tiangen Biotech, China).β-actin served as an internal control. All PCR reactions were performed in triplicate. Primer sequences and annealing temperature of mTOR, insulin receptor substrate 1 (IRS-1), PI3K, PKB, and GLUT4 are presented in Table 1. Quantitative real-time PCR analysis was carried out using the $2^{-\Delta\Delta Ct}$ method.

Table 1. Primer sequences.

Gene	Primer sequence	Annealing temperature (°C)
β-actin	F 5'CCTGTACGCCAACACAGTGC3'	58
	R 5'ATACTCCTGCTTGCTGATCC3'	
mTOR	F 5'GACGGTGTAGAACTTGGAGAA3'	58
	R 5'TGAGATGTCGCTTGCTTGA3'	
IRS-1	F 5'TGTGCCAAGCAACAAGAAAG3'	58
	R 5'ACGGTTTCAGAGCAGAGGAA3'	
Akt	F 5'GTCCTATTGTCGTGCATGTGG3'	58
	R 5'CTCTTCAAGCCTGAGGTTTCC3'	
PI3K	F 5'AGGCTGTGATTGGGCGTA3'	58
	R 5'AAGCAACCTCAAAGGGAAA3'	
GluT4	F 5'GCCAAGAAGGAGCCCTAACTT3'	58
	R 5'TGTCTCAGCCCATCCTCAAGTA3'	

Western blotting

Frozen abdominal muscle samples were homogenized in cold cell lysis buffer (Sigma-Aldrich, USA) supplemented with a protease and phosphatase inhibitor cocktail and phenylmethanesulfonylfluoride (PMSF). Crude extract was cleared from debris by centrifugation (800 g, 10 min, and 4°C), and supernatant was separated by centrifugation (14000 g, 15 min, 4°C). Lysate was collected, and protein concentration was determined by bicinchoninic acid assay (Sigma, USA). From each sample, 50 µg was denatured in 4× Laemmli buffer [250 mM Tris-hydrochloride pH 6.8, 40% glycerol, 8% sodium dodecyl sulfate (SDS), 0.01% bromphenol blue, and 20% β-mercaptoethanol], run on 10% SDS-polyacrylamide gel, and transferred onto nitrocellulose membrane (Amersham, USA). Membranes were blocked with 5% milk in Tris Buffered Saline with Tween 20 (TBS-T) and subsequently incubated with primary antibody in TBS-T supplemented with 5% blocking milk in 1: 1000 dilution of rabbit anti-mTOR (#2972S, Cell Signaling, USA) and anti-p-mTOR (Ser2448) (#2971P, Cell Signaling, USA), anti-IRS-1(59G8) (#2390, Cell Signaling, USA) and anti-p-IRS-1 (Ser302) (#2384, Cell Signaling,

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

USA), anti-PI3Kp85 (#4292, Cell Signaling, USA) and anti-p-PI3K p85 (Tyr458) (#4228, Cell Signaling, USA), anti-AKT (4685S, Cell Signaling, USA) and anti-p-AKT(Thr308) (#2965P, Cell Signaling, USA), and anti-GluT4 (#2213S, Cell Signaling, USA) and anti-β-actin (sc-47778, Santa Cruz, USA). An IRDye 800CW (926-32210) (LI-COR, USA) was used as a secondary antibody. Proteins were visualized with 800 nM wave length on an Odyssey Luminescent Image Analysis system (LI-COR, USA).

Statistical analysis

Data were analyzed using SPSS 11.5 for windows (SPSS Inc, Chicago, Illinois, USA). Continuous data are presented as means ± standard deviation. Analysis of variance and Kruskal-Wallis were used to compare the groups. Pairwise comparisons were conducted using the least significant difference t-test or Dunnett T3 test. Chi-square test was used to analyze correlation in the dataset. P-values <0.05 were considered statistically significant.

Results

Animal weight

Before surgery, the weight of the animals in the 4 treatment groups were 180.72±15.09 g (PreOp group), 181.24±14.79 g (glucose group), 179.87±15.39 g (saline group), and 180.37±14.39 g (fasting group) (F=0.094, P=0.761).

Changes in blood glucose and serum insulin levels

There was no difference in preoperative blood glucose levels among the 4 treatment groups (P=0.968). However, blood glucose levels were increased at 30 min post-surgery and continued to increase thereafter. At 90–120 min after surgery, blood glucose levels were different among the 4 treatment groups (P<0.05). Blood glucose levels of the PreOp group were significantly lower than that of the fasting group at 30 to 120 min after surgery; the corresponding values were lower in the PreOp group than in the glucose and saline groups at 90 to 120 min after surgery, respectively, and higher in the fasting group than in the glucose and saline groups at 120 min after surgery (Figure 1A).

There was no difference in the preoperative serum insulin levels among the 4 treatment groups (P=0.778). After surgery, blood insulin levels showed the same trend as for blood glucose. Serum insulin levels of the PreOp group were significantly lower than that of the fasting group at 30, 90, and 120 min (P<0.05); the corresponding values were significantly lower in the PreOp group than in the glucose and saline groups at 60 and 120 min after surgery, respectively (Figure 1B).

HOMA-IR, HOMA-β, and HOMA-ISI

Before surgery, there was no difference in HOMA-IR among the 4 treatment groups (P=0.990). After surgery, HOMA-IR showed a rapid increase in the 4 treatment groups at 30 min. HOMA-IR of the PreOp group became significantly lower than the values of the other 3 groups at 60 min post-surgery (P<0.05), which suggested that the PreOp treatment reduced postoperative HOMA-IR (Figure 2A). On the other hand, HOMA-β of animals underwent a rapid decrease postoperatively, and HOMA-ISI were higher in the PreOp group *vs.* the other 3 groups after surgery (P<0.05), while HOMA-β in the PreOp group was higher than that in the other 3 groups at 30 and 120 min after surgery (P<0.05 and P<0.01, respectively, Figure 2B, 2C).

Serum leucine levels

HPLC results showed that there was no difference in preoperative serum leucine levels among the 4 treatment group: 0.34±0.05 μmol/L (PreOp group), 0.35±0.04 μmol/L (glucose group), 0.35±0.08 μmol/L (saline group), and 0.35±0.05 μmol/L (fasting group) (P>0.05, Figure 3A). Significant differences were observed in serum leucine levels between the PreOp group and the other 3 groups at 2 h post-surgery (F=13.886, P<0.001, Figure 3B).

mRNA expression of PI3K, AKT, mTOR, IRS-1, and GluT4 in muscles by RT-PCR

Results showed that the mRNA expression levels of PI3K, AKT, and IRS-1 in muscles of the PreOp group were significantly higher than those of the other 3 groups (all P<0.05), whereas mTOR expression was downregulated in the PreOp group compared with the other 3 groups (*P*<0.05). There was no difference in GLUT4 expression in rat muscles among the 4 groups (Figure 4).

Protein expression and phosphorylation of PI3K, AKT, mTOR, and IRS-1 in muscles by Western blot

There was no change in total PI3K, AKT, mTOR, and IRS-1 protein expression (all *P*<0.05). However, upregulation of phosphorylated-(p-) PI3K, p-AKT, p-IRS, and downregulation of p-mTOR were demonstrated in the PreOp group compared with that in the other groups (all *P*<0.05) (Figure 5). These results indicate that the PI3K/AKT/mTOR pathway might be involved in the postoperative development of IR.

Discussion

Following trauma, PIR frequently occurs as a reaction of the body to traumatic stress and manifestation of any impairment

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

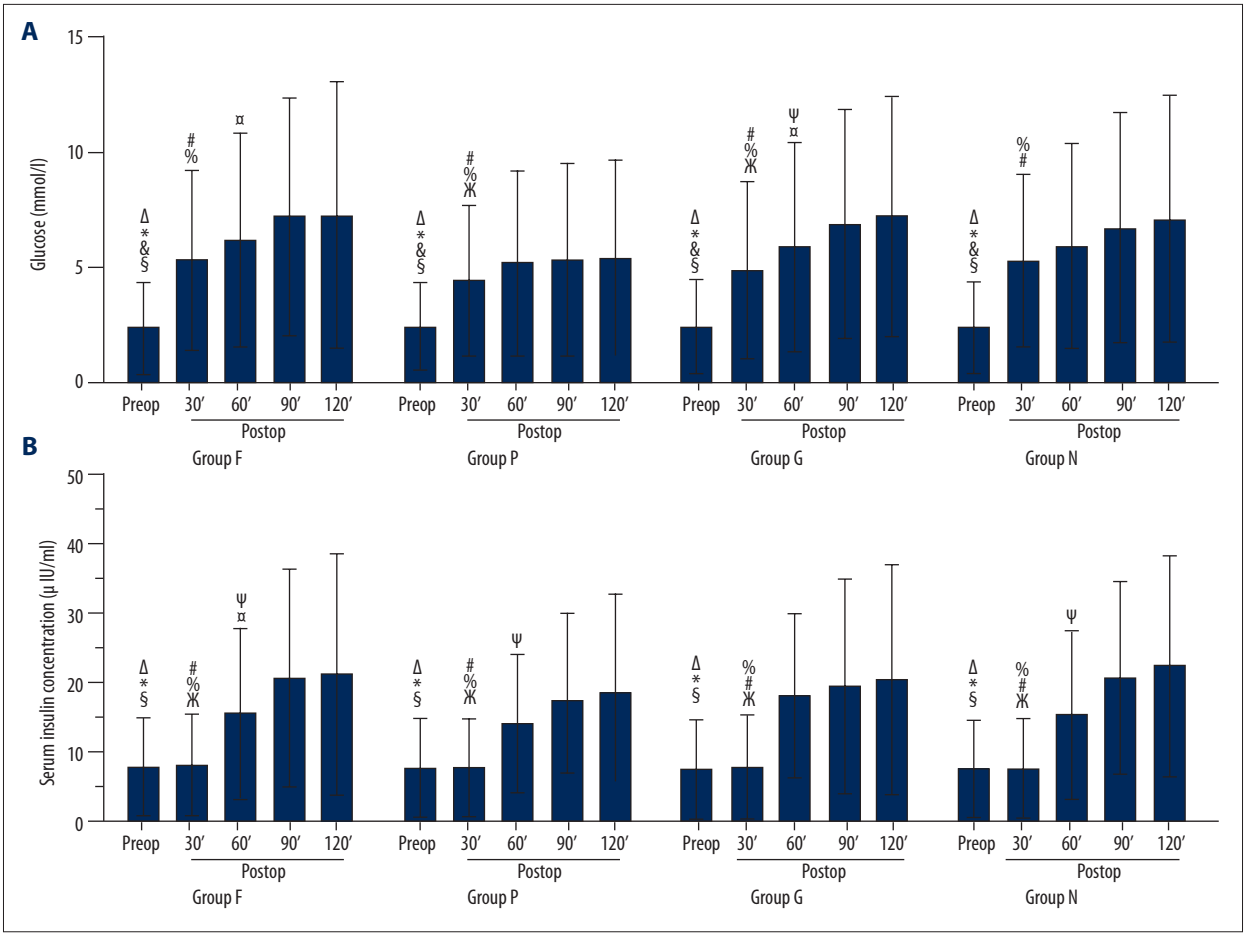

Figure 1. (**A**) Blood glucose levels in the PreOp (Group P), 5% glucose (Group G), saline (Group S), and fasting groups (Group F) before surgery, and 30, 60, 90, and 120 min after surgery in rats. * Preoperative *vs.* 30 min.; § Preoperative *vs.* 60 min; & Preoperative *vs.* 90 min; Δ Preoperative *vs.* 120 min; ж 30 min *vs.* 60 min; % 30 min *vs.* 90 min; # 30 min *vs.* 120 min; ¤ 60 min *vs.* 90 min; Ψ 60 min *vs.* 120 min. (**B**) Serum insulin levels in Groups P, G, S, and F before surgery, and 30, 60, 90, and 120 min after surgery in rats. * Preoperative *vs.* 30 min.; § Preoperative *vs.* 60 min; & Preoperative *vs.* 90 min; Δ Preoperative *vs.* 120 min; ж 30 min *vs.* 60 min; % 30 min *vs.* 90 min; # 30 min *vs.* 120 min; ¤ 60 min *vs.* 90 min; Ψ 60 min *vs.* 120 min.

of the body's reaction to physiological functions of insulin. In the present study, the established rat model exhibited significant PIR after SBR surgery. The PreOp OCH treatment attenuated the postoperative blood glucose and insulin levels, improved insulin secretion function of pancreas islets, and increased insulin sensitivity of peripheral tissues, thus contributing to the reduction of PIR. Despite a lack of substantial change in protein expression of the PI3K/AKT/mTOR signal pathway, the associated protein phosphorylation level underwent significant changes, suggesting activation of the PI3K/AKT/mTOR pathway. Additionally, the activation of IRS-1 phosphorylation was also observed. These results suggest that the OCH treatment may affect PIR via regulation of the PI3K/AKT/mTOR pathway.

Compared with the PreOp OCH treatment, preoperative oral administration of glucose solution or normal saline induced no significant improvement in PIR, possibly because OCH contained polysaccharides and disaccharides as well as fructose and electrolytes, which were able to promoting gastric emptying. Previous studies in healthy volunteers showed that oral administration of monosaccharide solution (e.g., 5% glucose) led to extension of gastric emptying time [20,21]; when the monosaccharide was replaced with polysaccharides to reduce the osmotic pressure, the gastric emptying time of OCH was significantly shortened [22–24]. Therefore, preoperative oral administration of sugar-rich solution generally uses polysaccharides (dextrin-maltose mixture), which leads to substantial reduction of the osmotic pressure compared with an equal amount of pure glucose or other monosaccharides, thereby accelerating the rate of gastric emptying.

Under normal circumstances, insulin from pancreatic β cells approaches the target organ via a short cycle, then accelerating glucose metabolism in target cells. IR is defined as a

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

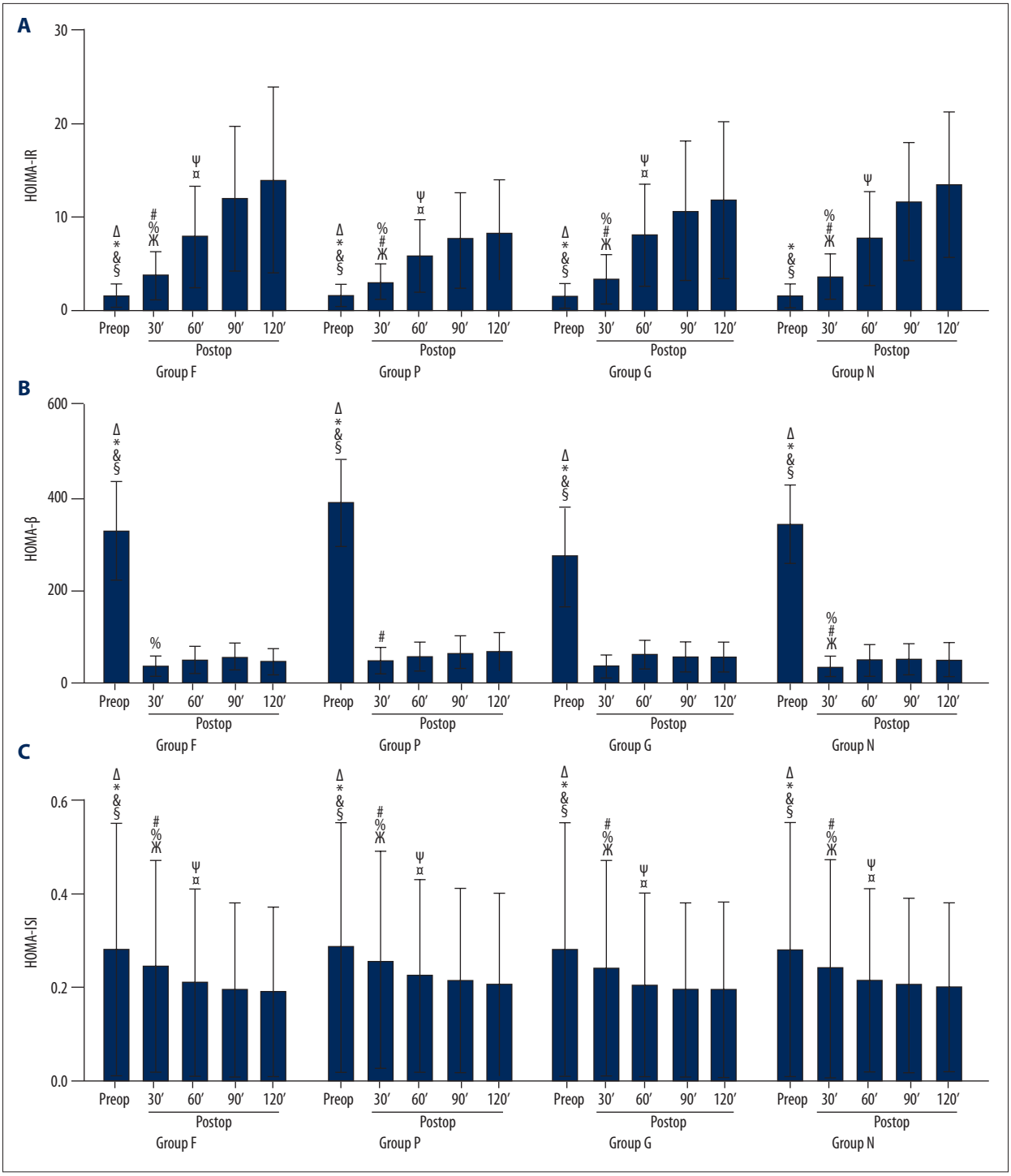

Figure 2. (**A**) HOMA-IR in Groups P, G, S, and F before surgery, and 30, 60, 90, and 120 min after surgery in rats. * Preoperative *vs.* 30 min.; § Preoperative *vs.* 60 min; & Preoperative *vs.* 90 min; Δ Preoperative *vs.* 120 min; ж 30 min *vs.* 60 min; % 30 min *vs.* 90 min; # 30 min *vs.* 120 min; ¤ 60 min *vs.* 90 min; Ψ 60 min *vs.* 120 min. (**B**) HOMA-β in Groups P, G, S, and F before surgery, and 30, 60, 90, and 120 min after surgery in rats. * Preoperative *vs.* 30 min.; § Preoperative *vs.* 60 min; & Preoperative *vs.* 90 min; Δ Preoperative *vs.* 120 min; ж 30 min *vs.* 60 min; % 30 min *vs.* 90 min; # 30 min *vs.* 120 min; ¤ 60 min *vs.* 90 min; Ψ 60 min *vs.* 120 min. (**C**) HOMA-ISI in Groups P, G, S, and F before surgery, and 30, 60, 90, and 120 min after surgery in rats. * Preoperative *vs.* 30 min.; § Preoperative *vs.* 60 min; & Preoperative *vs.* 90 min; Δ Preoperative *vs.* 120 min; ж 30 min *vs.* 60 min; % 30 min *vs.* 90 min; # 30 min *vs.* 120 min; ¤ 60 min *vs.* 90 min; Ψ 60 min *vs.* 120 min.

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

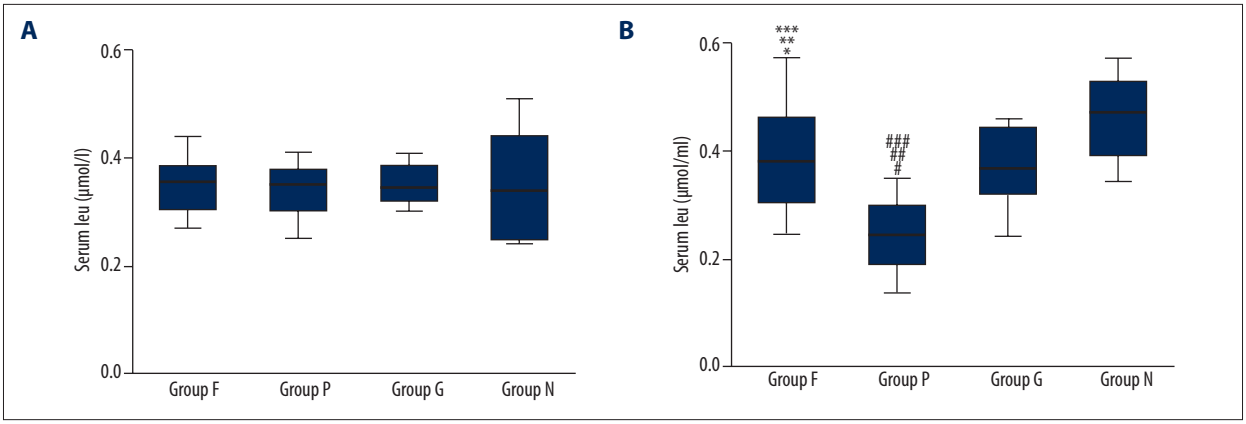

Figure 3. Serum leucine levels in Groups P, G, S, and F. (**A**) before surgery and (**B**) 120 min after surgery in rats. * F group *vs.* P group; ** F group *vs.* G group; *** F group *vs.* N group; # comparison among all 4 groups; ## P group *vs.* G group; ### P group *vs.* N group.

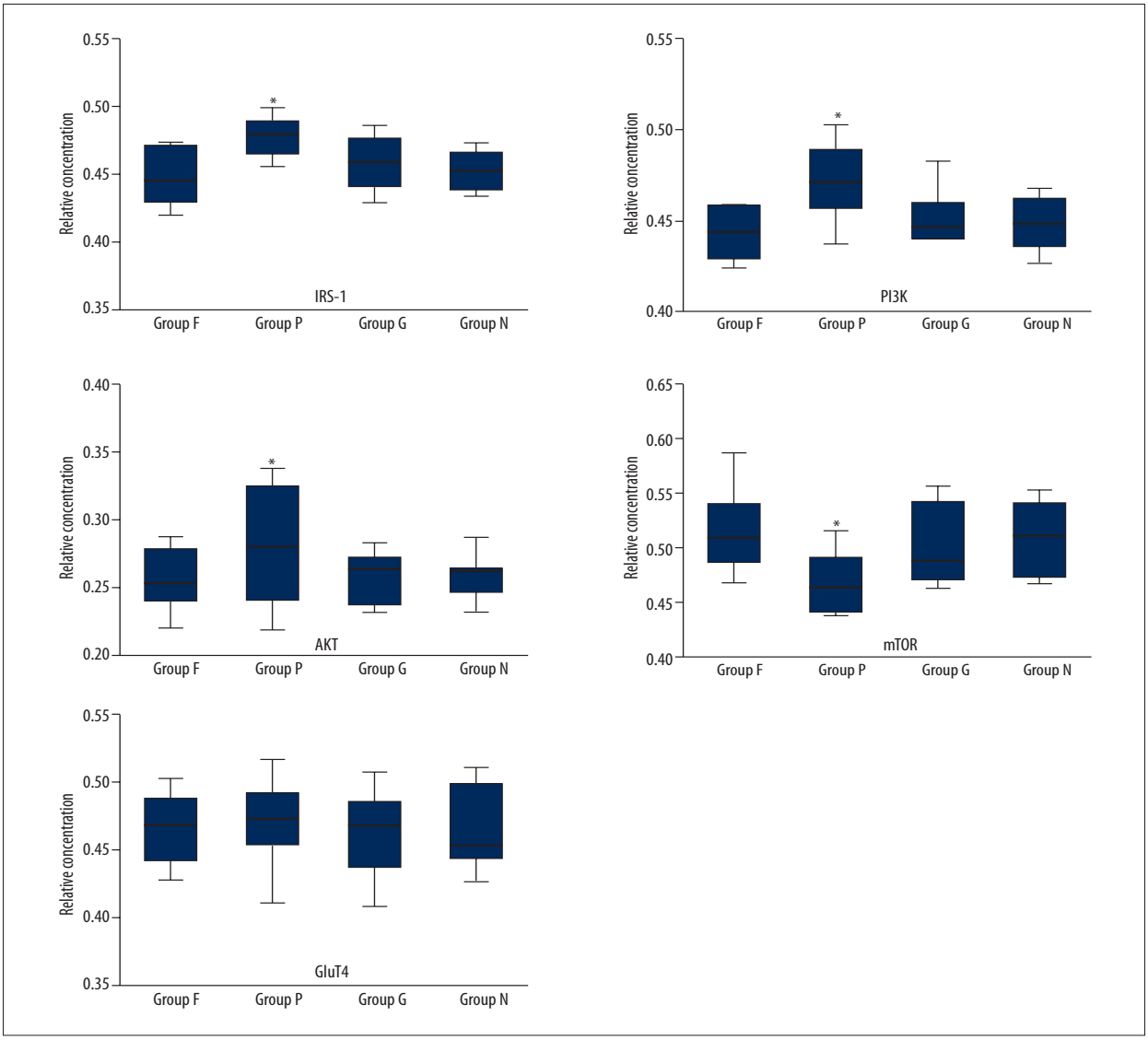

Figure 4. Messenger RNA expression of IRS-1, PI3K, AKT, mTOR, and Glut4 in skeletal muscles of rats 2 h after surgery.

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

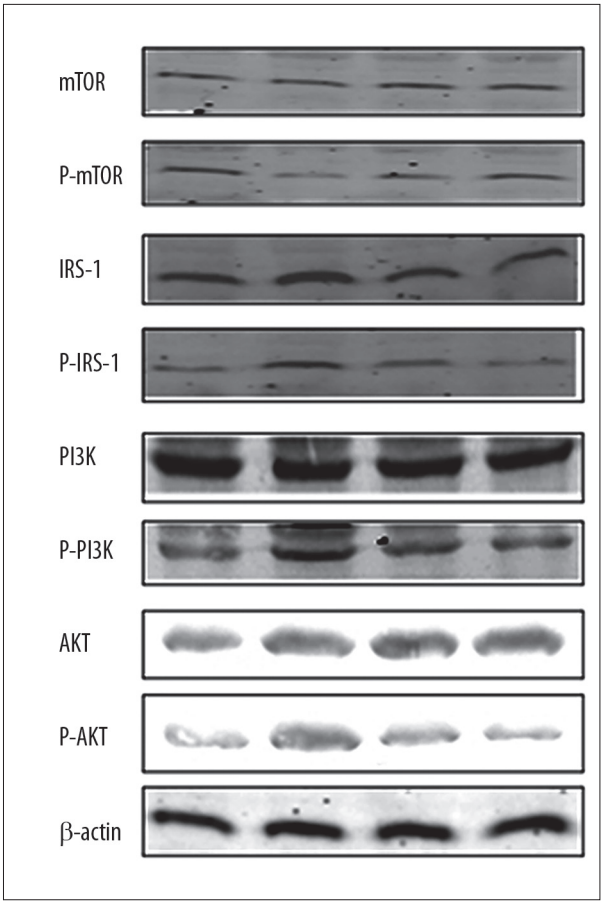

Figure 5. Total protein and phosphorylated levels of mTOR, IRS-1, PI3K, and AKT in skeletal muscle of rats 2 h after surgery.

phenomenon in which a certain amount of insulin cannot achieve the expected biological effects; that is, only a normal or higher level of insulin achieves a low biological effect. IR is an important cause of high blood glucose levels after trauma, mainly in peripheral tissues, especially muscle tissues. In addition to pathological hyperglycemia and impaired glucose tolerance, IR can cause increased catabolism, negative nitrogen balance, reduction of lean tissues, impaired would healing, and elevation of infection rate. These can seriously affect the environmental stability *in vivo*. The above phenomenon is referred to as diabetes mellitus [8]. In the present study, a significant decrease in HOMA-ISI and HOMA-β was observed post-surgery, indicating that the pancreatic secretion function was suppressed at an early stage after trauma. HOMA-β of the PreOp group exhibited a small range of reduction and rapid recovery, demonstrating that animals of the PreOp group had the least impairment of secretion function and fastest recovery post-surgery. HOMA-ISI became obviously lower than the baseline values at 30 min post-surgery, suggesting that insulin sensitivity *in vivo* was suppressed at an early stage after trauma. However, HOMA-ISI of animals was significantly higher in the PreOp group compared with the other 3 groups, indicating that the PreOp OCH treatment might enhance insulin sensitivity via a specific pathway and thereby improving PIR.

Posttraumatic IR involves complex mechanisms. Recently, the roles of the IRS-1/PI3K/AKT system in posttraumatic IR have received more attention. Tyrosine phosphorylation of IRS-1 is the first step for most biological effects of insulin and the core step of insulin signal transduction. Active IRS-1 in cells functions as an adaptor protein; the cells react with a variety of downstream signal proteins to induce cascade reactions and mediate the physiological activities of insulin in cells. In this process, activation of PI3K and AKT play an important role. The PI3K/PKB system is an independent pathway, which is involved in signal transduction of a variety of growth factors and has extensive biological effects [25]. After trauma, tyrosine phosphorylation of IRS-1 is suppressed; thus, the inhibition of serine phosphorylation associated with IRS-1 protein transduction is enhanced, resulting in IR. Regulating protein expression in the IRS-1/PI3K/Akt pathway can lead to reduction of IR [26] and acceleration of glucose transport in skeletal muscle [27]. The present study demonstrated that OCH treatment inhibited the mTOR pathway to activate the PI3K/AKT-independent pathway. These results are supported by previous findings from China [28].

A previous study reported that mTOR and Akt contributed to IR. The basal phosphorylation levels of mTOR and Akt were higher in the insulin-resistant plantaris muscles of obese rats compared with that found in their lean counterparts [29]. mTOR can be regulated by the level of serum amino acids [30–32], especially leucine [33]. Therefore, similar results were obtained with the analysis of serum leucine levels. However, the relationship between OCH and serum leucine levels needs further investigation.

The present study is not without limitations. These results were obtained in a rat model, and some studies might be needed in humans to confirm these results. In addition, we explored only 1 pathway, while many others might be more or less involved in PIR. Most importantly, the present study did not explore the mechanisms leading to PIR, which are not well established.

Conclusions

Surgical trauma may induce IR in peripheral skeletal muscle tissues. Preoperative OCH administration can reduce postoperative serum leucine concentration to inhibit the mTOR pathway and activate IRS-1/PI3K/Akt phosphorylation. Through these 2 pathways, it is possible that the postoperative insulin sensitivity is enhanced and IR is improved. Further studies

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

are needed to better understand how PreOp changes in the basal phosphorylation of mTOR, and how Akt may contribute to pathophysiology of IR after surgery.

References:

1. Ljungqvist O, Soreide E: Preoperative fasting. Br J Surg, 2003; 90: 400–6
2. Svanfeldt M, Thorell A, Hausel J et al: Randomized clinical trial of the effect of preoperative oral carbohydrate treatment on postoperative whole-body protein and glucose kinetics. Br J Surg, 2007; 94: 1342–50
3. Ljungqvist O, Nygren J, Thorell A: Modulation of post-operative insulin resistance by pre-operative carbohydrate loading. Proc Nutr Soc, 2002; 61: 329–36
4. Nygren J, Thorell A, Jacobsson H et al: Preoperative gastric emptying. Effects of anxiety and oral carbohydrate administration. Ann Surg, 1995; 222: 728–34
5. Noblett SE, Watson DS, Huong H et al: Pre-operative oral carbohydrate loading in colorectal surgery: a randomized controlled trial. Colorectal Dis, 2006; 8: 563–69
6. McMahon M, Marsh H, Rizza R: Comparison of the pattern of postprandial carbohydrate metabolism after ingestion of a glucose drink or a mixed meal. J Clin Endocrinol Metab, 1989; 68: 647–53
7. Nygren J, Soop M, Thorell A et al: Preoperative oral carbohydrate administration reduces postoperative insulin resistance. Clin Nutr, 1998; 17: 65–71
8. Carlson GL: Insulin resistance in sepsis. Br J Surg, 2003; 90: 259–60
9. Kammoun HL, Kraakman MJ, Febbraio MA: Adipose tissue inflammation in glucose metabolism. Rev Endocr Metab Disord, 2014; 15: 31–44
10. Wang ZG, Wang Q, Wang WJ, Qin HL: Randomized clinical trial to compare the effects of preoperative oral carbohydrate versus placebo on insulin resistance after colorectal surgery. Br J Surg, 2010; 97: 317–27
11. Proud CG: Regulation of protein synthesis by insulin. Biochem Soc Trans, 2006; 34: 213–16
12. Taniguchi CM, Emanuelli B, Kahn CR: Critical nodes in signalling pathways: insights into insulin action. Nat Rev Mol Cell Biol, 2006; 7: 85–96
13. Tesseraud S, Metayer S, Duchene S et al: Regulation of protein metabolism by insulin: value of different approaches and animal models. Domest Anim Endocrinol, 2007; 33: 123–42
14. Salles J, Chanet A, Giraudet C et al: 1,25(OH)2-vitamin D3 enhances the stimulating effect of leucine and insulin on protein synthesis rate through Akt/PKB and mTOR mediated pathways in murine C2C12 skeletal myotubes. Mol Nutr Food Res, 2013; 57: 2137–46
15. Yoshizawa F, Mochizuki S , Sugahara K: Differential dose response of mTOR signaling to oral administration of leucine in skeletal muscle and liver of rats. Biosci Biotechnol Biochem, 2013; 77: 839–42
16. Obata K, Ogata M, Matsumoto T et al: The effects of glucose on plasma amino acids and pyruvate during upper abdominal surgery. Anesth Analg, 1993; 76: 357–61
17. Niemczyk S, Szamotulska K, Giers K et al: Homeostatic model assessment indices in evaluation of insulin resistance and secretion in hemodialysis patients. Med Sci Monit, 2013; 19: 592–98
18. Stepien M, Rosniak-Bak K, Paradowski M et al: Waist circumference, ghrelin and selected adipose tissue-derived adipokines as predictors of insulin resistance in obese patients: preliminary results. Med Sci Monit, 2011; 17(11): PR13–18
19. Fan H, Pan Q, Zhang P, Liu J et al: Influence of islet function on typing and prognosis of new-onset diabetes after intensive insulin therapy. Med Sci Monit, 2013; 19: 787–93
20. Brener W, Hendrix TR, McHugh PR: Regulation of the gastric emptying of glucose. Gastroenterology, 1983; 85: 76–82
21. Johanson C: Influence of the composition of food on the gastric emptying pattern. Monogr Mount Sinai J Med, 1976; 43: 45–57
22. Foster C, Costill DL, Fink WJ: Gastric emptying characteristics of glucose and glucose polymer solutions. Res Q Exerc Sport, 1980; 51: 299–305
23. Elias E, Gibson GJ, Greenwood LF et al: The slowing of gastric emptying by monosaccharides and disaccharides in test meals. J Physiol, 1968; 194: 317–26
24. Sole CC, Noakes TD: Faster gastric emptying for glucose-polymer and fructose solutions than for glucose in humans. Eur J Appl Physiol Occup Physiol, 1989; 58: 605–12
25. Hemmings BA: Akt signaling: linking membrane events to life and death decisions. Science, 1997; 275: 628–30
26. Zhang Y, Hai J, Cao M et al: Silibinin ameliorates steatosis and insulin resistance during non-alcoholic fatty liver disease development partly through targeting IRS-1/PI3K/Akt pathway. Int Immunopharmacol, 2013; 17: 714–20
27. Zeng XQ, Zhang CM, Tong ML et al: Knockdown of NYGGF4 increases glucose transport in C2C12 mice skeletal myocytes by activation IRS-1/PI3K/AKT insulin pathway. J Bioenerg Biomembr, 2012; 44: 351–55
28. Xiao F, Huang Z, Li H et al: Leucine deprivation increases hepatic insulin sensitivity via GCN2/mTOR/S6K1 and AMPK pathways. Diabetes, 2011; 60: 746–56
29. Katta A, Kakarla S, Wu M et al: Altered regulation of contraction-induced Akt/mTOR/p70S6k pathway signaling in skeletal muscle of the obese Zucker rat. Exp Diabetes Res, 2009; 2009: 384683
30. Sabers CJ, Martin MM, Brunn GJ et al: Isolation of a protein target of the FKBP12-rapamycin complex in mammalian cells. J Biol Chem, 1995; 270: 815–22
31. Sabatini DM, Erdjument-Bromage H, Lui M et al: RAFT1: a mammalian protein that binds to FKBP12 in a rapamycin-dependent fashion and is homologous to yeast TORs. Cell, 1994; 78: 35–43
32. Brown EJ, Albers MW, Shin TB et al: A mammalian protein targeted by G1-arresting rapamycin-receptor complex. Nature, 1994; 369: 756–58
33. Lynch CJ, Fox HL, Vary TC et al: Regulation of amino acid-sensitive TOR signaling by leucine analogues in adipocytes. J Cell Biochem, 2000; 77: 234–51

Conflicts of interest

The authors declare that they have no conflicts of interest.

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]