# Effects of a carbohydrate, glutamine and antioxidant enriched oral nutritional supplement on major surgery induced insulin resistance: a randomized pilot study

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## ABSTRACT

### Background

Insulin resistance after surgery hampers recovery. Oxidative stress is shown to be involved in the occurrence of postoperative insulin resistance. Preoperative carbohydrate-rich oral nutritional supplements reduce, but do not prevent insulin resistance. The aim of the present study was to investigate the effect of a carbohydrate, glutamine and antioxidant enriched preoperative oral nutritional supplement on postoperative insulin resistance.

### Methods

A double blind randomized controlled pilot study in eighteen rectal cancer patients, who received either the supplement (S) or the placebo (P) 15, 11 and 4 hours preoperatively, was conducted. Insulin sensitivity was studied prior to surgery and on the first postoperative day using a hyperinsulinemic euglycemic two-step clamp.

### Results

Hepatic insulin sensitivity (insulin-mediated suppression of glucose production) decreased significantly after surgery in both groups, with no differences between the groups. Peripheral insulin sensitivity (glucose rate of disappearance, Rd) was significantly decreased after surgery in both groups (**S**: 37.2 [19.1-50.9] vs. 20.6 [13.9-27.9]; **P**: 23.8 [15.7-35.5] vs. 15.3 [12.6-19.1] μmol/kg·min), but less pronounced in the supplemented group (p=0.04). The percentage decrease in glucose Rd did not differ between the groups. Adipose tissue insulin sensitivity (insulin-mediated suppression of plasma free fatty acids) decreased to the same extent after surgery in both groups.

### Conclusion

Rectal cancer surgery induced profound insulin resistance affecting glucose and fatty acid metabolism. The preoperative nutritional supplement somewhat attenuated, but did not prevent postoperative peripheral insulin resistance.

## CLINICAL RELEVANCY STATEMENT

The occurrence of postoperative insulin resistance affects postoperative recovery negatively. This study gave improved insight in postoperative insulin resistance, by showing reduced insulin action in liver, muscle and adipose tissue. Enhanced awareness on postoperative insulin resistance may guide clinicians in their perioperative decisions.

## INTRODUCTION

Surgery induced insulin resistance is most pronounced on the first postoperative day, its degree correlates with the magnitude of the procedure and it increases the risk of postoperative morbidity [1]. Several studies showed that reduction of postoperative insulin resistance, with tight regulation of glucose metabolism [2] or by giving a preoperative carbohydrate-rich beverage [3], may improve clinical outcome. Other supplements, such as antioxidants or glutamine, known to modulate insulin sensitivity may also improve postoperative insulin sensitivity.

Postoperative insulin resistance is related to the immune-inflammatory response to surgical trauma and tissue damage, leading to increased free radical production, lipid peroxidation and oxidative stress [4]. Several authors have suggested a relation between oxidative stress and insulin resistance. For instance, Urakawa et al. showed a significant correlation between the plasma levels of $F_2$-isoprostanes, a marker of lipid peroxidation, and insulin sensitivity in obese and non-obese men [5]. On the other hand, antioxidant agents promote insulin sensitivity in experimental studies. Haber et al. showed that a decrease in insulin-stimulated glucose uptake after a high glucose infusion was prevented by simultaneous infusion of N-acetylcysteine or taurine in rats [6]. In addition, the antioxidant vitamin E attenuated the $H_2O_2$ induced reduction in insulin-stimulated glucose transport in cultured rat L6 muscle cells [7]. Therefore, reduction of oxidative stress could be an additional target to improve postoperative insulin resistance.

Glutamine, a conditionally essential amino acid in situations of catabolic stress such as surgery and a precursor of the antioxidant glutathione, is of interest in modulating postoperative insulin resistance [8, 9]. After severe trauma, glutamine supplementation prevented the deterioration of insulin sensitivity [8].

The combined effect of carbohydrates, glutamine and antioxidants on postoperative insulin sensitivity has not been studied. We therefore set out to study the effect of a novel glutamine, carbohydrate and antioxidant containing oral nutritional supplement on postoperative insulin resistance in rectal cancer patients using a two-step hyperinsulinemic euglycemic clamp. The novel supplement was compared to a placebo. To note, preOp® (Nutricia, Zoetermeer, the Netherlands) was not used as a placebo,

since the usage, amount and composition of carbohydrates in preOp® is slightly different from the carbohydrates in our novel mix of supplements. To have a reliable comparison, a placebo without carbohydrates was chosen.

## METHODS

### Patients

Patients undergoing rectal cancer surgery at the Northwest Clinics (Alkmaar, the Netherlands) were included in the study. Inclusion criteria were: age between 18 and 80 years, patients with a rectal tumor with an indication for either an elective low anterior resection (LAR) with an ileostoma or an abdominoperineal resection (APR) with a colostomy, patients who received preoperative radiotherapy (5x5 Gy) or chemo-radiotherapy, and written informed consent. Exclusion criteria were: severe malnutrition according to the Nutritional Risk Screening, NRS 2002 [10], severe renal insufficiency (creatinine clearance <30 mL/min), diabetes mellitus, concomitant thyroid or corticosteroid medication, impaired oral intake, severe cardiovascular dysfunction, respiratory dysfunction, ongoing infection, psychiatric diseases, suspicion of drug or alcohol abuse, pregnancy, known or suspected allergy to any component of the investigational product, and participating in another trial.
The study was approved by the loco-regional Medical Ethics Committee and was conducted according to the Declaration of Helsinki (as revised in 1983). The study is registered at www.clinicaltrials.gov (NCT00730808).

### Study design

The study was a single center prospective, placebo-controlled, double blind, randomized pilot study. At the outpatient clinic, patients eligible were approached for participation in the study. After inclusion, the patients were prospectively assigned to one of the two groups, equally distributing patients scheduled for LAR and APR between the groups.
In the week prior to surgery, glucose and lipid metabolism were studied in the basal state and during a two-step hyperinsulinemic euglycemic clamp after an overnight fast. The day before surgery, patients were admitted to the hospital for preoperative bowel preparation. After the bowel preparation patients were only allowed to drink clear fluids. A 250 mL preoperative oral nutritional supplement (Fresenius Kabi Deutschland GmbH) or placebo (same volume and taste; see Table 1) was given 15, 11 and 4 hrs before surgery. Timing of the last study drink before surgery was planned 4 hrs before surgery, since Lobo et al. revealed a delayed gastric emptying with this novel nutritional supplement compared to a clear carbohydrate drink [11]. We could achieve timely administration of the drinks, since all patients were scheduled as first to be operated on

at their day of surgery. A three dosage schedule was chosen to mimic the two dosage schedule of preOp® (Nutricia, Zoetermeer, the Netherlands) and to provide sufficient amount of supplements. For glutamine it is shown that a short duration of administration can alter insulin sensitivity. Borel et al. showed that simultaneous infusion of glutamine enhanced insulin sensitivity during a hyperinsulinemic euglycemic clamp versus saline infusion [12]. Torres-Santiago et al. gave insulin dependent diabetes mellitus patients enteral glutamine in three doses of 0.25 mg/kg versus placebo, and showed an effect on glucose homeostasis in less than 24 hours [13].

**Table 1:** Content of the supplement and placebo

| | **Supplement per 250 ml** | **Placebo per 250 ml** |
|---|---|---|
| Glutamine | 15 g | |
| Antioxidants | | Orange juice concentrate<br>Modified starch<br>Sodium saccharin<br>Color E 104: Quinoline Yellow<br>Color E 110: Sunset Yellow FCF |
| ▪ Green tea extract | 1 g | |
| ▪ Vitamin C | 750 mg | |
| ▪ Vitamin E | 250 mg | |
| ▪ Beta-carotene | 5 mg | |
| ▪ Selenium | 150 µg | |
| ▪ Zinc | 10 mg | |
| Carbohydrates | 50 g | |

The patients underwent a LAR or an APR for rectal cancer under combined general and epidural anesthesia for per- and postoperative pain relief. To minimize differences between surgeries, all interventions were carried out with a minimally invasive technique by the same three experienced gastro-intestinal surgeons. And, no intravenous glucose was given postoperatively.

The patients had no special diet after surgery, but most of them only ate light meals postoperatively on the day of surgery. The day after surgery, glucose and lipid metabolism were studied for a second time, again after an overnight fast. This first postoperative day was chosen for two reasons: first, because postoperative insulin resistance is considered most pronounced on the first day after surgery [1]; second, to minimize the differences in dietary intake between the patients. At this first postoperative day dietary intake after surgery is still highly comparable between the patients.

Blood samples were taken in the post-absorptive state at the outpatient clinic and on the first, third, fifth and seventh postoperative day.

### Hyperinsulinemic euglycemic clamp study design

After admission to the Metabolic Unit of the MCA at 07:30 a.m., a catheter was inserted into an antecubital vein for infusion of the stable isotope-labeled glucose ([6.6-$^{2}H_{2}$] glucose; >99% enriched; Cambridge Isotopes, Andover, USA), insulin and glucose

20%. Another catheter was inserted in a retrograde fashion into a contralateral hand vein and the hand was kept in a thermo-regulated (60ºC) Plexiglas box for arterialized venous blood sampling. Saline was infused as NaCl 0.9% at a rate of 50 mL/h to keep the catheters patent.

At T=0h (08:00 a.m.), a blood sample was drawn for determination of background enrichment. Then, a primed continuous infusion of [6,6-$^2H_2$]glucose was started (prime: 11 µmol/kg; continuous: 0.11 µmol/kg·min) and continued until the end of the study. After an equilibration period of two hours, three blood samples were drawn for the determination of isotope enrichments, glucoregulatory hormones and free fatty acids (FFA) concentrations. Thereafter (T=2h) a two-step hyperinsulinemic euglycemic clamp was started: step 1 included an infusion of insulin at a rate of 10 mU/m$^2$·min (Actrapid 100 IU/ml; Novo Nordisk Farma B.V., Alphen aan den Rijn, the Netherlands) to assess hepatic insulin sensitivity. Infusion of glucose 20% was started to maintain a plasma glucose level of 5 mmol/L. [6,6-$^2H_2$] glucose was added to the glucose 20% solution to achieve glucose enrichments of 1% to approximate the values for enrichment reached in plasma and thereby minimizing changes in isotopic enrichment due to changes in the infusion rate of exogenous glucose [14]. Plasma glucose levels were measured every five minutes. After two hours (T=4h), five blood samples were drawn at five minute intervals for determination of glucose concentrations and isotopic enrichments. Another blood sample was drawn for determination of glucoregulatory hormones and FFA. Hereafter, insulin infusion was increased to a rate of 40 mU/m$^2$·min (step 2) to assess peripheral insulin sensitivity. After two more hours (T=6h), blood sampling was repeated.

**Laboratory measurements**

Plasma substrates and hormones: Plasma glucose concentrations were determined upon collection using the Biosen C-line GP (EKF Diagnostic®, Magdeburg, Germany). The [6,6-$^2H_2$]glucose enrichment was measured as described previously [15]. Insulin was measured by a chemiluminescent immunoassay on an Immulite 2000 analyzer (Siemens Medical Solutions Diagnostics, New York, USA). Cortisol was measured by a chemiluminescent immunoassay on an Advia Centaur analyzer (Bayer Diagnostics, New York, USA). FFA were determined spectrophotometrically using an enzymatic method (RX Daytona NEFA kit, Randox Laboratories Ltd., Country Antrim, UK). Glucagon was determined by a radioimmunoassay (Linco, St Charles, MO).

Inflammatory parameters: IL-6 was measured by an ELISA technique (Pelikine human IL-6, Sanquin, Amsterdam, The Netherlands). CRP was measured on a Synchron analyzer (Beckman Coulter, Fullerton, California). WBC was measured on a Sysmex XE2100 analyzer (Sysmex Corporation, Kobe, Japan). LBP was measured by an automated chemiluminescent immunometric assay (Immulite®; DPC, Los Angeles,

CA, USA). MPO was determined by an ELISA technique (Elizan, MPO, Zentech S.A., Angleur, Belgium).

Antioxidant/oxidant parameters: Glutamine and glutamate were determined by reversed-phase HPLC as previously described [16]. GSH-peroxidase determination was performed using RANSEL reagent (Randox, Crumlin, UK) based on the method of Paglia and Valentine [17], in which the enzyme catalyses the oxidation of GSH into GSSG by Cumene hydroperoxide and the reduction of GSSG by GSH reductase in the presence of NADPH is measured at 340 nm. Total GSH was measured by reverse-phase HPLC on a Dionex system (Dionex, Voisins Le Bretonneux, France) by means of electrochemical detection. Vitamin E and ß-carotene were determined as described by Miller and Yang [18]. Zinc and selenium were determined using Zeeman corrected atomic absorption spectrometry. The flame was used for zinc determination. A graphite furnace and Palladium-modifier were used for the determination of selenium. TEAC was measured as described by Miller et al. [19]. Plasma $F_2$-Isoprostane: The total, i.e. free and esterified, concentration of iPF2α-VI was determined by liquid chromatography tandem mass spectrometry (LC-MS/MS) as described by Fischer et al. [20]. Plasma MDA concentrations were determined using HPLC with fluorescence detection as described by Van De Kerkhof et al. [21].

**Calculations and statistical analyses**

Sample size estimation of the number of patients needed for the present study was based on the results of a previous study, where a preoperative carbohydrate drink was used in elective colorectal surgery patients and a similar hyperinsulinemic euglycemic clamp method was used, and a minimum of 7 patients in each group was chosen in order to have a 80% power of detecting difference in insulin resistance of 25% with a standard deviation of 16% at a significance level of 0.05 [3]. As the present study has a slightly different patient population and a different beverage, we included 10 patients in each group.

Endogenous glucose production (EGP) and peripheral glucose uptake (rate of disappearance: Rd) were calculated using modified forms of the Steele Equations as described previously [14].

All single group comparisons were analyzed with non-parametric analyses, due to small sample size and probability of unequal distribution. Comparisons between groups were analyzed using the Mann-Whitney U test. Comparisons within groups were analyzed with the Wilcoxon Signed Rank test. The SPSS software (version 19.0, SPSS Inc., Chicago, IL) was used for statistical analyses.

Differences over time in postoperative inflammatory parameters and antioxidant/oxidant parameters between the supplemented and placebo group were analyzed using general estimating equations (GEE). GEE is a linear regression technique suitable for analyzing

data from a longitudinal study in which outcome variables are repeatedly measured in each individual. The difference between the supplemented and placebo group over time were analyzed in two ways. In the first analysis the average difference over time between the groups was analyzed, while the second analysis assessed whether the differences between the groups changed over time. This was investigated by adding an interaction term between time and group to the GEE model. All GEE analyses were adjusted for baseline differences between the groups in each particular outcome variable. GEE analyses were performed with STATA® (version 11.0), and for all statistical analyses a p-value <0.05 was considered statistically significant.

## RESULTS

### Patients

Thirty-two patients were eligible for inclusion, of whom twenty were included (Figure 1). Of the twelve patients that were not included, eight did not want to participate, two were not included due to logistic reasons, one patient deceased before participation, and one patient chose to undergo surgery in another hospital. Of the twenty patients studied, two were excluded from the final analyses, one because of receiving preOp® (Nutricia, Zoetermeer, the Netherlands) preoperatively instead of the study drink or placebo, and one because of newly diagnosed diabetes mellitus with a fasted glucose level higher than 7.0 mmol/L. There were no differences in patient characteristics between the groups (Table 2). The supplement and placebo were well tolerated by the patients and no side effects were observed.

### Hepatic insulin sensitivity

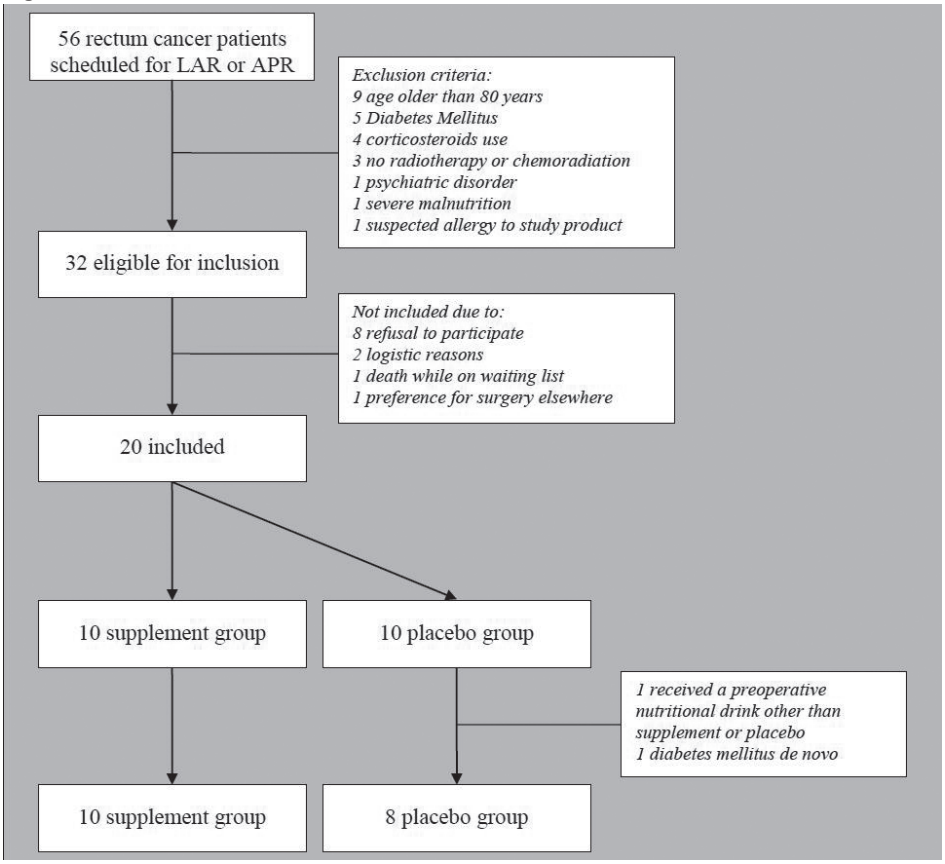
Data on basal endogenous glucose production (EGP) and hepatic insulin sensitivity are presented in Table 3. EGP and hepatic insulin sensitivity (expressed as insulin-mediated suppression of EGP) did not differ between the groups, neither in the postoperative basal state, nor in step 1 and step 2 of the postoperative clamp.

Postoperative basal EGP was higher and hepatic insulin sensitivity was lower after surgery in the supplemented group. While, in the placebo group basal EGP and hepatic insulin sensitivity assessed during step 1 of the clamp did not differ from the preoperative condition. During step 2, EGP suppression was significantly lower in both groups after surgery, with no differences between the groups.

### Peripheral insulin sensitivity

Results on peripheral insulin sensitivity (expressed as glucose Rd) are shown in Table 3 and Table 4. In both the preoperative and postoperative state, in step 2 the glucose Rd

**Figure 1:** Flow Chart inclusions




*LAR = low anterior resection; APR = abdominoperineal resection.*

**Table 2:** Patient characteristics

| | Supplement | Placebo | p-value |
|---|---|---|---|
| **Male / Female** | 7 / 3 | 6 / 2 | 0.82 |
| **Age** (yr) | 60 [53-68] | 62 [49-79] | 0.72 |
| **Height** (m) | 1.77 [1.67-1.93] | 1.74 [1.65-1.90] | 0.72 |
| **Weight** (kg) | 80.5 [58.0-91.3] | 81.7 [71.3-97.4] | 0.79 |
| **BMI** (kg/m$^2$) | 25.0 [20.8-28.1] | 26.2 [23.3-31.1] | 0.33 |
| **FFM** (%) | 79 [52-90] | 78 [61-87] | 0.72 |
| **FM** (%) | 21 [10-48] | 22 [13-39] | 0.72 |
| **NRS 2002 total score** | | | 0.81 |
| **1** | 8 | 6 | |
| **2** | 2 | 2 | |
| **Type of surgery: LAR / APR** | 7 / 3 | 7 / 1 | 0.39 |

*BMI = body mass index; FFM = fat free mass; FM = fat mass; NRS = Nutritional Risk Screening (total score <3 means no need for nutritional support); LAR = low anterior resection; APR = abdominoperineal resection; data are expressed as median [range] or as quantity. There were no significant differences between the supplement and the placebo group.*

**Table 3.** Pre- and postoperative glucose and fatty acid metabolism & Insulin-mediated suppression of EGP and FFA levels

| | group | Preoperative | | | Postoperative | | |
|---|---|---|---|---|---|---|---|
| | | **Basal** | **step 1** | **step 2** | **basal** | **step 1** | **step 2** |
| **Glucose** | S | 5.3 [5.0-5.7] | 5.0 [4.8-6.0] | 5.1 [4.8-5.8] | 6.0 [5.1-7.0]* | 5.5 [4.8-6.1] | 5.0 [3.5-5.7] |
| (mmol/L) | P | 5.7 [5.2-6.8]‡ | 5.1 [4.9-5.6] | 5.0 [4.7-5.0] | 6.4 [4.8-7.1] | 5.5 [5.0-6.3] | 5.0 [4.8-5.2] |
| **EGP** | S | 11.5 [9.3-14.2] | 5.7 [4.0-8.8] | 1.2 [0.0-3.4] | 13.4 [11.8-14.9]* | 9.5 [6.3-10.9]* | 3.8 [0.6-8.3]* |
| (µmol/kg·min) | P | 11.7 [10.2-12.9] | 7.8 [3.6-9.1] | 1.3 [0.0-2.7] | 12.5 [9.9-15.7] | 8.9 [4.4-11.3] | 3.5 [1.2-5.2]* |
| **Rd** | S | - | 12.6 [8.5-19.1] | 37.2 [19.1-50.9] | - | 10.7 [8.9-13.9] | 20.6 [13.9-27.9]* |
| (µmol/kg·min) | P | - | 9.9 [8.6-11.2]‡ | 23.8 [15.7-35.5]‡ | - | 9.4 [7.9-12.5] | 15.3 [12.6-19.1]‡* |
| **FFA** | S | 0.61 [0.40-0.97] | 0.18 [0.07-0.37] | 0.05 [0.04-0.09] | 0.61 [0.41-0.79] | 0.23 [0.13-0.43] | 0.08 [0.06-0.14] |
| (mmol/L) | P | 0.73 [0.34-1.14] | 0.21 [0.08-0.43] | 0.06 [0.04-0.11] | 0.65 [0.50-0.94] | 0.30 [0.22-0.89] | 0.09 [0.06-0.24]* |

| | group | Preoperative | Preoperative | Postoperative | Postoperative |
|---|---|---|---|---|---|
| | | **Δ basal-step 1** | **Δ basal-step 2** | **Δ basal-step 1** | **Δ basal-step 2** |
| **Insulin-mediated suppression EGP** (%)§ | S | 53 [30-64] | 89 [74-100] | 31 [22-48]* | 72 [38-95]* |
| | P | 37 [21-65] | 88 [77-100] | 28 [11-56] | 74 [59-88]* |
| **Insulin-mediated suppression FFA** (%)§ | S | 74 [38-88] | 92 [87-94] | 57 [29-76]* | 88 [77-90]* |
| | P | 68 [21-85] | 92 [79-95] | 51 [5-66]* | 89 [52-89]* |

*S = supplement; P = placebo; EGP = endogenous glucose production; FFA = free fatty acids; data are expressed as median [range], * p-value < 0.05 vs. preoperative using Wilcoxon Signed Rank Test; ‡ p-value < 0.05 vs. supplement using Mann-Whitney U Test; § there were no significant differences between the groups.*

was higher in the supplemented group. Postoperative peripheral insulin sensitivity was significantly lower in both groups. In terms of absolute values the postoperative drop in glucose Rd was less pronounced in the supplemented group (Table 3). The relative decrease in Rd after surgery, expressed as percentage reduction was not different between the groups (Table 4).

**Adipose tissue insulin sensitivity**

Data on insulin sensitivity of adipose tissue are presented in Table 3. Although suppression of FFA was significantly lower in both groups in the postoperative condition, adipose tissue insulin sensitivity (expressed as insulin mediated suppression of FFA production) did not differ after surgery between the groups.

**Glucoregulatory hormones**

Glucoregulatory hormone levels are shown in Table 5. Levels of insulin, glucagon and cortisol were not different between the supplemented and the placebo group, neither in the basal state, nor during step 1 or step 2 of the clamp in both, preoperative and postoperative conditions.

However, within group analysis showed that in both groups, postoperative glucagon levels in the basal state and in step 1 and step 2 were significantly higher compared to the preoperative state. Basal postoperative cortisol levels were significantly higher in both groups, while during the clamp postoperative cortisol levels were only higher in the supplemented group.

**Table 4.** Reduction in insulin-mediated pre and postoperative glucose Rd

| | Group | ∆ Pre - postoperative step 1 | ∆ Pre - postoperative step 2 |
|---|---|---|---|
| **Reduction in Rd** (%) | S | 11.9 [-23.1 - 39.4] | 40.7 [-21.3 - 63.6] |
| | P | 4.3 [-15.3 - 22.1] | 28.2 [-16.2 - 60.5] |

*S = supplement; P = placebo; Data are expressed as percentage reduction. There were no significant differences between the groups.*

**Table 5:** Glucoregulatory hormones

| | group | Preoperative | | | Postoperative | | |
|---|---|---|---|---|---|---|---|
| | | **Basal** | **step 1** | **step 2** | **basal** | **step 1** | **step 2** |
| **Insulin** | S | 45 [<15-150] | 96 [66-149] | 306 [368-582] | 74 [31-100] | 96 [21-144] | 298 [169-419]* |
| (pmol/L) | P | 72 [23-124] | 96 [62-152] | 351 [256-459] | 50 [<15-110] | 101 [41-136] | 354 [199-525] |
| **Cortisol** | S | 327 [177-462] | 332 [240-465] | 304 [181-439] | 621 [234-823]* | 478 [337-1012]* | 426 [272-997]* |
| (nmol/L) | P | 199 [124-463] | 309 [120-384] | 235 [167-464] | 440 [287-974]* | 405 [178-772] | 348 [182-593] |
| **Glucagon** | S | 20 [<15-65] | 22 [<15-49] | 15 [<15-40] | 72 [<15-187]* | 57 [26-100]* | 51 [<15-77]* |
| (ng/L) | P | 36 [<15-87] | 27 [<15-90] | 19 [<15-64] | 81 [<15-162]* | 84 [<15-276]* | 59 [<15-326]* |

*S = supplement; P = placebo; data are expressed as median [range]; * p-value < 0.05 vs. preoperative using Wilcoxon Signed Rank Test; there were no significant differences between the groups*

### Inflammatory and antioxidant/oxidant parameters

Inflammatory and antioxidant/oxidant parameters are shown in Table 6. Surgery induced an inflammatory response as shown by increased CRP, WBC and IL-6 levels on the first postoperative day in both groups. GEE analysis showed that, in the supplement group, the increase in CRP levels over time was less pronounced. This suggests an attenuated inflammatory response in the supplemented group.

A state of increased oxidative stress with higher postoperative MDA levels was present in both groups. The supplement had no effect on MDA and GSH levels. Compared to preoperative levels, plasma concentrations of glutamine, glutamate, beta-carotene, zinc, $F_2$-isoprostane dropped in both groups on the first postoperative day to a similar extent. Selenium and TEAC levels decreased in the placebo group on the first postoperative day compared to the preoperative state, whereas no difference was observed in the supplemented group. In the postoperative period, the selenium levels of both groups converged, since the GEE analyses showed a significant difference in the average difference over time analysis as well as in the change over time analysis.

## DISCUSSION

Our results show that uncomplicated rectal cancer surgery induces insulin resistance, consisting of reduced insulin action in liver, muscle and adipose tissue. A preoperative drink containing carbohydrates, glutamine and antioxidants could not prevent the postoperative decline in insulin stimulated glucose and fatty acid metabolism, although the higher absolute glucose Rd in the supplemented group suggests some attenuation of postoperative peripheral insulin resistance.

**Table 6:** Inflammatory and antioxidant/oxidant parameters

| | group | Preoperative | Postoperative | | | | GEE analysis | |
|---|---|---|---|---|---|---|---|---|
| | | | day 1 | day 3 | day 5 | day 7 | *1‡* | *2‡* |
| **CRP** (mg/L) | S | 3 [1-25] | 117 [40-210]* | 85 [9-322] | 62 [14-210] | 76 [10-252] | *0.577* | *0.003* |
| | P | 3 [1-8] | 114 [82-159]* | 145 [107-372] | 68 [38-142] | 25 [14-96] | | |
| **WBC** (10^9/L) | S | 3.8 [2.8-5.7] | 7.3 [4.6-9.5]* | 6.8 [4.3-53.0] | 4.8 [2.7-7.5] | 6.9 [2.5-13.8] | *0.422* | *0.904* |
| | P | 4.2 [2.8-5.3] | 8.2 [5.4-11.9]* | 6.0 [3.3-11.3] | 5.3 [3.4-8.5] | 5.7 [4.3-9.4] | | |
| **IL-6** (pg/mL) | S | 1.9 [0.7-5.0] | 60.6 [25.7-407.7]* | 9.0 [4.6-128.1] | 13.7 [4.3-43.2] | 11.3 [4.5-88.5] | *0.298* | *0.914* |
| | P | 1.9 [0.8-3.5] | 79.7 [45.0-124.4]* | 26.1 [11.3-35.2] | 14.4 [4.1-32.0] | 9.4 [4.9-16.2] | | |
| **LBP** (µg/mL) | S | 18 [7-37] | 51 [26-193]* | 48 [28-183] | 48 [19-110] | 49 [16-95] | *0.222* | *0.617* |
| | P | 16 [11-22] | 55 [36-112]* | 56 [22-83] | 45 [14-73] | 35 [18-62] | | |
| **MPO** (ng/mL) | S | 31 [18-65] | 70 [24-193]* | 49 [24-178] | 48 [24-79] | 64 [34-139] | *0.114* | *0.195* |
| | P | 23 [16-49] | 36 [20-47] | 54 [0-111] | 35 [15-109] | 30 [24-65] | | |
| **Glutamine** (µmol/L) | S | 566 [497-644] | 433 [326-633]* | 535 [374-677] | 552 [398-769] | 610 [390-882] | *0.063* | *0.805* |
| | P | 548 [522-648] | 386 [316-516]* | 483 [365-612] | 510 [356-571] | 598 [527-631] | | |
| **Glutamate** (µmol/L) | S | 68 [46-79] | 41 [24-55]* | 40 [21-62] | 41 [28-78] | 60 [41-100] | *0.848* | *0.945* |
| | P | 74 [50-92] | 43 [21-67]* | 31 [29-60] | 42 [18-106] | 53 [33-143] | | |
| **Beta-carotene** (µmol/L) | S | 0.50 [0.23-1.70] | 0.28 [0.17-0.91]* | 0.31 [0.16-0.97] | 0.24 [0.13-0.93] | 0.18 [0.06-1.00] | *0.774* | *0.145* |
| | P | 0.42 [0.20-1.10] | 0.24 [0.11-0.62]* | 0.24 [0.08-0.59] | 0.22 [0.08-0.67] | 0.18 [0.13-0.59] | | |
| **Vitamin E** (µmol/L) | S | 38 [18-48] | 24 [14-29]* | 29 [16-38] | 28 [19-33] | 26 [15-34] | *0.636* | *0.929* |
| | P | 41 [31-45] | 26 [20-33]* | 28 [24-34] | 27 [23-36] | 30 [21-37] | | |
| **Selenium** (µmol/L) | S | 1.02 [0.69-1.20] | 0.92 [0.56-1.30] | 0.88 [0.54-1.20] | 0.87 [0.55-1.30] | 0.75 [0.60-1.10] | *0.003* | *0.000* |
| | P | 1.05 [0.78-1.20] | 0.72 [0.60-0.87]* § | 0.77 [0.63-0.91] | 0.78 [0.62-0.96] | 0.79 [0.69-1.00] | | |
| **Zinc** (µmol/L) | S | 10.4 [9.4-34.0] | 5.0 [3.5-16.0]* | 6.7 [3.9-22.0] | 8.2 [5.4-29.0] | 9.6 [4.3-22.0] | *0.646* | *0.605* |
| | P | 9.7 [8.4-11.0] | 5.0 [2.7-6.2]* | 6.3 [5.9-8.6] | 7.4 [6.8-11.0] | 8.2 [7.1-11.0] | | |
| **Glutathione** (µmol/L) | S | 452 [367-678] | 562 [266-792] | 543 [303-811] | 520 [310-720] | 496 [242-712] | *0.247* | *0.442* |
| | P | 730 [354-1036] | 630 [402-957] | 661 [371-1073] | 598 [342-1106] | 601 [459-896] | | |

**Table 6:** Inflammatory and antioxidant/oxidant parameters (*continued*)

| | group | Preoperative | Postoperative | | | | GEE analysis | |
|---|---|---|---|---|---|---|---|---|
| | | | day 1 | day 3 | day 5 | day 7 | *1‡* | *2‡* |
| **GSH-Px activity** (µmol/min/mL) | S | 1.47 [0.48-2.48] | 1.08 [0.15-1.82] | 1.37 [0.14-2.85] | 1.00 [0.43-3.08] | 1.45 [0.20-5.45] | *0.612* | *0.073* |
| | P | 1.48 [0.20-2.59] | 1.13 [0.50-3.90] | 1.39 [0.80-3.58] | 0.86 [0.13-1.59] | 1.16 [0.18-2.01] | | |
| **TEAC** (mmol/L) | S | 1.3 [1.2-1.7] | 1.3 [1.0-1.7] | 1.3 [1.0-1.7] | 1.3 [1.1-1.8] | 1.4 [1.1-1.8] | *0.399* | *0.338* |
| | P | 1.3 [1.2-2.0] | 1.1 [0.9-1.7]* | 1.3 [1.1-1.7] | 1.4 [1.0-1.7] | 1.3 [1.1-1.9] | | |
| **F2-Isoprostane** (pg/mL) | S | 80.4 [58.4-126.4] | 60.9 [43.4-96.4]* | 78.3 [57.0-110.8] | 79.3 [53.2-142.0] | 86.6 [49.8-186.0] | *0.790* | *0.581* |
| | P | 79.7 [57.2-117.4] | 53.6 [37.6-81.0]* | 73.4 [55.4-112.0] | 85.5 [53.2-194.8] | 82.2 [53.4-135.0] | | |
| **MDA** (µmol/L) | S | 4.96 [4.70-6.06] | 6.86 [5.81-8.69]* | 6.37 [4.81-7.72] | 6.44 [5.61-7.57] | 6.30 [4.57-7.64] | *0.738* | *0.732* |
| | P | 5.45 [5.03-6.13] | 7.03 [5.34-8.94]* | 6.70 [5.36-7.63] | 7.00 [4.89-7.81] | 6.78 [5.56-7.83] | | |

*S = supplement; P = placebo; CRP = C-reactive protein; WBC = white blood cell count; IL-6 = interleukin 6; LBP = lipopolysaccharide binding protein; MPO = myeloperoxidase; GSH-Px = glutathione-peroxidase; TEAC = total endogenous antioxidant capacity; MDA = malondialdehyde; data are expressed as median [range], * p-value < 0.05 day 1 postoperative vs. preoperative using Wilcoxon Signed Rank Test, § p-value < 0.05 vs. supplement at day 1 postoperative using Mann-Whitney U Test; 1‡ p-value of an average difference over time between supplement and placebo group, corrected for baseline using GEE-analysis. 2‡ p-value of a change over time between supplement and placebo group, using GEE analysis.*

It is noteworthy that although our patients were operated on by minimally invasive techniques, the trauma response in rectal cancer surgery, in terms of postoperative insulin resistance, is profound. Thus, despite minimally invasive techniques and using an advanced recovery program, surgeons should be aware of the profound metabolic consequences of rectal cancer surgery. A significant stress response was induced as shown by the elevated postoperative levels of cortisol, glucagon, CRP and IL-6, accompanying the profound postoperative insulin resistance. Only a few studies in colorectal surgery patients reported on postoperative insulin sensitivity. Svanfeldt et al. showed a negative effect of colorectal surgery on hepatic insulin sensitivity using a hyperinsulinemic euglycemic clamp method [22]. In a study of Nygren et al. the euglycemic hyperinsulinemic clamp detected postoperative insulin resistance of glucose and lipid metabolism after major surgery for benign colorectal disease [3]. But no previous study has reported on insulin sensitivity of liver, muscle and adipose tissue simultaneously in patients undergoing rectal cancer surgery.

In healthy individuals, infusion of insulin at a low concentration suppresses hepatic glucose production completely [23]. In our patients, we showed no complete suppression of hepatic glucose production with a similar low insulin concentration infusion. This indicates profound hepatic insulin resistance in our study patients. Recently, it has been shown that lower insulin suppression of FFA contributes significantly to hepatic insulin resistance [24], which in part could be an explanation of lower insulin action in the liver after surgery. This is in line with the lack of effect of the supplementation of antioxidants in hepatic insulin sensitivity. Of note, the supplemented group had a significantly higher EGP after surgery, which could not be explained by differences in the postoperative levels of glucoregulatory hormones, but other hormones like epinephrine (not measured) might have been involved in the higher EGP in the basal state of the supplemented group [25]. It can be concluded that the supplement did not inhibit the postoperative reduction in hepatic insulin sensitivity.

Postoperative peripheral insulin sensitivity was profoundly reduced in the placebo and supplemented group. In terms of absolute Rd values, postoperative peripheral insulin sensitivity was higher in the supplemented group (Table 3). Although this shows an effect of the supplement, the lack of difference in terms of relative reduction from preoperative values between the groups suggests otherwise (Table 4). This apparent contradiction is explained by the higher peripheral glucose sensitivity in the supplemented group both before and after surgery. Noteworthy, preoperative glucose Rd values were measured before supplementation was started. Thus, despite proper randomization, the groups had different peripheral insulin sensitivity at the start of the study that may have worked through after surgery. However, it is unknown whether insulin sensitivity before surgery influences the extent of its postoperative decline. Looking at the study of Nygren et al. on the effect of a preoperative carbohydrate beverage versus fasting in patients

undergoing comparable stressful benign colorectal surgery, the significant preoperative differences in glucose Rd did not work through after surgery because no difference in postoperative glucose Rd was noted. The reported lower relative postoperative reduction in peripheral insulin sensitivity in the supplemented group therefore is only explained by the higher preoperative Rd in the fasted group [3]. It can be argued, whether in the absence of a difference in postoperative Rd there is an effect of the intervention at all. Thus, in terms of differences in absolute postoperative Rd values, the supplement attenuated peripheral insulin resistance, but in terms of relative percentage change it did not. These considerations question the value of the current preoperative randomization and stratification in studies investigating insulin sensitivity. It would be best to have groups that are highly comparable in preoperative insulin sensitivity before randomization starts. We acknowledge the practical and financial difficulty of achieving this but it should be considered for future studies.

Elevated plasma FFA concentrations reduce insulin-mediated glucose uptake, stimulate EGP and are used as a marker of insulin resistance [24, 26]. Therefore, the postoperative reduction in insulin suppression of FFA might have contributed to the overall decline in insulin sensitivity in the study patients.

The composition of the oral supplement drink was designed to modulate the inflammatory and oxidative stress response after surgery. However, except for selenium, the preoperative drink was not able to prevent the postoperative drop in plasma levels of the supplemented trace elements. Although plasma levels may not reflect biological availability, the lack of any effect on markers of oxidative stress and antioxidant capacity compared to placebo indicates that the preoperative drink in the concentrations used had no relevant effect on oxidative stress.

Glutamine was added to the supplement because it influences insulin secretion and it contributes to better glycemic control [27]. For instance, in trauma patients intravenously administered alanyl-glutamine improves insulin-mediated glucose uptake 8. Therefore, preoperative oral administration of glutamine was expected to attenuate postoperative insulin resistance. However, even at a dose higher than the recommended dose of 0.5g/kg bodyweight [28], it did not prevent the postoperative drop in plasma glutamine. This seems to be in contrast with the study of Awad et al., who showed that a similar supplement in patients undergoing laparoscopic cholecystectomy resulted in higher postoperative glutamine plasma levels compared to a placebo group [29]. Apparently, glutamine consumption is higher after rectal cancer surgery, explaining the lack of effect on postoperative glutamine plasma levels in the present study. Additionally, it is unclear if supplementing glutamine for only 15 hours before surgery is sufficient enough to elicit an effect on postoperative insulin sensitivity. In burned children it was shown that a 48 hour glutamine supplementation period was not sufficient to have an effect on protein synthesis [30]. However, for effects on insulin sensitivity, rapid effects of intravenous

glutamine have been found by Borel et al., who showed enhanced insulin sensitivity by infusing glutamine during a hyperinsulinemic euglycemic clamp compared to saline infusion in dogs [12]. Further studies in humans using supplements via the enteral route are needed to show whether the period of supplementation of glutamine influences postoperative insulin sensitivity. And, the contribution of glutamine in the present oral supplement on Rd cannot be deducted from our study design, since we studied a mix of supplements together.

As for glutamine, an effect of selenium supplementation on postoperative insulin sensitivity was expected, since selenium may act as an insulin-mimetic micronutrient and has anti-oxidative capacity as a constituent of ROS-detoxifying seleno-enzymes [31]. The plasma selenium levels in our supplemented group remained in the normal range after supplementation on the first postoperative day, compared to a decrease in the placebo group. This is in line with results showed by Braga et al. in cancer patients undergoing elective pancreaticoduodenectomy, who received a similar preoperative nutritional supplement as in the present study [32].

Finally, it has been shown that inhibiting redox signaling by supraphysiological amounts of vitamin C (1 g/day) and vitamin E (400 IU/day) impairs insulin sensitivity [33]. In the present study, postoperative plasma levels of vitamin E were in the (sub)normal range and an inhibiting effect on insulin action is unlikely, but may have obscured an effect of other additives like glutamine and carbohydrates.

**Limitations of the study**

The used oral supplement contained carbohydrates, antioxidants and glutamine. Therefore, we can only draw conclusions on the combined effect on insulin sensitivity. Also, some of the supplements could have counteract possible positive or negative effect(s) of the others, since antioxidants are known to be able to act as an oxidant as well.

Second, the supplement was administered three times preoperatively, mimicking the usage of preOp® (Nutricia, Zoetermeer, the Netherlands). It can be debated if the lack of a clinically significant effect of the supplement on postoperative insulin resistance is due to dosage and/or duration of the treatment. However, with just two administrations before surgery, the carbohydrate rich preOp® already attenuated the postoperative insulin resistance in benign colorectal surgery when compared to 24 hours fasted controls [3]. With three preoperative administrations in less than 24 hours in the present study a small but significant attenuation of peripheral insulin resistance was found. The used combination with glutamine and antioxidants was expected to result in a more significant effect. For these supplements to achieve a more profound effect on insulin sensitivity a longer period of administration may have been necessary. A topic that warrants future studying.

Third, one can also debate about the timing of the drinks, and especially of the last drink before surgery. The last drink of preOp® is given two hours before surgery to induce insulin release until surgery. In this way, the extra circulating insulin will overcome the developing insulin resistance by forcing glucose into the cell. In our study, due to the earlier timing of the last drink before surgery, this effect of the carbohydrates may have been less. This may explain the small effect on peripheral insulin sensitivity.
Fourth, to make a placebo identical in color and flavor to the supplement, we had to make concessions about its metabolic activity. Since the placebo contains orange juice concentrate and modified starch, although in very small amounts, it still may have influenced insulin sensitivity. However, we strongly felt that a placebo drink was necessary, because preoperative fasting is not done anymore. Thus, a possible effect on insulin resistance by the placebo drink cannot be discarded and may have reduced the difference with the effect induced by the supplement. This is an interesting issue, because the effect of the carbohydrate rich preOp® drink on peripheral insulin resistance in benign colorectal patients was found in comparison to fasted controls, not receiving a placebo drink [3]. In the type of patients having similar surgical trauma as in the present study there is no information in comparison to a placebo drink. However, using a placebo control, the preOp® drink, in patients undergoing elective total hip replacement Soop et al. were able to show better preservation of insulin sensitivity using the preOp® drink [34]. In the era where we have abandoned preoperative fasting, selecting the proper content of a placebo drink in studies on insulin sensitivity should be debated.

**Conclusion**

Surgery for rectal cancer induces a profound state of early postoperative insulin resistance with reduced insulin action in liver, muscle and adipose tissue. An oral preoperative supplement, containing carbohydrates, glutamine and antioxidants, could not prevent this decline in insulin sensitivity, although a slight attenuation in the decrease of postoperative peripheral insulin sensitivity was found.

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