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Original article

Preoperative carbohydrate supplementation attenuates post-surgery insulin resistance via reduced inflammatory inhibition of the insulin-mediated restraint on muscle pyruvate dehydrogenase kinase 4 expression

Petter Fosse Gjessing [a, b, *, 1], Dumitru Constantin-Teodosiu [c, 1], Martin Hagve [b], Dileep N. Lobo [d], Arthur Revhaug [a, b], Øivind Irtun [a, b]

[a] Department of Digestive Surgery, University Hospital of North Norway, Tromsø, Norway
[b] Laboratory of Surgical Research, Institute of Clinical Medicine, University of Tromsø, Tromsø, Norway
[c] School of Life Sciences, University of Nottingham Medical School, Nottingham NG7 2UH, United Kingdom
[d] Division of Gastrointestinal Surgery, Nottingham Digestive Diseases Centre, National Institute for Health Research Biomedical Research Unit, Nottingham University Hospitals, Queen's Medical Centre, United Kingdom

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SUMMARY

Background & aims: We hypothesized that the so far poorly understood improvement in postoperative insulin sensitivity, when surgery is preceded by a carbohydrate (CHO) drink, occurs via attenuation of skeletal muscle inflammatory responses to surgery, improved insulin signaling and attenuated expression of muscle pyruvate dehydrogenase kinase (PDK) 4.

Methods: Vastus lateralis muscle biopsies, collected before and after major abdominal surgery and during postoperative hyperinsulinaemic-euglycaemic clamping from 16 pigs randomized to either 200 ml of a CHO-supplemented drink 2 h before surgery (CHO, 25 g; $n = 8$), or preoperative overnight fasting (fasted; $n = 8$), were analyzed by fast qRT-PCR and IR-Western blotting.

Results: During clamping, expression of IKKβ, SOCS3 and the ratio of phosphorylated/total JNK2 proteins were lower in the CHO group than in the fasted group (−1.0 vs. 2.9-fold, $P < 0.001$; −0.6 vs. 3.2-fold, $P < 0.01$; and −0.5 vs. 1.1-fold, $P < 0.02$, respectively). Furthermore, the ratio of Ser^{307}-phosphorylated (inhibition)/total IRS1 protein was reduced only in the CHO group (−2.4 fold, $P < 0.02$), whereas FOXO1 phosphorylation (inactivation), which correlated negatively with PDK4 mRNA ($r^2 = 0.275$, $P < 0.05$), was lower in the CHO group than in the fasted group (−1.1-fold, $P > 0.05$ vs. −2.3-fold, $P = 0.05$). Post-surgery, PDK4 mRNA increased ~20-fold ($P < 0.01$) in both groups, but was reversed to a greater extent by insulin in the CHO group (−40.5 vs. −22.7-fold, $P < 0.05$), resulting in 5-fold lower PDK4 protein levels, which correlated negatively with insulin-stimulated whole-body glucose disposal rates ($r^2 = -0.265$, $P < 0.05$).

Conclusions: Preoperative carbohydrate supplementation was found to ameliorate postoperative insulin sensitivity by reducing muscle inflammatory responses and improved insulin inhibition of FOXO1-mediated PDK4 mRNA and protein expression after surgery.

1. Introduction

The surgery-mediated rise in circulating cortisol and pro-inflammatory cytokines has been advocated as a major factor contributing to the onset of postoperative acute insulin resistance. However, in accordance with human data [1], we have previously shown in a major abdominal surgery pig model that preoperative carbohydrate (CHO) administration in the immediate preoperative phase improves postoperative insulin-stimulated whole-body

Non-standard abbreviations: CHO, carbohydrate; FOXO1, forkhead transcription factor 1; IKKβ, inhibitor κB kinase; IL-6, interleukin 6; IRS1, insulin receptor substrate 1; JNK, c-Jun N-terminal kinase; PDC, pyruvate dehydrogenase complex; PDK4, pyruvate dehydrogenase kinase 4; SOCS3, suppressor of cytokine signaling 3; TNF-α, tumor necrosis factor α.

* Corresponding author. Department of Digestive Surgery, University Hospital of North Norway, 9038 Tromsø, Norway. Tel.: +47 07766; fax: +47 77626605.
E-mail address: petter.fosse.gjessing@unn.no (P.F. Gjessing).

[1] PFG and DC-T are joint first authors.

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oxidative glucose disposal independently from cortisol levels and other postoperative counter-regulatory hormone levels [2]. Central to CHO oxidation is the activity of pyruvate dehydrogenase complex (PDC), which acts as a gatekeeper to the CHO-derived pyruvate flux into mitochondria. PDC activity is controlled by pyruvate dehydrogenase kinase (PDK), which phosphorylates and inactivates PDC, thereby inhibiting CHO oxidation [3]. Of the four PDK protein isoforms (PDK1–4), the activity of PDK4 is several-fold greater than any other member of this protein family [4]. Interestingly, elevation in muscle PDK4 mRNA and protein expression in response to glucocorticoids is reinforced by enhanced binding kinetic of forkhead transcription factor 1 (FOXO1) to its promoter on the PDK4 gene [5]. On the other hand, activation of insulin signaling via insulin receptor substrate 1 (IRS1)/Akt1 axis inhibits (through phosphorylation) FOXO1-mediated PDK4 expression [5].

Inhibition of the insulin signaling pathway through phosphorylation of Ser^{307} on IRS1 by serine/threonine kinases inhibitor κB kinase (IKKβ) and c-Jun N-terminal kinase (JNK), which are activated by pro-inflammatory cytokines, is a well-recognized cause of chronic insulin resistant states such as type 2 diabetes mellitus. Similar alterations in skeletal muscle insulin signaling are seen after surgery [6] when circulating IL-6 concentrations correlate well with both the extent of tissue trauma [7] and with the magnitude of postoperative peripheral insulin resistance [8]. Surgery also induces skeletal muscle expression of suppressor of cytokine signaling 3 (SOCS3) [9], which binds to IRS1 and targets it for proteasome degradation [10]. Collectively, these data suggest that there is a link between trauma at the site (e.g. abdominal) of surgery-initiated systemic inflammation and peripheral muscle insulin resistance. A further indication in support of this contention comes from recent evidence showing that both postoperative circulating IL-6 concentrations and insulin resistance are significantly reduced when surgery is preceded immediately by CHO oral supplementation [11].

Therefore, we hypothesized that preoperative oral CHO supplementation reduces the magnitude of surgery-mediated inflammatory responses and impairment of insulin signal transduction in skeletal muscle, thereby ameliorating insulin inhibition of muscle PDK4 protein expression. Particularly, the present study would examine the outcome of preoperative oral CHO supplementation on inflammatory mediators (JNK1/2, IKKβ and SOCS3), insulin signaling (IRS1, Akt1, FOXO1), all known to be involved in expression of PDK4 gene and protein in muscle tissue, at pre- and post-surgery, and post-surgery hyperinsulinaemic-euglycaemic clamping time points.

2. Materials and methods

2.1. Ethical approval

All experiments were conducted in compliance with the institutional animal care guidelines and the National Institute of Health's (NIH) *Guide for the Care and Use of Laboratory Animals* [DHHS Publication no. (NIH) 85-23, Revised 1985].

2.2. Study design

This randomized, single-blinded, controlled study describes the molecular events of a larger study investigating the effects of a single-dose of preoperative oral CHO loading on the development of postoperative hepatic and peripheral insulin resistance, glycogen content and levels of free fatty acids, and counter-regulatory hormones in pigs [2]. Muscle tissue collected during these experiments was utilized for the present gene and protein expression measurements. The author performing the analyses (DC-T) was blinded to the treatment allocations.

2.3. Animals and interventions

Yorkshire/Landrace pigs ($n = 16$) weighing 29.5 (25.8–33.0) kg were submitted to a 12:12-hr light–dark cycle, a standardized diet and ad libitum access to water for a week prior to the experiments, which were commenced between 06:00 and 07:00 a.m. Pigs were randomized to two surgery groups receiving either 200 ml preOp®/25 g CHO (12.6 g/100 ml carbohydrate, 79% polysaccharides, 260 mOsmol/kg, Nutricia, Zoetermeer, The Netherlands) 2 h prior to surgery (CHO; $n = 8$) or were fasted overnight (fasting; $n = 8$). Following sedation by intramuscular injection of 15 mg/kg ketamine, 1 mg/kg midazolam and 1 mg atropine and mask inhalation of 4% isoflurane in 100% oxygen, animals were orotracheally intubated. Gas anesthesia (minimal alveolar concentration of 0.8–1.5% mixed with 40–60% oxygen) in combination with infusion of 0.02 mg/kg/hr fentanyl and 0.3 mg/kg/hr midazolam was continued throughout the experiments and adjusted according to respiration, repeated blood gas analyses (ABL 800 FLEX; Radiometer, Copenhagen, Denmark) and snout reflex tests. After a right sided thoracotomy and a midline laparotomy with total colectomy followed by closure of the incisions, hepatic and peripheral insulin sensitivity was assessed by D-[6,6-$^2H_2$]glucose infusion in combination with hyperinsulinaemic-euglycaemic clamping with labeled glucose infusate. For more details please refer to our in-depth method description [12]. Open muscle biopsies were sampled from vastus lateralis muscle at the commencement and immediately after surgery, and during the end of the second of two consecutive 2 h long hyperinsulinaemic-euglycaemic clamps (insulin infusions: 0.4 and 1.2 mU/kg/min; s-insulin: ~15 and 40 μU/ml for a total of 4 h) [2]. Muscle samples were snap-frozen in liquid nitrogen and stored at −80 °C until further analysis was undertaken.

2.4. Real-time PCR measurement

Total RNA was isolated from frozen wet muscle (~30 mg) using Tri Reagent (Sigma Aldrich, UK), according to the manufacturer's protocol. Total RNA quantification, first-strand cDNA synthesis and real-time PCR protocols were carried out as previously described [13]. PDK4 Taqman primer/probe sets were obtained from Life Technologies (UK). The housekeeping gene hydroxymethylbilane synthase (HMBS) was used as an internal control. Relative quantification of gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method. The preoperative values were used as calibrator with a value of 1 within each group.

2.5. Protein extraction and Western blotting measurements

Cytosolic and nuclear proteins were extracted from ~ 30 mg frozen wet muscle tissue using a modified method by Blough [14]. Muscle samples were lysed in the presence of phosphatase and protease inhibitors and protein content was quantitated using the Bradford assay. Protein lysates were run on a 4–12% Bis–Tris acrylamide gel (Life Technologies, UK) for 2 h at constant voltage (200 V) and transferred to a polyvinylidenedifluoride membrane (PVDF) in ice-cold buffers (4 °C) overnight at constant current (100 mA), as described previously [13]. The protein transfer was tested using Ponceau S red staining, before blocking the membrane in bovine serum albumin-tris buffer saline tween (TBS-T) for 1 h at room temperature. The membranes were then incubated with the primary antibodies overnight at 4 °C. The following day, the membranes were washed in TBS-T, and incubated with an IRDye 800 labeled anti-goat secondary antibody and further quantitated