

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



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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 IKK β , 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 Ser³⁰⁷-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.

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Non-standard abbreviations: CHO, carbohydrate; FOXO1, forkhead transcription factor 1; IKK β , inhibitor κ B 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 .

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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

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 Ser³⁰⁷ on IRS1 by serine/threonine kinases inhibitor κ B kinase (IKK β) 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, IKK β 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-[6,6-²H₂]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 μ U/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 $2^{-\Delta\Delta\text{Ct}}$ 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

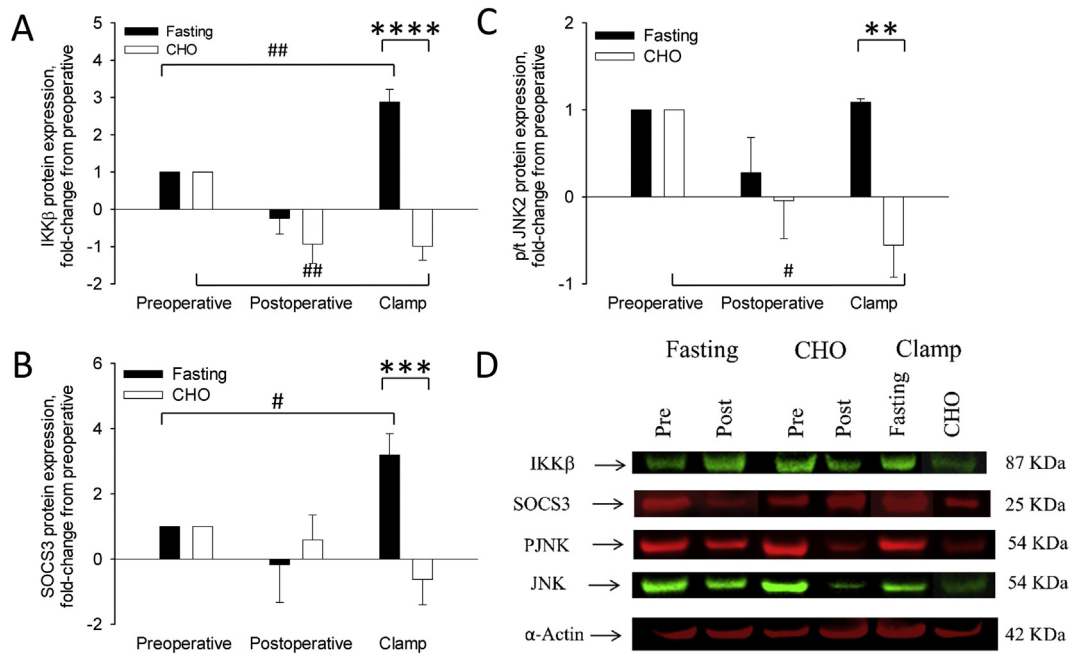


Fig. 1. Tissue markers of inflammation. Mean inhibitor κ B kinase (IKK β ; Fig. 1A), suppressor of cytokine signaling 3 (SOCS3; Fig. 1B) and ratio of phosphorylated to total c-Jun N-terminal kinase 2 (JNK2; Fig. 1C) protein expression presented as fold-change from preoperative values. Bars are SEM. *Significant difference by Mann Whitney *U*-test, #Significant difference by Wilcoxon *t*-test; one symbol, $P < 0.05$; two symbols, $P < 0.02$; three symbols, $P < 0.01$; four symbols, $P < 0.001$. Typical Western blots depicting IKK β , SOCS3 and JNK2 protein bands with their corresponding molecular weights are presented in Fig. 1D.

by using an Odyssey® Infrared Imaging System (LI-COR, Biosciences, NE, USA). The band intensities were adjusted by subtracting the background and normalized to α -actin and lamin protein controls for cytosolic and nuclear proteins, respectively. Antibodies were purchased from Cell Signaling Technology (Danvers, MA, USA): total insulin receptor substrate 1 (tIRS1), phosphorylated insulin receptor substrate 1 (Ser³⁰⁷; pIRS1), total Akt1 (tAkt1), phosphorylated Akt1 (Ser⁴⁷³; pAkt1), total forkhead transcription factor 1 (tFOXO1), phosphorylated forkhead transcription factor 1 (Ser²⁵⁶; pFOXO1); from Abcam (Cambridge, UK): total c-Jun N-terminal kinase 1/2 (tJNK1/2), phosphorylated c-Jun N-terminal kinase 1/2 (Thr¹⁸⁰/Tyr¹⁸²; pJNK1/2), suppressor of cytokine signaling 3 (SOCS3), inhibitor κ B kinase (IKK β) and pyruvate dehydrogenase kinase 4 (PDK4).

2.6. Statistics

Sample size was estimated from a two-sample means test with a 95% degree of confidence for two groups with comparable standard deviations and a 2-fold mean difference and with the α and β values fixed at 0.05 and 0.2 (power 0.8), respectively. Within each group the preoperative level of expression was given a value of 1, and fold-changes in mRNA and protein expression after surgery and during clamping were calculated, relative to preoperative. Comparison of gene and protein data between and within groups was carried out by Mann–Whitney *U* and Wilcoxon *t*-tests, respectively. Insulin sensitivity data were analyzed by Student's independent *t*-test. Data are presented as mean \pm SEM. Differences were considered significant at $P < 0.05$.

3. Results

3.1. Tissue markers of inflammation

During the hyperinsulinaemic-euglycaemic clamp, expression of IKK β (Fig. 1A), SOCS3 (Fig. 1B) and ratio of phosphorylated to total

JNK2 (Fig. 1C) proteins decreased significantly in the CHO group and increased significantly 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, across groups). No significant change in phosphorylated to total JNK1 was seen (data not shown). Typical Western blots depicting IKK β , SOCS3 and JNK2 protein bands with their corresponding molecular weights are presented in Fig. 1D.

3.2. IRS1, Akt1 and FOXO1 activity

The ratio of phospho Ser³⁰⁷ IRS1 to total IRS1 was reduced during clamping in the CHO group (−2.4-fold, $P < 0.02$, Fig. 2A), whereas no significant change was seen in the fasting group. A significant decrease in the phospho Ser²⁵⁶ FOXO1 to total FOXO1 ratio was seen during clamping in the fasted group (−2.3-fold, $P = 0.05$), but not in the CHO group (−1.1-fold, $P > 0.05$; Fig. 2B), demonstrating inhibitory action of insulin on FOXO1 in both groups, albeit to a greater extent in the preoperatively CHO treated animals. Total IRS1 protein was not changed by surgery and was not different between groups (data not shown). Typical Western blots depicting IRS1 and FOXO1 proteins are presented in Fig. 2C. The restraining action of insulin via Akt1 on FOXO1 activity, i.e. ratio pFOXO1/tFOXO1 was confirmed by a strong correlation ($P < 0.001$) between the fold-change in phosphorylated to total Akt1 (i.e. activation) protein to fold-change phosphorylated to total FOXO1 (i.e. inactivation) protein from preoperative during insulin stimulation (Fig. 2D).

3.3. PDK4 gene and protein expression

Following surgery muscle PDK4 mRNA expression increased similarly in the CHO and fasting groups (20.6- and 18-fold, respectively; both $P < 0.01$; Fig. 3A), whereas during the 4 h of hyperinsulinaemic-euglycaemic clamping the inhibitory effect of insulin on muscle PDK4 mRNA expression in the CHO group was almost twice as great than in the fasting group compared to the

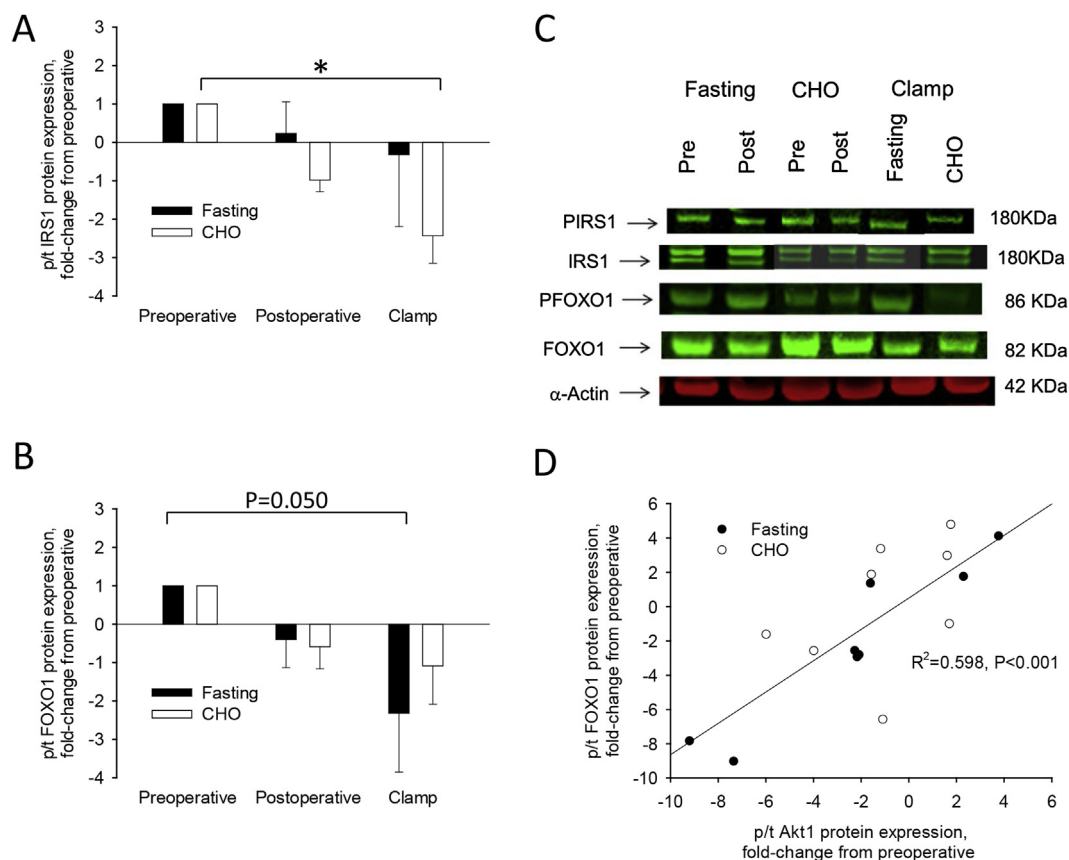


Fig. 2. Insulin signaling. Mean ratio of Ser³⁰⁷ phosphorylated to total insulin receptor substrate 1 (IRS1, Fig. 2A) and Ser²⁵⁶ phosphorylated to total forkhead transcription factor 1 (FOXO1, Fig. 2B) presented as fold-change from preoperative values. Bars are SEM. *Significant difference by Wilcoxon *t*-test, $P < 0.02$. Typical Western blots depicting IRS1 and FOXO1 protein bands with their corresponding molecular weights are presented in Fig. 2C. The Pearson's correlation between fold-change from preoperative levels in Ser⁴⁷³ phosphorylated to total Akt1 and Ser²⁵⁶ phosphorylated to total forkhead transcription factor 1 (FOXO1) in skeletal muscle during hyperinsulinaemic-euglycaemic clamping is presented in Fig. 2D ($n = 16$).

postoperative values (−40.5 vs. −22.7-fold, $P < 0.05$; Fig. 3B). Consequently, PDK4 protein expression measured during the clamp was almost 5-fold lower in the CHO-loaded group than in the fasted group when compared with the postoperative values (−1.6 vs. 3.7-fold, $P < 0.01$; Fig. 3C).

3.4. Relationship between p/tFOXO1 and PDK4 gene expression

The fold-change in ratio of phosphorylated to total FOXO1 during the clamp was significantly inversely correlated to level of PDK4 mRNA expression (Fig. 4).

3.5. Relationship between postoperative peripheral insulin sensitivity and PDK4 protein expression

The rate of insulin-mediated whole-body glucose disposal (WGD) during the postoperative clamp was negatively correlated to the level of muscle PDK4 protein expression (Fig. 5).

3.6. Postoperative insulin sensitivity

Insulin-stimulated whole-body glucose disposal at the time point when the insulin-stimulated biopsies were collected ($\sim 40 \mu\text{U/ml}$ serum insulin), was higher in the CHO group than in the fasted group (12.2 ± 1.3 vs. 9.0 ± 0.6 mg/kg/min, $P < 0.05$).

4. Discussion

The present study has demonstrated that oral supplementation with a formulated CHO drink 2 h preoperatively reduces the skeletal muscle inflammatory responses to major abdominal open surgery, as was highlighted by our observations of lower expression of IKK β , SOCS3, and p/t JNK2 proteins in one of the biggest skeletal muscles, i.e. vastus lateralis. Furthermore [1], the level of inhibitory phosphorylated Ser³⁰⁷ IRS1 was reduced in the CHO group [2], the changes in muscle FOXO1 activity were highly correlated with muscle PDK4 mRNA expression, and [3] the insulin stimulated whole-body glucose disposal rates, which were higher in the CHO than in the fasting group, were negatively correlated with the expression of muscle PDK4 protein during post-surgery hyperinsulinaemic-euglycaemic clamping. Since the glycogen content in skeletal muscle and liver tissue failed to increase during the postoperative clamps in both groups as we have recently reported [2], this would indicate that preoperative CHO supplementation cannot restore the abolishment of insulin stimulated non-oxidative glucose disposal in the form of glycogen synthesis after surgery. This finding bears resemblance to previous human abdominal and hip-replacement surgery studies [1,15], which by using similar clamping methods to the present study, showed that the predominant effect of preoperative CHO supplementation on post-surgery insulin stimulated whole-body glucose disposal rates was mainly associated with increasing glucose oxidation. Therefore, it is pertinent to suggest that reduced inflammatory mediation on

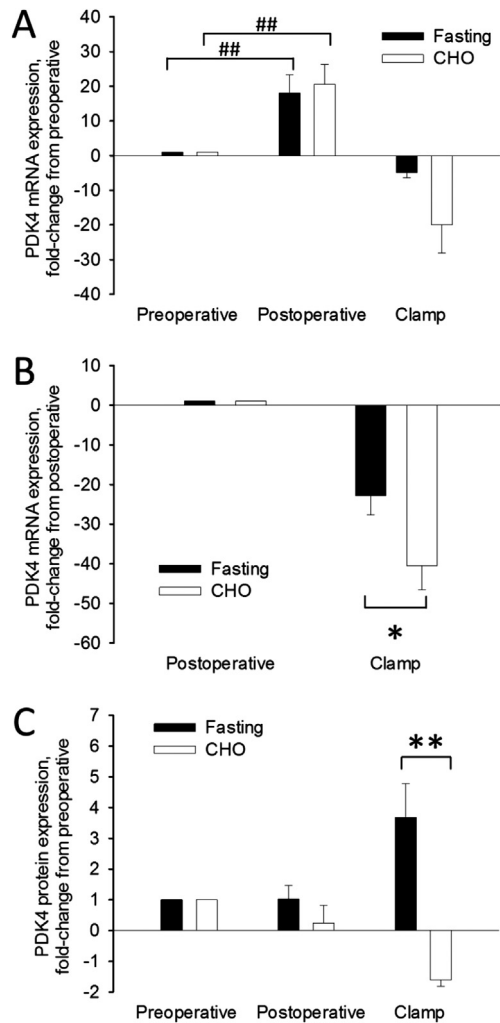


Fig. 3. PDK4 gene and protein expression. Mean pyruvate dehydrogenase kinase 4 (PDK4) gene expression reported as fold-change from pre- and post-operative values and expression PDK4 protein reported as fold-change from preoperative are presented in Fig. 3A, B and 3C, respectively. Bars are SEM. *Significant difference by Mann Whitney U-test, #Significant difference by Wilcoxon *t*-test, one symbol, $P < 0.05$; two symbols, $P < 0.01$.

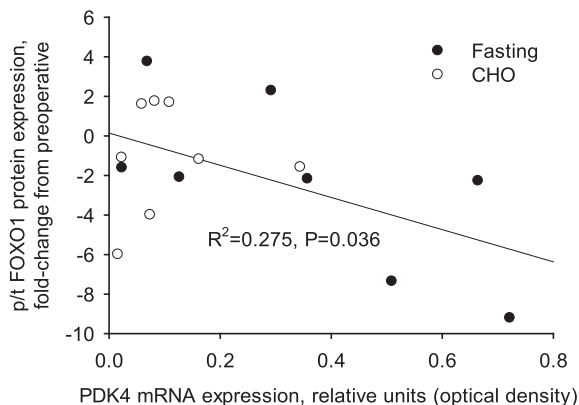


Fig. 4. Relationship between p/tFOXO1 and PDK4 gene expression. The Pearson's correlation between fold-change from preoperative levels in pyruvate dehydrogenase kinase 4 (PDK4) gene expression and Ser²⁵⁶ phosphorylated to total forkhead transcription factor 1 (FOXO1) in skeletal muscle during hyperinsulinaemic-euglycaemic clamping ($n = 16$).

insulin signal transduction in the preoperatively CHO supplemented animals led to an improvement in insulin inhibition of FOXO1-mediated skeletal muscle PDK4 protein expression and increased oxidative glucose disposal rates post-surgery. The changes in the phosphorylation status/expression of these muscle proteins constitute the core of the mechanism, which we believe is behind the protective role of preoperative CHO supplementation in reducing the development of postoperative insulin resistance (Fig. 6).

In line with recent evidence of rapid up-regulation of inflammatory mediators following various forms of trauma [9,16–18], abdominal surgery led postoperatively to an increase in expression of muscle IKK β , JNK2 and SOCS3, all known for their ability to inhibit insulin signaling via their interaction with IRS1, in the fasted animals (Fig. 1A, B and C). Previous studies have shown that activation of JNK and IKK β were associated with increased inhibitory Ser^{307/312} IRS1 phosphorylation in the liver immediately after trauma and hemorrhage in rodents [18], and in skeletal muscle following tumor necrosis factor α (TNF α) infusion in healthy humans [19]. Equally, expression of skeletal muscle SOCS3 was rapidly up-regulated after major abdominal surgery [9] and after lipopolysaccharide-induced endotoxaemia in rodents [20].

The molecular events underlying the attenuation of the postoperative increase in IKK β , JNK2 and SOCS3 in the CHO group compared to the fasted group (Fig. 1) are not readily available from the current data. A possible explanation could be found in the potent and fast-acting anti-inflammatory properties that physiological levels of hyperinsulinaemia exhibits [21]. Thus, the attenuation of inflammatory responses in the CHO group might have not been directly related to the CHO load itself, but was rather a result of modulation of the inflammatory response to surgery by anti-inflammatory properties of the concurrent preoperative hyperinsulinaemia. Importantly, the dosage of ~ 0.85 g CHO/kg body-weight supplemented in the current study is equivalent to the standard morning CHO dose, which is grade A recommended as part of the preoperative care of most surgical patients [22] and which has been repeatedly shown to reduce the development of postoperative insulin resistance in clinical trials [23] and the postoperative circulating IL-6 concentrations in patients undergoing moderate and major abdominal surgery [11]. In further support of this contention, the level of hyperinsulinaemia elicited by the CHO load in the current study is similar to those seen in human studies [2,24] and has been shown to display potent and fast-acting anti-inflammatory properties by inhibition of nuclear factor κ B in

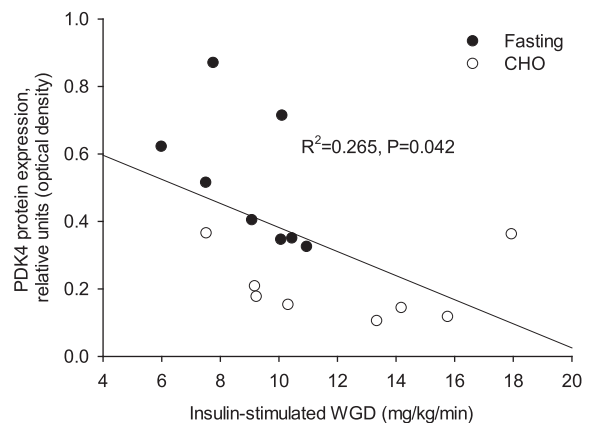


Fig. 5. Relationship between postoperative peripheral insulin sensitivity and PDK4 protein expression. The Pearson's correlation between pyruvate dehydrogenase kinase 4 (PDK4) protein expression and insulin-mediated whole-body glucose disposal rate during hyperinsulinaemic-euglycaemic clamping ($n = 16$).

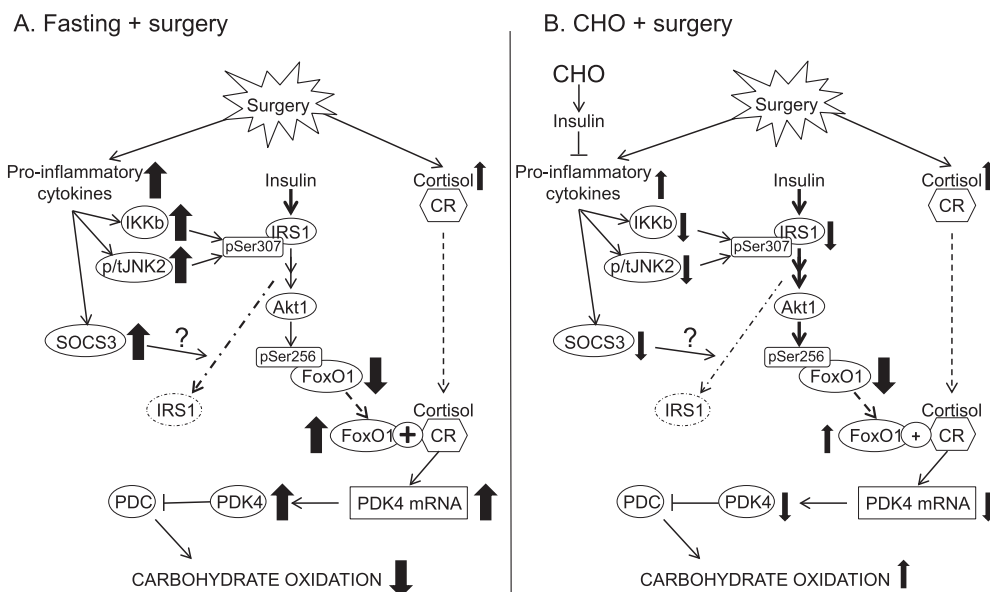


Fig. 6. Proposed mechanism of action. Proposed mechanism underlying the prophylactic effect of preoperative carbohydrate loading (CHO) on inflammatory interference with insulin regulation of pyruvate dehydrogenase kinase 4 (PDK4) expression in muscle after surgery. **A:** PDK4 expression in muscle is increased after surgery both by a cortisol-induced increase in transcription and reduced insulin-mediated inhibitory phosphorylation (pSer²⁵⁶) of forkhead transcription factor 1 (FOXO1), which increases the PDK4 gene responsiveness to glucocorticoids. Insulin action is inhibited by the serine/threonine kinases inhibitor κ B kinase (IKK β) and c-Jun N-terminal kinase (JNK) which are activated by cytokines after surgery, resulting in inhibitory phosphorylation (pSer³⁰⁷) of insulin receptor substrate 1 (IRS1) and impaired downstream propagation of insulin signaling. IKK β and JNK also induce expression of suppressor of cytokine signaling 3 (SOCS3) which targets IRS1 for degradation. **B:** CHO improves insulin-mediated inhibitory phosphorylation of FOXO1 and regulation of PDK4 expression in muscle after surgery by reducing postoperative IKK β , JNK and inhibitory phosphorylation of IRS1. We propose that these effects occur due to anti-inflammatory properties of hyperinsulinaemia following CHO supplementation in the immediate preoperative phase \uparrow/\uparrow and \downarrow/\downarrow arrows indicate upregulation/increased activity and downregulation/decreased activity, while arrow thickness indicates the magnitude of the effect.

mononuclear cells [21] and reducing the expression of pro-inflammatory cytokines in endotoxaemic human macrophages [25]. Secondly, plasma concentrations of TNF- α and IL-6 in pigs were reduced in response to lipopolysaccharide (LPS) infusion-induced systemic inflammation if the LPS infusion was preceded by a low dose hyperinsulinaemic-euglycaemic clamp [26]. Lastly, increased circulating insulin inhibits expression of skeletal muscle PDK4 [27], which in turn inhibits, through the phosphorylation of three serine sites on the component E1, the PDC activity [4]. Since PDC is the rate limiting step in CHO oxidation its malfunction has been therefore advocated as causative to induction of peripheral insulin resistance after surgery [28] together with reduced insulin-stimulated glycogen synthase activity and GLUT-4 translocation [29]. Currently, muscle PDK4 protein expression was not different between groups at the preoperative time-point (data not shown). However, after postoperative insulin infusion the expression of PDK4 protein was significantly lower in the CHO group than in the fasted group, and this was associated with a greater sensitivity of muscle glucose disposal, mainly through oxidation, as was reported in the present results section and in our recent published study [2]. An additional drive, although limited, to the increase in whole-body glucose disposal after CHO, and therefore a conceivable limitation of the present study, could have been accounted for by the effect of insulin on adipose tissue.

SOCS3 induces insulin resistance by competitively binding to the docking site of the insulin receptor and by targeting IRS1 for ubiquitin-mediated degradation [10]. Nevertheless, despite lower expression of SOCS3 protein in the CHO group during the clamp (Fig. 1B), the change in total IRS1 protein content across groups was not significantly different. On a different note, however, the ratio of Ser³⁰⁷ phosphorylated to total IRS1 was reduced postoperatively in the CHO group and further during the clamp, whereas no change was seen in the fasted group (Fig. 2A). This is in support of our hypothesis and was probably an important underlying factor to the

improvement in the downstream IRS1 signaling in the CHO group during the clamp, as reflected by the lower fall of the phosphorylated (inactive) to total ratio of FOXO1 and by stronger inhibitory drive of insulin (via the IRS1-Akt1-FOXO1 pathway) on PDK4 mRNA and protein expression compared with the fasted group (Figs. 2 and 3). Since PDK4 protein expression was significantly lower in the CHO group than in the fasted group during clamping (Fig. 3C), but not during the surgery, consequently an increase in muscle PDC activity in the former group could have accounted for the improvement in the postoperative peripheral oxidative glucose disposal in this group [2].

In conclusion, the present results indicate that oral CHO supplementation in the immediate preoperative phase reduces the skeletal muscle inflammatory responses to surgery-mediated trauma, the interference of inflammatory mediators with insulin downstream signal transduction, and thereby improves the ability of insulin to down-regulate FOXO1-mediated PDK4 transcription in skeletal muscle. Collectively, these responses may account for the protective role of preoperative CHO supplementation on development of postoperative insulin resistance.

Author contributions

PFG, DC-T, DNL, AR and ØI: generated the experimental hypothesis and study design, wrote and edited the manuscript; PFG and MH: performed the animal experiments; PFG and DC-T: generated and analyzed the experimental data; PFG: had primary responsibility for final content of the manuscript; and all authors: read and approved the final manuscript.

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Conflict of interest

No conflicts of interest, financial or otherwise, are declared by the authors.

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