Perioperative infusion of glucagon like peptide-1 prevents insulin resistance after surgical trauma in female pigs

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ABSTRACT

Insulin resistance is an independent negative predictor of outcome after elective surgery and increases mortality among surgical patients in intensive care. The incretin hormone glucagon like peptide-1 (GLP-1) potentiates glucose-induced insulin release from the pancreas, but may also increase insulin sensitivity in skeletal muscle and directly suppress hepatic glucose release. Here, we investigated whether a perioperative infusion of GLP-1 could counteract the development of insulin resistance after surgery. Pigs were randomly assigned to 3 groups; surgery/control, surgery/GLP-1 and sham/GLP-1. Both surgery groups were subjected to major abdominal surgery. Whole body glucose disposal (WGD) and endogenous glucose release (EGR) were assessed pre- and postoperatively using D-[6,6-2H2]-glucose infusion in combination with hyperinsulinemic euglycemic step-clamping. In the surgery/control group, peripheral insulin sensitivity (i.e. WGD) was reduced by 44% relative to preoperative conditions, whereas the corresponding decline was only 9% for surgery/GLP-1 (P < 0.05). Hepatic insulin sensitivity (i.e. EGR) remained unchanged in the surgery/control group, but was enhanced after GLP-1 infusion in both surgery and sham animals (40% and 104%, respectively, both P<0.05). Intraoperative plasma glucose increased in surgery/control (~20%), but remained unchanged in both groups receiving GLP-1 (P < 0.05). GLP-1 diminished an increase in postoperative glucagon levels, but did not affect skeletal muscle glycogen or insulin signalling proteins after surgery. We show that GLP-1 improves intraoperative glycemic control, diminishes peripheral insulin resistance after surgery and suppresses EGR. This study supports the use of GLP-1 to prevent development of postoperative insulin resistance.
Insulin resistance arises after any trauma, including surgery. It is an independent negative predictor of outcome and length of hospital stay for elective surgical patients (1,2), and it is associated with increased mortality among those in intensive care (3). In the immediate phase after surgery insulin resistance is of peripheral origin, and as skeletal muscle accounts for ~80% of insulin stimulated glucose disposal it is considered the main site of impaired insulin response. While different means to improve insulin sensitivity and maintain glycemic control are generally recommended as part of elective surgery (4,5), tight glycemic control with insulin infusion reduces mortality for intensive care surgical patients (3).

The incretin hormone glucagon like peptide-1 (GLP-1) is released postprandially from the small intestine and augments glucose-dependent release of insulin from pancreatic β-cells during hyperglycemia, inhibits glucagon release and stimulates β-cell proliferation (6). Incretin based therapies are now widely used in treatment of chronic insulin resistance and are increasingly being acknowledged to exhibit extra-pancreatic properties. GLP-1 has been shown to directly suppress hepatic endogenous glucose release (EGR) (7,8) and to improve insulin mediated glucose uptake in peripheral tissues (9–12). The latter is suggested to result from a direct effect of GLP-1 on myocytes by increasing glycogen synthesis through activation of intracellular insulin signalling (13–15), although such direct effects remain controversial (16–21).

Thus, as GLP-1 may improve both insulin sensitivity and glycemic control (22) with practically no risk of hypoglycemia, it is a potential in-hospital therapeutic agent for acute conditions with reduced insulin sensitivity. The use of GLP-1 in surgical and ICU patients has not been put into clinical practise, although small scale clinical studies show promising results for improving glycemic control (23–28). No study has examined if GLP-1 also can diminish peripheral trauma-induced insulin resistance. The gold standard for measuring insulin
sensitivity is the hyperinsulinemic euglycemic clamp (HEC) (29) and when combined with a glucose tracer, it can estimate both basal and insulin-stimulated hepatic and peripheral insulin sensitivity (30,31).

Here, we utilized the euglycemic clamp technique to investigate if a perioperative infusion of GLP-1 could improve peripheral and hepatic insulin sensitivity after surgical trauma, with whole body glucose disposal (WGD) during HEC, as the study’s primary outcome. Secondly, we evaluated intraoperative glycemic control, alterations in hormone release, glycogen content, intracellular insulin signalling and pancreatic β-cell insulin secretion capacity after GLP-1 infusion to investigate the nature of such an effect.
MATERIALS AND METHODS

Animals, anesthesia and instrumentation. The protocols were approved by the committee of the Norwegian Experimental Animal Board and 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 [Dept of Health and Human Services Publication no. (NIH) 85-23, revised 1985]. Female Yorkshire/Landrace hybrid pigs weighing ~29 kg were acclimatized at the animal research facilities for one week on a standardized diet and ad libitum access to water, but they were fasted 12 hours before the experiments. Experiments were commenced at 7 a.m. During all experiments, animals were sedated before orotracheal intubation and commencement of gas anaesthesia with isoflurane mixed with nitrous oxide/oxygen (40/60 %) together with infusions of fentanyl and midazolam (0.02 and 0.3 mg/kg/hr, respectively), as earlier described (31). An infusion of 0.9% sodium chloride (Braun) at an initial load of 30 mL/kg/hr for the first 30 min was administered and continued at 10 mL/kg/hr throughout the experiment. Respiration was monitored through a Capnomac instrument (Datex, Tewksbury, MA, USA) and anaesthesia was adjusted according to blood gas analysis (ABL 800 FLEX; Radiometer, Copenhagen, Denmark) and snout reflex tests. Invasive arterial pressure and heart rate was monitored together with body temperature for surveillance. Normal porcine body core temperature at 38.5 C° was maintained with heating blankets. At the end of the experiment on day two, the animals were euthanized with an infusion of pentobarbital (20 mg/kg).

Study design and surgical intervention. Group sizes were estimated with power analysis on glucose clamp data from pilot experiments with GLP-1, as well as considering required group sizes in earlier clamp protocols (32). The pigs were examined in two consecutive experiments on separate experimental days (see figure 1). On experimental day one, animals were anesthetized, and preoperative basal glucose turnover and peripheral and
hepatic insulin sensitivity were assessed by tracer infusion followed by two-step hyperinsulinemic-euglycemic clamping (HEC). Pancreatic insulin secretion capacity was assessed by a hyperglycemic clamp. Thereafter, the pigs were allowed 5 days for recovery and metabolic normalization. On experimental day two, animals were blindly randomized to receive surgery/control (n=7), surgery/GLP-1 (n=7) or a non-surgical sham/GLP-1 (n=5) intervention. Preoperative biopsies were then harvested immediately after onset of anaesthesia. GLP-1 infusion was commenced 15 min before onset of surgery at a rate of 10 pmol/min/kg, shown to be safe and appropriate for porcine metabolism in pilot experiments and earlier studies (33,34). The infusion was discontinued after 145 min at the end of surgery. The two surgery groups were subjected to a midline laparotomy with resection of 1.5 m small bowel with primary anastomosis 1.5 m proximal to the ileocecal junction (duration ~2 hours) to avoid affecting the GLP-1 producing L-cells in the terminal ileum of the pig (35). Incisions were closed and postoperative basal glucose turnover, insulin sensitivity (HEC) and insulin secretion capacity (hyperglycemic clamp) were measured in the immediate postoperative phase. The sham/GLP-1 group received the same anaesthetic regimen, infusions and instrumentation within the same timeframe, but did not undergo surgery. Pre-, intra- and postoperative arterial blood samples (serum and plasma) and tissue biopsies from skeletal muscle and liver where collected serially throughout the experiments (figure 1).

Tracer infusion and hyperinsulinemic-euglycemic step clamp (HEC). Basal glucose turnover was assessed during the last 30 min of a 90 min primed (6 mg/kg), continuous (0.12 mg/kg/min) infusion of D-[6,6-2H2]-glucose (basal period) (32). Thereafter, two consecutive 120 min hyperinsulinemic euglycemic (~4.5 mmol/L) clamps with labelled glucose infusate (2.1 % atom percent enrichment, APE%) were performed. Insulin was infused at rates of 0.4 mU/kg/min (low insulin clamp, HEC 1) and 1.2 mU/kg/min (high insulin clamp, HEC 2) to differentiate between hepatic and peripheral insulin sensitivity as previously described (31).
Glucose infusion rates (GIR) of the labelled infusate were calculated as an average of the last 40 min of each clamp. Tracer enrichment in arterial blood was measured by liquid chromatography with tandem mass spectrometry (LC-MS/MS) (31). Calculations of whole-body glucose disposal (WGD) and endogenous glucose release (EGR) were performed based on modified versions of Steele’s equation (30). After estimation of basal glucose turnover rates, the increase in WGD (peripheral insulin sensitivity) and reduction in EGR (hepatic insulin sensitivity) during HEC were calculated, both pre- and postoperatively (31).

Hyperglycemic clamp. A glucose bolus (300 mg/kg) was administered over 45 seconds and glucose was clamped for 120 min at ~15 mmol/L as described with modifications (29). Insulin was measured every 2 min for the first 10 min, and thereafter every 10 min. 1st and 2nd phase insulin secretion were calculated as area under the curve (AUC) for the first 10 min (AUC$_{0-10}$) and for the remaining of the clamp (AUC$_{10-120}$), respectively.

Hormones, free fatty acids and tissue glycogen. Serum insulin and plasma glucagon during clamps by RIA methods (Linco research, Inc., St. Charles, MO, USA). C-peptide was measured with a porcine ELISA kit (Mercodia, Uppsala, Sweden)(36). Serum cortisol was determined by electrochemiluminescence immunoassay (Roche Diagnostics, Basel, Switzerland)(37). Plasma FFA was measured using a colorometric assay kit (Wako Diagnostics, Richmond, VA, USA). Glycogen content in muscle and liver biopsies was determined as glucose units after hydrolysing macroglycogen and acid-insoluble proglycogen, using a hexokinase reagent kit (Horiba ABX, Montpellier, France).

Western Blots. Skeletal muscle tissue (~30 mg) was grinded and diluted in RIPA buffer, containing phosphatase and protease inhibitors (Roche, Basel, Switzerland). The protein concentrations were measured using a DC protein assay (Bio-rad, Hercules, CA, USA) with antibodies (Cell Signaling, Beverly, MA, USA) against total AKT(38), p-AKT (Ser473)(39), PI3K p85(40) and the secondary antibody anti-rabbit(41) anti-Actin (42) Sigma,
Saint Louis, MO, USA) was used as loading control. Captured protein image was quantified using Image Studio (LI-COR, Lincoln Neb, USA) and expressed as fold-change from preoperative values.

**Inflammation.** Pre- and postoperative plasma cytokines were analysed using a multiplex cytokine assay (Bio-Rad Laboratories Inc., Hercules, CA, USA) analysing TNFα, IL-4, IL-6, IL-8, IL-10, IL1β, IFNα and IFNγ on a Multiplex Analyser (Bio-Rad Laboratories) according to manufactures instructions. Porcine high sensitivity C-reactive protein (hs-CRP) with an ELISA kit (MyBioSource, San Diego, CA, USA) according to manufactures instructions.

**Statistics.** All values are displayed as mean ± SEM. Pre- to postoperative changes within the same group were analysed by Student’s dependent t-test. Relative changes from preoperative values (% or fold change) were calculated, and one-way ANOVA was used to detect differences between the groups with Dunnet’s post-hoc test. For comparison of repeated measures of hormones and glucose intraoperatively and for FFA profiles, two-way repeated measures ANOVA multiple comparisons was applied with Dunnet’s post-hoc test. Differences were considered significant at P < 0.05.
RESULTS

Hemodynamic measurements and monitoring. Pre- vs. intra- vs. postoperative hemodynamic measurements were stable and not different between groups including average heart rate (surgery/control; 95±5 vs. 112±5 vs. 96±6; surgery/GLP-1; 96±2 vs. 114±4 vs. 115±5; sham/GLP-1 95±4 vs. 109±5 vs. 102±7 beats/min) and mean arterial pressure (surgery/control; 73±5 vs. 67±2 vs. 69±4; surgery/GLP-1; 77±4 vs. 71±3 vs. 71±3; sham/GLP-1 73±4 vs. 71±2 vs. 71±2 mmHg). There were no further changes in hemodynamics during the glucose clamps (data not shown). Respiratory pre-, intra- and postoperative pCO₂ values were stable and unchanged in and between groups (ranging from 4.9-5.0 kPa), and as expected slightly increased during the clamps with no difference between groups (ranging from 5.9-6.0 kPa).

Basal glucose turnover and glucose kinetics during two-step euglycemic clamping (HEC). Pre- and postoperative values for EGR and WGD during the basal period are displayed in table 1. No differences in basal EGR and WGD were found within or between the groups. Values for EGR and WGD during HEC are displayed in table 2 and the insulin-mediated response is shown in figure 2. In the surgery/control group, there was a reduction in both GIR (33%, P < 0.01) and insulin stimulated WGD (44%, P < 0.01) during HEC 2 showing peripheral insulin resistance, while there was near normalization of total and peripheral insulin sensitivity in the surgery/GLP-1 group. GIR and WGD remained unchanged in the sham/GLP-1 group. During HEC 1 (low insulin), insulin stimulated suppression of EGR was unchanged from preoperative values in the surgery/control group, but significantly more suppressed in surgery/GLP-1 (40%, P < 0.05), and even more profoundly in sham/GLP-1 (104%, P < 0.05). During HEC 2 (high insulin), EGR was still more suppressed in surgery/GLP-1 (31%, P < 0.05), while no difference in EGR suppression was observed in the sham/GLP-1 group. Comparing group differences in total and peripheral
insulin sensitivity, GIR and WGD in surgery/control were significantly more reduced than the two GLP-1 groups (P < 0.05, figure 3).

**Insulin, glucose and counter-regulatory hormones.** Intraoperative levels of glucose, insulin, cortisol and glucagon are shown in figure 4. Glucose increased ~20% in surgery/control but was kept stable at baseline levels and was significantly lower in the GLP-1 receiving groups (P < 0.05). In surgery/control, insulin remained unchanged, but there was a significant elevation of insulin levels in surgery/GLP-1 (P < 0.05). No group difference in sham/GLP-1 was found, but insulin increased within the group from 30-60 min after start of GLP-1 infusion (P < 0.05). Cortisol was unaffected by GLP-1, though the surgery groups had higher cortisol levels than sham/GLP-1 (P < 0.05). Intraoperative glucagon levels were unchanged in all groups. Pre- and postoperative levels of insulin, glucose and glucagon during the basal period are displayed in table 1. Despite increased intraoperative levels of circulating insulin during GLP-1 infusion, levels were equal in the postoperative phase within and between the groups. A postoperative increase in glucagon levels was seen in surgery/control (3.3-fold, P < 0.05), but was not evident in the GLP-1 groups. Insulin and counter-regulatory hormones were equal during HEC, confirming stable clamping conditions.

**Free fatty acids.** Arterial plasma free fatty acid (FFA) concentrations were unchanged from preoperative levels and remained within the same range in all three groups during the postoperative basal period (surgery/control 228±54 vs. 285±51; surgery/GLP-1 252±42 vs. 295±41; sham/GLP-1 176±16 vs. 178±39 μmol/L), and were equally suppressed during HEC 1 (surgery/control 94±24; surgery/GLP-1 85±26; sham/GLP-1 114±30 μmol/L) and HEC 2 (surgery/control 49±45; surgery/GLP-1 48±23; sham/GLP-1 23±10 μmol/L).

**Tissue glycogen content.** Tissue glycogen levels are shown in table 3. As expected, surgical trauma led to a depletion of hepatic glycogen (82% reduction in both surgery groups,
P < 0.05), but GLP-1 did not affect glycogen levels. Hepatic glycogen was slightly increased during HEC in the surgery/control group (26 %, P < 0.05) with a similar trend in the surgery/GLP-1 group (P < 0.14). Muscle glycogen content did not change in response to surgery nor GLP-1 in any of the groups.

*Insulin signalling proteins.* Phosphorylated Akt (p-Akt) and PI3K p85 protein expression are shown in figure 5. As expected, Akt phosphorylation was elevated in response to insulin infusion during the clamp. GLP-1 had no effect on Akt phosphorylation. GLP-1 did not affect PI3K p85 expression in surgery/control or surgery/GLP-1, but significantly increased insulin stimulated PI3K-p85 in the sham/GLP-1 group (P < 0.05) with a similar trend in non-insulin stimulated expression (P=0.06).

*Hyperglycemic clamp.* There was no difference in 1st phase insulin secretion from pre- to postoperative clamps in neither surgery/control (172±52 vs. 129±12 µU*min/mL), surgery/GLP-1 (232±56 vs. 197±43 µU*min/mL) or sham/GLP-1 (156±25 vs. 126±47 µU*min/mL), nor were there any difference between the groups. 2nd phase insulin secretion was also unaffected by GLP-1, but higher circulating concentrations of insulin were seen after surgery (surgery/control 2563±667 vs. 3906±687 µU*min/mL, P < 0.05; surgery/GLP-1, 2993±440 vs. 5892±1392 µU*min/mL, P < 0.05), but not in the sham/GLP-1 group (3706±544 vs 3241±666 µU*min/mL), most likely caused by the previously described reduction in insulin clearance in the immediate phase after surgery in pigs (31).

*Inflammatory biomarkers.* IFNα was significantly reduced compared to preoperative measurements in the surgery/GLP-1 group (1.74±0.87 vs. 0.64±0.36 pg/mL, P < 0.05), but remained unchanged in the surgery/control (0.97±0.55 vs. 0.98±0.78 pg/mL) and sham/GLP-1 group (0.47±0.20 vs. 0.43±0.24 pg/mL). A postoperative increase in IL-6 was only detectable in half of the pigs undergoing surgery and no differences were detected between the groups.
There was no difference in IL-12 concentrations within or between the groups. TNFα, IL-10, IL-4, IL1β and IFNγ was below detection limits in all groups. There were no differences from pre- to postoperative levels in hs-CRP within or between groups (surgery/control; 12.07±2.50 vs. 11.84±2.53 surgery/GLP-1; 12.89±2.44 vs. 13.33±3.14 sham/GLP-1 15.28±3.10 vs. 14.94±2.54 mg/L).
DISCUSSION

In the present study, we show that infusion of GLP-1 during major abdominal surgery prevents development of peripheral insulin resistance and increases hepatic insulin sensitivity in the immediate postoperative phase.

Incretin-based therapies improve glycemic control in ICU patients (23,24,26), but have also been shown to be beneficial for elective surgical patients with preoperative metabolic risk factors (27) or for those undergoing major surgical procedures with high risk of hyperglycemia (25,28). Further, postoperative insulin resistance is associated with adverse postoperative outcome (44,45) while good intraoperative glycemic control improves insulin sensitivity after surgery (5). A crucial point during glucose regulation in acute insulin resistance is avoidance of hypoglycemia which augments morbidity and mortality (46). Thus, GLP-1, at least in theory, represents a safer approach to achieve glycemic control during and after surgery which can ease, decrease or even eliminate the use insulin infusions, reduce complications and contribute to improved postoperative outcome.

Previous studies have suggested that GLP-1 may increase peripheral glucose uptake in healthy subjects through insulin-independent mechanisms (47,48), and studies in depancreatized dogs have shown that GLP-1 potentiates insulin-stimulated glucose utilization (49). However, several clamp studies on healthy human subjects shown conflicting results with no change in glucose uptake during superimposed GLP-1 infusion (8,17,19–21). A more recent study confirmed this finding during euglycemia, but showed that GLP-1 increases glucose uptake during hyperglycemia (50). Moreover, in most clamp studies on subjects with compromised insulin sensitivity (due to diabetes mellitus or high-fat feeding), GLP-1 improved oxidative glucose disposal (9-12), although some studies does argue against this (51,52). During experimentally induced acute insulin resistance, GLP-1 restored the associated reduced glucose disposal (12). Hence, the present results support the notion that
GLP-1 has a positive effect on WGD, but that this effect is limited to subjects were insulin sensitivity is already compromised, as after surgical trauma.

GLP-1 infusion has been observed to reduce hepatic EGR, a finding which was previously attributed entirely to the regulation of pancreatic hormone release (16,18,52,53). However, more recent investigations show that even short-time treatment with GLP-1 augments the suppressive effect of insulin on EGR (7), as supported by our findings. Other clamp-studies have failed to demonstrate a similar effect on EGR (52,54). However, this might have been due to infusion of too high insulin doses during HEC with near complete suppression of EGR, potentially masking an effect of GLP-1 on hepatic insulin sensitivity (8).

Our results support this view, as the difference in EGR in the sham/GLP-1 group was evident only during low insulin infusion, while higher insulin concentrations led to near total and equal suppression of EGR after GLP-1 infusion.

The mechanisms underlying the effects of GLP-1 on peripheral insulin sensitivity have rarely been addressed in in vivo studies. Results from in vitro studies indicate that direct stimulation of the GLP-1 receptor (GLP-1R) which is expressed in several tissues, including myocytes may play a role (55). The prevailing view suggests a direct effect through regulating the PI3K-Akt pathway, leading to increased glucose uptake and tissue glycogen content, as shown in cell studies and in a clamp study on GLP1-R knockout mice (14,15,56). However, other in vivo studies have failed to demonstrate any effect of GLP-1 on glycogen storage in peripheral tissues (57,58). Trauma does lead to depletion of hepatic glycogen (59), as it did in both surgery groups in our study. Thus, our results did not demonstrate any effect of GLP-1 on glycogen storage, and as no effect of GLP-1 was seen on Akt activation, this questions a direct effect on myocytes in the present study. A slight increase in PI3K expression was evident in the sham/GLP-1 group as earlier reported (56), but not in the surgery/GLP-1 group. Hence, GLP-1 could have an effect on PI3K regulation as has been demonstrated in in vitro
studies, but this effect does not seem to be involved in increasing WGD after surgery in our
study. Taken together, our data indicate that GLP-1 exerts its effects on muscle glucose
uptake and oxidative disposal through other mechanisms than through the previously
suggested direct myocyte regulation.

Improved intraoperative glycemic control should be ascribed mainly to the increased
intraoperative insulin levels induced by GLP-1, possibly aided by increased microcirculatory
perfusion (12). Dysregulated glucose homeostasis increases inflammation, while
hyperglycemic metabolic stress would lead to increased levels of reactive oxygen species,
both known to induce insulin resistance and both shown to be counteracted by GLP-1 (60,61).
Thus, improved intraoperative glycemic control itself could indirectly improve postoperative
insulin sensitivity, as demonstrated by Blixt et al. (5). As a direct effect does not appear to
explain the improved insulin sensitivity, improved glycemic control seems to be the main
factor contributing to improved insulin sensitivity in our study as well. Although there were
no major changes in plasma cytokines in the present study, the reduced IFNα levels in the
GLP-1/surgery group indicate an immunomodulatory mechanism. Finally, it has also been
suggested that GLP-1 can modulate the release of FFA, which indirectly could induce insulin
resistance in skeletal muscle. Results from human studies did not find GLP-1 to exert these
properties (62), which is in concert with our findings.

In the postoperative phase, endogenous insulin remained at preoperative levels in all
groups, and as levels of insulin during clamping were equal and our HEC method suppresses
pancreatic function to near completeness (31), the clamp results were not affected by
differences in endogenous insulin release. The moderate postoperative increase in glucagon
levels was antagonized by GLP-1 treatment. This is in agreement with a clinical study on
elective surgical patients with diabetes (28), and could partly explain the positive effect on
postoperative EGR. However, as the EGR was in fact more supressed after GLP-1 infusion
compared to values before the intervention in the normal sensitive GLP-1/sham group, this
indicates, at least in part, a direct effect of GLP-1 on the liver, as glucagon levels were
unchanged in this group. As expected, GLP-1 did not affect postoperative pancreatic insulin
secretion capacity, an effect more likely to occur after long-lasting GLP-1 therapy (6).

This study has limitations. Paired experiments were performed to minimize metabolic
variance and while the effects on insulin sensitivity were quite clear, it is difficult to eliminate
that any other differences have been overlooked with limited group sizes. The anaesthesia
protocol was designed to minimize any metabolic effect, but it should be noted that isoflurane
is known to affect glucose metabolism to a certain degree. The increase in glucose disposal
without any effect on tissue glycogen content indicates that glucose oxidation was increased
by GLP-1. However, glucose oxidation rates were not directly measured and an increase in
alternative non-oxidative glucose disposal cannot be excluded. Specific studies on GLP-1
with e.g. indirect calorimetry combined with HEC and extended insulin signalling pathway
measurements are needed to further determine the fate of the disposed glucose. Finally, we
did not observe any clear effect on all inflammatory biomarkers. As earlier acknowledged in
anesthetized pigs (63), basal cytokine levels are suppressed and low, hence studies with
longer observational time and tissue specific analyses are necessary to determine any certain
anti-inflammatory effect of GLP-1.

In conclusion, we show that GLP-1 along with improving glycemic control almost
completely abolishes postoperative peripheral insulin resistance, probably due to maintained
oxidative glucose disposal, but not through a direct effect on the Akt-PI3K pathway or
glycogen content in skeletal muscle. A favourable antagonistic effect on glucagon release and
an increase in hepatic insulin sensitivity from preoperative levels was also seen after surgery.
This study suggests that use of GLP-1 for prevention and treatment of trauma-induced insulin
resistance could provide beneficial metabolic effects for surgical patients.
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**FIGURE LEGENDS**

**Figure 1. Study design.**
Time course of the two experimental days for instrumentation (instr.), pre- and postoperative metabolic basal metabolism, hyperinsulinemic euglycemic clamps (HEC) and hyperglycemic clamp (HC). Time points of essential pre-, intra- and post-operative blood sampling (▲) and biopsy harvesting (●) are shown. During basal period, HEC and HC, plasma glucose was measured continuously, and blood samples for hormones and FFA was collected serially (every 30 min) indicated by grey arrow.

**Figure 2. Insulin-mediated response during hyperinsulinemic euglycemic clamping.**
Changes from basal glucose turnover in pre- (white bars) and postoperative (black bars) endogenous glucose release (EGR) and whole body glucose disposal (WGD) in response to insulin, as determined by two-step hyperinsulinemic (0.4 and 1.2 mg insulin/kg/min) euglycemic clamping. Values are mean ± SEM. *, P < 0.05; **, P < 0.01 vs. preoperative (Student’s dependent t-test).

**Figure 3. Pre- to post-operative changes in total and peripheral insulin sensitivity.**
Percent changes (from pre- to postoperative measurements) within each group for steady state glucose infusion rate (SS GIR) and whole body glucose disposal (WGD), as measured during high insulin clamp. Values are mean ± SEM. *, P < 0.05; **, P < 0.01 vs. preoperative (one-way ANOVA).
Figure 4. Intraoperative glucose and hormone levels.

Intraoperative changes from preoperative levels in (a) serum glucose (%) change, (b) serum levels of insulin and (c) cortisol and (d) plasma glucagon. Values are mean ± SEM. Repeated measures two-way ANOVA was applied with †P < 0.05, ‡P < 0.01 vs. preoperative levels within the group and *P < 0.05 in difference by group (and time).

Figure 5. Pre- to post-operative changes in insulin signalling proteins.

Postoperative protein expression of (a) p/t-Akt and (b) PI3K p85 in snap-frozen biopsies from skeletal muscle harvested during the non-insulin stimulated (non-ins) basal period and during insulin-stimulated (ins-stim) clamp, expressed as fold-change from preoperative measurements. Values are mean ± SEM. *, P < 0.05 vs. surgery/control (one-way ANOVA).
Table 1. Pre- and postoperative basal glucose kinetics and pancreatic hormones.

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<th>Surgery / control</th>
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<th>Sham / GLP-1</th>
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<td></td>
<td>6.12±0.55</td>
<td>7.01±0.32</td>
<td></td>
</tr>
</tbody>
</table>

Serum glucose, insulin, glucagon, whole body glucose disposal (WGD) and endogenous glucose release (EGR) during pre- and postoperative basal period. Data are displayed as Mean ± SEM. *, P < 0.05 vs. preoperative (Student’s dependent t-test).
Table 2. Glucose kinetics during hyperinsulinemic euglycemic step clamp (HEC).

<table>
<thead>
<tr>
<th></th>
<th>Surgery / control</th>
<th>Surgery / GLP-1</th>
<th>Sham / GLP-1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Preop</td>
<td>Postop</td>
<td>Preop</td>
</tr>
<tr>
<td>S-glucose, mmol/L</td>
<td>4.46±0.09</td>
<td>4.39±0.04</td>
<td>4.62±0.20</td>
</tr>
<tr>
<td>Insulin, µU/mL</td>
<td>9.10±0.71</td>
<td>9.75±0.64</td>
<td>10.12±0.56</td>
</tr>
<tr>
<td>SS GIR, mg/kg/min</td>
<td>3.92±0.72</td>
<td>4.64±0.97</td>
<td>3.98±1.14</td>
</tr>
<tr>
<td>WGD, mg/kg/min</td>
<td>7.17±0.94</td>
<td>6.93±0.85</td>
<td>6.98±0.86</td>
</tr>
<tr>
<td>EGR, mg/kg/min</td>
<td>3.32±0.52</td>
<td>2.22±0.41</td>
<td>3.00±0.51</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Surgery / GLP-1</th>
<th>Sham / GLP-1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Preop</td>
<td>Postop</td>
</tr>
<tr>
<td>S-glucose, mmol/L</td>
<td>4.54±0.03</td>
<td>4.44±0.05</td>
</tr>
<tr>
<td>Insulin, µU/mL</td>
<td>20.57±0.51</td>
<td>23.09±1.14</td>
</tr>
<tr>
<td>SS GIR, mg/kg/min</td>
<td>16.55±1.59</td>
<td>11.30±0.99**</td>
</tr>
<tr>
<td>WGD, mg/kg/min</td>
<td>17.66±1.73</td>
<td>11.82±1.11**</td>
</tr>
<tr>
<td>EGR, mg/kg/min</td>
<td>1.11±0.32</td>
<td>0.52±0.32</td>
</tr>
</tbody>
</table>
Glucose kinetics during two-step hyperinsulinemic euglycemic clamp (HEC) for low insulin HEC 1 (0.4 mU/kg/min) and high insulin HEC 2 (1.2 mU/kg/min). Serum glucose during last 40 min of each clamp (steady state), insulin during steady state, steady state glucose infusion rate (SS GIR), whole body glucose disposal (WGD) and endogenous glucose release (EGR). Data are displayed as Mean ± SEM. *, P<0.05; **, P < 0.01 vs. preoperative (Student’s dependent t-test).
Table 3. Tissue glycogen content.

<table>
<thead>
<tr>
<th></th>
<th>Surgery / control</th>
<th>Surgery / GLP-1</th>
<th>Sham / GLP-1</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Skeletal Muscle (µmol/g)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preop</td>
<td>39.2±1.1</td>
<td>38.5±1.9</td>
<td>43.8±4.4</td>
</tr>
<tr>
<td>Postop</td>
<td>41.2±4.6</td>
<td>34.9±6.0</td>
<td>38.4±3.3</td>
</tr>
<tr>
<td>Postop clamp</td>
<td>36.8±5.0</td>
<td>35.7±2.5</td>
<td>41.6±3.9</td>
</tr>
</tbody>
</table>

| **Liver (µmol/g)**    |                   |                 |              |
| Preop                 | 94.6±20.0         | 92.0±23.8       | NM           |
| Postop                | 14.3±5.8*         | 15.7±3.8*       | NM           |
| Postop clamp          | 30.7±5.5†         | 25.12±4.5       | NM           |

Pre- and postoperative basal and insulin stimulated (clamp) tissue glycogen levels in skeletal muscle and liver. Data displayed as mean ± SEM. *, P < 0.05 vs. preoperative; †, P < 0.05 vs. postoperative basal (Student’s dependent t-test). NM: Not measured.
Figure 3

SS GIR

(% change from preop)

(a)

Surgery / control
Surgery / GLP-1
Sham / GLP-1

WGD

(% change from preop)

(b)

Surgery / control
Surgery / GLP-1
Sham / GLP-1

**

*
Figure 4

(a) Glucose (% change from preop)

(b) Insulin (μU/mL)

(c) Glucagon (pmol/L)

(d) Cortisol (nmol/L)

* indicates significant difference from baseline.

Legend:
- Surgery / control
- Surgery / GLP-1
- Sham / GLP-1
Figure 5

(a) Non-insulin stimulated
Insulin stimulated

Insulin stimulated

(b) Non-insulin stimulated
Insulin stimulated

p-Akt protein expression
(fold change from preop)

PI3K protein expression
(fold change from preop)

p-Akt
Actin

Insulin stimulated

Surgery / control
Surgery / GLP-1
Sham / control
Sham / GLP-1

Preop
Non-ins
Ins-stim
Preop
Non-ins
Ins-stim
Preop
Non-ins
Ins-stim

Surgery / control
Surgery / GLP-1
Sham / GLP-1

Preop
Non-ins
Ins-stim
Preop
Non-ins
Ins-stim

PI3K p85
Actin

Surgery / control
Surgery / GLP-1
Sham / GLP-1