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Lack of Preservation of Insulin Gene Expression by a Glucagon-Like Peptide 1 Agonist or a Dipeptidyl Peptidase 4 Inhibitor in an In Vivo Model of Glucolipototoxicity

Ghislaine Fontés, PhD^{1,2}, Derek K. Hagman, PhD^{1,2}, Martin G. Latour, PhD¹, Meriem Semache, MSc^{1,3}, and Vincent Poitout, DVM, PhD^{1,2,3}

¹ Montréal Diabetes Research Center, CRCHUM, University of Montreal, QC, Canada

² Department of Medicine, University of Montreal, QC, Canada

³ Department of Biochemistry, University of Montreal, QC, Canada

Abstract

Prolonged exposure of pancreatic beta-cells to elevated levels of glucose and fatty acids adversely affects insulin secretion and gene expression.

Aim—To examine whether the GLP-1 agonist exenatide or the inhibitor of the GLP-1 degrading enzyme dipeptidyl peptidase 4 (DPP-4) sitagliptin rescue insulin gene expression in rats infused for 72-h with glucose + Intralipid, independently from their glucose-lowering action.

Methods—Wistar rats were infused alternatively with glucose or Intralipid for cycles of 4h each for a total of 72h. The animals received exenatide (5 µg/kg/day IV) or sitagliptin (5 mg/kg/day IV) continuously starting 4 days prior to and continuing throughout the 3-day infusion period.

Results—Plasma glucose, fatty acids, insulin and C-peptide levels were unaffected by exenatide or sitagliptin treatment during the infusion period. Insulin mRNA levels increased in response to the glucose infusion, but this increase was abolished in islets from rats receiving glucose + Intralipid. Neither exenatide nor sitagliptin administration rescued insulin mRNA in glucose + Intralipid infused rats.

Conclusions—Neither a GLP-1 agonist nor a DPP-4 inhibitor, at doses that do not alter blood glucose levels, prevented the inhibition of insulin gene expression in this in vivo model of glucolipototoxicity.

Keywords

Insulin gene; GLP-1; exenatide; sitagliptin

Corresponding Author: Ghislaine Fontés, PhD, CRCHUM – Technopole Angus, 2901 Rachel Est, Montréal, QC, H1W 4A4 – CANADA, Tel: (514) 890-8000 Ext: 23653, Fax: (514) 412-7648, ghislaine.fontes@crchum.qc.ca.

Conflict of interest

The authors declare that they have no conflict of interest.

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INTRODUCTION

Type 2 diabetes mellitus is due to defective insulin secretion and peripheral insulin resistance. Whereas the initial beta-cell defect underlying the etiology of T2DM is unknown, it has been clearly demonstrated that insulin secretion declines after the onset of the disease (1). Chronic hyperglycemia (glucotoxicity) (2), chronic dislipidemia (lipotoxicity) (3), and the association of both (glucolipotoxicity) (4;5), have been proposed to contribute to the deterioration of beta-cell function after the onset of type 2 diabetes.

In vitro studies have shown that chronically elevated levels of fatty acids, in conjunction with high glucose, inhibit insulin secretion and insulin gene expression and promote beta-cell death (reviewed in (6)). Under glucolipotoxic conditions, we have shown that palmitate inhibition of insulin gene expression in isolated rat islets is mediated at the transcriptional level (7) via decreased function of the transcription factors PDX-1 and MafA (8). More recently, we have shown that alternate infusions of glucose for 4h and Intralipid + heparin for 4h, for a total of 72-h, in normal Wistar rats led to reduced insulin mRNA levels, nuclear exclusion of PDX-1, and a marked decrease in PDX-1 occupancy of the endogenous insulin promoter (9). However, no significant differences in insulin secretion between the groups were detected either in hyperglycemic clamps at the end of infusion or in static incubations of isolated islets. We concluded from these results that fatty-acid inhibition of the insulin gene occurs in vivo prior to any changes in insulin secretion.

The incretin hormone glucagon-like peptide-1 (GLP-1) has been shown to exert beneficial effects on beta-cell function, including stimulation of glucose-induced insulin secretion and insulin gene expression, enhancement of beta-cell proliferation and differentiation, and inhibition of beta-cell apoptosis. These pleiotropic effects of the hormone have been proposed to contribute to the long-term regulation of insulin secretion by maintaining a functional beta-cell mass (reviewed in (10)). GLP-1 has been shown to promote insulin biosynthesis (11) and insulin gene expression (12;20) through, at least in part, enhancement of PDX-1 expression (13–15). GLP-1 also promotes the nuclear translocation of PDX-1 (21), resulting in increased insulin, Glut 2 and glucokinase expression (13;22). Beneficial effects of GLP-1 on beta-cell mass have been demonstrated in vitro (16), whereas GLP-1 agonists have been used in vivo in several rodent models of obesity and type 2 diabetes (17;18). However, the potential contribution of preserved insulin biosynthesis to the overall improvement of glycemic control was not directly assessed. Based on our previous report (9), we hypothesized that increasing GLP-1 receptor signaling might enhance insulin gene expression in the glucose + Intralipid rat infusion model. The aim of this study was therefore to examine whether administration of the GLP-1 analogue exenatide or the inhibitor of the GLP-1-degrading enzyme dipeptidyl-peptidase (DPP) IV sitagliptin during a 72-h infusion of glucose + Intralipid prevents the reduction of insulin gene expression, independently from the effect of the drugs on glucose levels.

MATERIALS AND METHODS

Reagents

Dextrose (50 and 70% solutions) was obtained from McKesson Canada Corp (Montreal, QC) and sterile 0.9% saline was from Baxter (Mississauga, ON). All other reagents (analytical grade) were from Sigma, unless otherwise noted.

Rat infusion and treatment protocol

All procedures were approved by the Institutional Committee for the Protection of Animals at the Centre Hospitalier de l'Université de Montréal. 250–300 g male Wistar rats (Charles River,

St.-Constant, QC) were housed under controlled temperature (21°C) and a 12-h light-dark cycle with unrestricted access to water and standard laboratory chow. As previously described (9), indwelling catheters were inserted into the left carotid artery and right jugular vein at day 0. After a 2-day recovery, the animals were randomized into three treatment groups, receiving saline vehicle as a control, exenatide (5 µg/kg/day) or sitagliptin (5 mg/kg/day) as a continuous IV infusion for 4 days (pre-infusion period), as illustrated in Supplementary Fig. 1A. These doses were selected so that blood glucose levels would not be different from those in the vehicle-treated groups during the infusions, thus enabling us to assess direct effects of the drugs on insulin gene expression independently from blood glucose control. After the pre-infusion period, each treatment group was randomized into 3 infusion groups, receiving alternate and cyclical infusions of either 0.9% saline (SAL), 50% glucose (GLU), or glucose plus Intralipid with heparin (20 U/ml) (GLU+IL), in addition to the drug treatment (Suppl. Fig. 1A). The infusion profile consisting of alternate 4-h cycles for a total of 72 h is illustrated in Suppl. Fig. 1B. Infusion pumps were controlled as previously described (9). The initial number of animals per group is indicated in Suppl. Fig. 1A. Based on our previous experience and the existing literature, we anticipated that the variance of insulin mRNA measurements would be approximately 10%. With type I and type II errors of 5 and 10%, respectively, the number of replicate animals per group necessary to detect a statistically significant difference of 25% was estimated to be n=6. Therefore, each drug treatment group was assigned more than 6 animals (8–13), and the control, saline-treated group was assigned approximately twice as many animals as each of the sitagliptin- or exenatide-treated group to increase statistical power.

Islets isolation and analytical measurements

Samples for glucose and NEFA determinations were collected throughout the infusion period. At the end of the infusion, animals were anesthetized by IP injection of a 100 mg/ml ketamine hydrochloride (Bimeda-MTC Animal Health Inc., Cambridge, ON)/20mg/ml xylazine (Bayer Inc., Toronto, ON) mixture and sacrificed for islet isolation. Islets were isolated by collagenase digestion and dextran density gradient centrifugation as described previously (7). Plasma glucose and NEFA levels were measured enzymatically using colorimetric kits (Wako Chemicals, Neuss, Germany). Plasma C-peptide and glucagon were measured by radioimmunoassay (LINCO Research, St. Charles, MO). Plasma insulin was measured by ELISA (LINCO Research), and active GLP-1 levels were measured using a GLP-1 (Active) ELISA kit (LINCO Research), which does not cross react with exendin-4. Isolated islets were collected for measurement of insulin mRNA. Total RNA was extracted from aliquots of 100 islets and cDNA was synthesized as described previously (19). PCR was carried out using the Faststart DNA Master PLUS SYBR Green Kit (Roche). Primers for insulin gene (Fwd 5'-AAGCTCTCTACCTGGTGTGTGG-3' and Rev 5'-GTAGAGAGAGCAGATGCTGGTG-3') or β -actin (Fwd 5'-TGAAGTGTGACGTTGACATCC-3' and Rev 5'-ACAGTGAGGCCAGGATAGAGC-3') were designed using Primer3. Results are expressed as the ratio of target mRNA to β -actin mRNA.

Expression of data and statistics

Data are expressed as mean \pm SE. Inter-group comparisons were performed by ANOVA followed by two-by-two comparisons using the one-way ANOVA followed by Tukey's t-test, as appropriate. $P < 0.05$ was considered significant.

RESULTS

Metabolic parameters in 72-h infused rats

Blood samples were collected throughout the 72-h infusion period to measure circulating glucose and Non Esterified Fatty Acid (NEFA) levels. Glycaemia increased to ~200 mg/dl in the GLU and GLU+IL groups during glucose cycles (Fig. 1B), returning to basal levels during

SAL or Intralipid cycles (Fig. 1A). In the SAL group, blood glucose remained at basal levels (~ 100 mg/dl) throughout the infusion (Fig. 1A&B). By design, treatment with exenatide or sitagliptin did not affect glucose levels in any of the infusion groups (Fig. 1A&B). Circulating NEFA levels reached ~ 3 mmol/l during the Intralipid infusion cycles and returned to basal values between cycles (Fig. 1C&D). Treatment with exenatide or sitagliptin did not affect NEFA levels in any of the infusion groups (Fig. 1C&D). Intravenous administration of either exenatide (5 µg/kg/day) or sitagliptin (5 mg/kg/day) did not significantly affect total caloric intake, calculated by taking into account calories from food intake plus calories contained in the infusions (GLU or GLU+IL) (Fig. 2A). In the SAL group, body weight was slightly but not significantly increased at the end of the 72-h infusion period in vehicle-, exenatide-, and sitagliptin-treated animals as compared to pre-infusion values (Fig. 2B). This increase was not observed in animals infused with GLU or GLU+IL (Fig. 2B).

Circulating hormone levels in 72-h infused rats

As shown in Fig. 3A, infusion with GLU or GLU+IL did not alter circulating levels of GLP-1 in vehicle-treated animals. When the 3 infusion groups were analyzed separately, there was a slight but not significant increase in circulating GLP-1 levels in animals treated with exenatide in all 3 groups. When animals from the 3 infusion groups were pooled, exenatide treatment significantly increased circulating GLP-1 levels (3.42 ± 0.24 in vehicle group versus 5.1 ± 0.6 in exenatide-treated group, $n=9$, $P<0.001$) (Fig. 3B). Of note, this is not due to cross-reactivity of the ELISA kit used to measure GLP-1. In contrast, sitagliptin treatment did not modify circulating GLP-1 levels in any of the infusion groups (Fig. 3A&B). During the Intralipid cycles, a small but significant increase in C-peptide levels was observed in vehicle-treated animal infused with GLU+IL, as compared to other infusion groups (Fig. 3C). During the glucose cycles, C-peptide levels were markedly increased in animals infused with GLU or GLU+IL (Fig. 3D). Exenatide and sitagliptin treatments, at doses which did not alter blood glucose levels, had no significant effect on C-peptide secretion in any of the infusion conditions (Fig. 3C&D). At the end of the infusions, no significant effects of the infusion regimens or the drug treatments were detected on plasma insulin levels (Fig. 4A), whereas a significant decrease in plasma glucagon levels was observed in GLU- and GLU + IL-infused animals vs. the SAL-infused group (Fig. 4B). The lack of differences in insulin levels can be explained by the fact that in the GLU- and GLU + IL-infused groups, the glucose cycle had been ended 4 h prior to blood sampling, since the very last cycle of the infusion is either saline or Intralipid.

Insulin mRNA levels in isolated islets from 72-h infused rats

We next examined the ability of exenatide or sitagliptin treatment to prevent glucolipototoxicity induced by cyclical infusion of glucose and Intralipid on insulin gene expression. Islets were isolated at the end of the infusion period to measure insulin mRNA levels. As we previously reported (9), insulin mRNA levels were increased in islets from rats infused with GLU ($P<0.01$; Fig. 5), and this increase did not occur in animals co-infused with GLU+IL. However, neither exenatide nor sitagliptin treatment rescued insulin mRNA levels in the GLU+IL-infused animals.

DISCUSSION

This study was aimed to determine whether the GLP-1 agonist exenatide or the DPP IV inhibitor sitagliptin could rescue insulin gene expression in rats infused for 72-h with glucose + Intralipid, independently from their glucose-lowering action. We found that neither drug, at doses that do not alter blood glucose levels, prevented the inhibition of insulin gene expression in this in vivo model of glucolipototoxicity.

Intravenous administration of exenatide or sitagliptin did not significantly affect total caloric intake or body weight. The absence of weight loss and decreased food intake, commonly observed after administration of exenatide (23), is probably due the lower doses used in this study. In fact, a higher exenatide concentration (10 µg/kg) resulted in a marked decrease in body weight and food intake (data not shown). Also, in this study the drugs were administered for 5 days, in contrast to a recent study reporting weight loss and reduced food intake by exendin-4 or a DPP IV inhibitor in mice after 4 weeks of treatment (22).

Unexpectedly, we observed a significant increase in circulating GLP-1 levels after exenatide treatment, although sitagliptin treatment did not modify circulating GLP-1 levels in any of the infusion groups. This was not due to cross-reactivity of the ELISA kit used to measure GLP-1. Several studies have shown that inhibition of DPP IV leads to lower levels of endogenous total GLP-1 than those observed after pharmacological administration of GLP-1 receptor agonists (reviewed in (24)). DPP-4 inhibition increases the levels of endogenous intact incretins, such as active GLP-1 (reviewed in (25)), which in turn improves glucose tolerance in diabetic animal models (26–28). GLP-1 synthesis is known to be induced by enteral glucose, activated by other incretins, and inhibited by somatostatin. However, no evidence for a direct effect of GLP-1 or its agonists on its own production and secretion has been reported in the literature.

Insulin mRNA levels in the GLU+IL-infused animals were unchanged after treatment with either exenatide or sitagliptin, suggesting that neither a GLP-1 agonist nor a DPP IV inhibitor, at doses that do not change blood glucose or C-peptide levels, prevent the deleterious effects of GLU+IL on insulin gene expression in this mild model of glucolipotoxicity. Clearly, interpretation of these results is limited by the fact that we did not observe an effect of the GLP-1 agonist or the DPP IV inhibitor on insulin secretion and insulin gene expression. Therefore, we cannot exclude the possibility that higher concentrations of the drug would have led to prevention of glucolipotoxicity on insulin gene expression. Nevertheless, our results contrast with a previous report showing that a 5-day infusion of GLP-1 increased insulin gene expression and β-cell mass in old glucose-intolerant Wistar rats (29). However, in that study GLP-1 administration significantly lowered blood glucose levels, making it difficult to distinguish between the direct effects of the drug and the indirect effects of alleviating hyperglycaemia. In high-fat fed mice, treatment with exendin-4 for 8 weeks increased the expression of PDX-1, IRS2 and glucokinase (22). Further experiments using animal models of glucolipotoxicity and beta-cell failure are necessary to clearly identify a potentially beneficial effect of GLP-1 agonism or DPP IV inhibition on the preservation of insulin gene expression in vivo.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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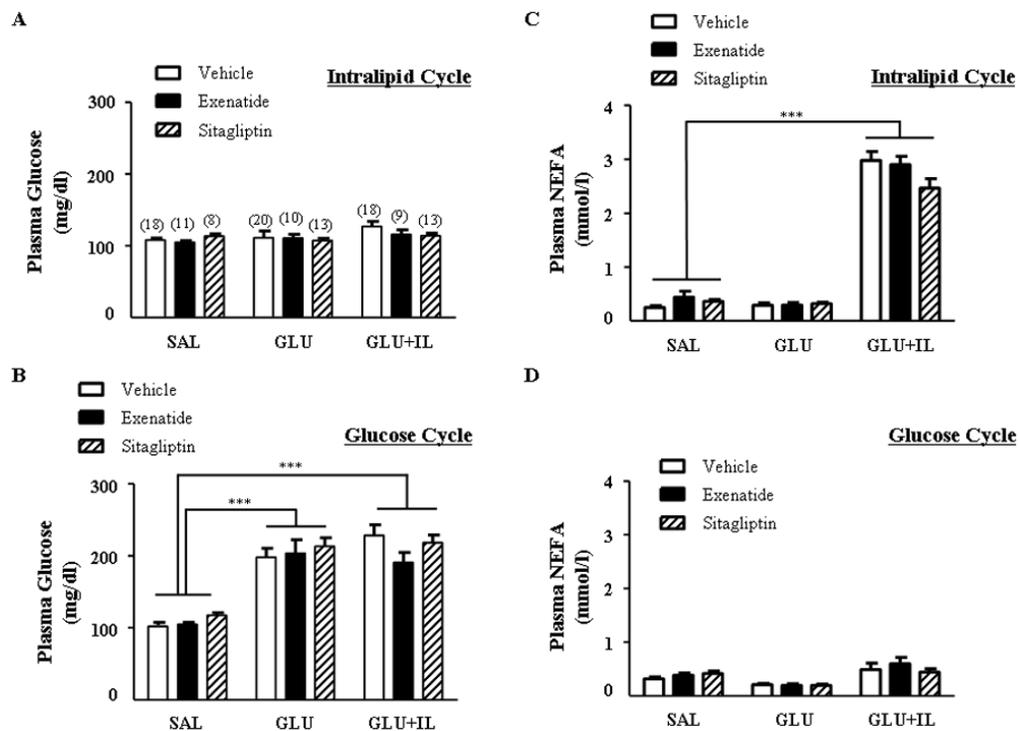
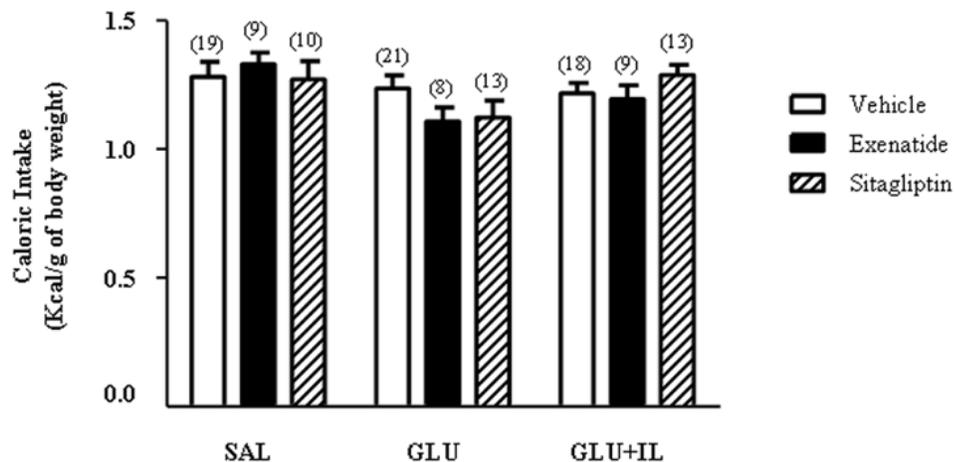


Figure 1. Metabolic parameters during glucose and intralipid cycles
 Circulating glucose (A, B) and Non Esterified Fatty Acids (NEFA; C, D) levels during the Intralipid (A, C) and glucose (B, D) infusion cycles. Results are expressed as the average NEFA or glucose levels during the corresponding cycles for the entire 72-h infusion period, and are mean ± SEM of (n) animals in each group as indicated on panel A. ***: P<0.001 vs. corresponding SAL control.

A



B

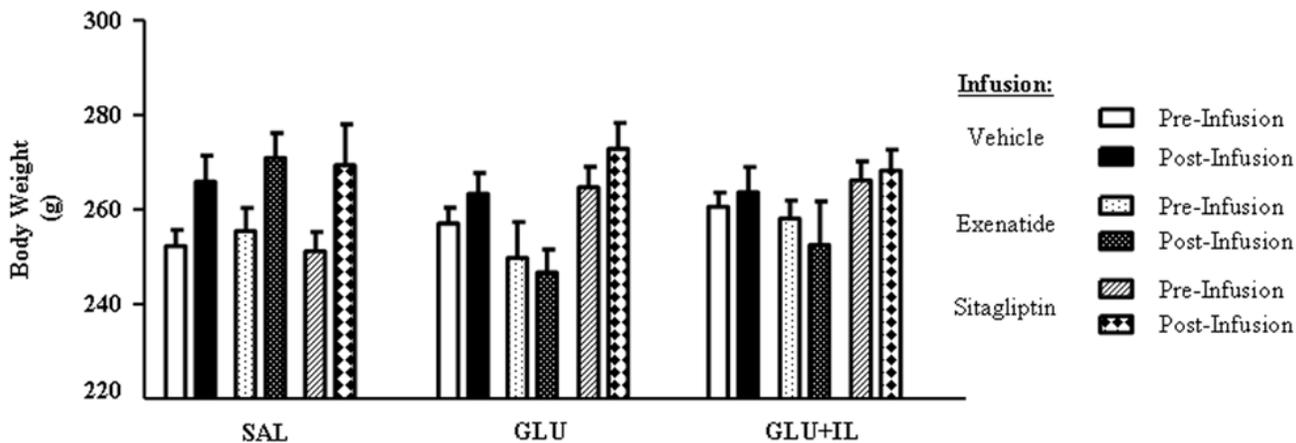


Figure 2.
A- Daily caloric intake during the 72-h infusion. Caloric intake was calculated by adding calories from food intake to calories received by the infusion regimen for the 72-h infusion period. **B-** Body weight before and after the 72-h infusion. Results are mean ± SEM of (n) animals in each group as indicated on panel A.

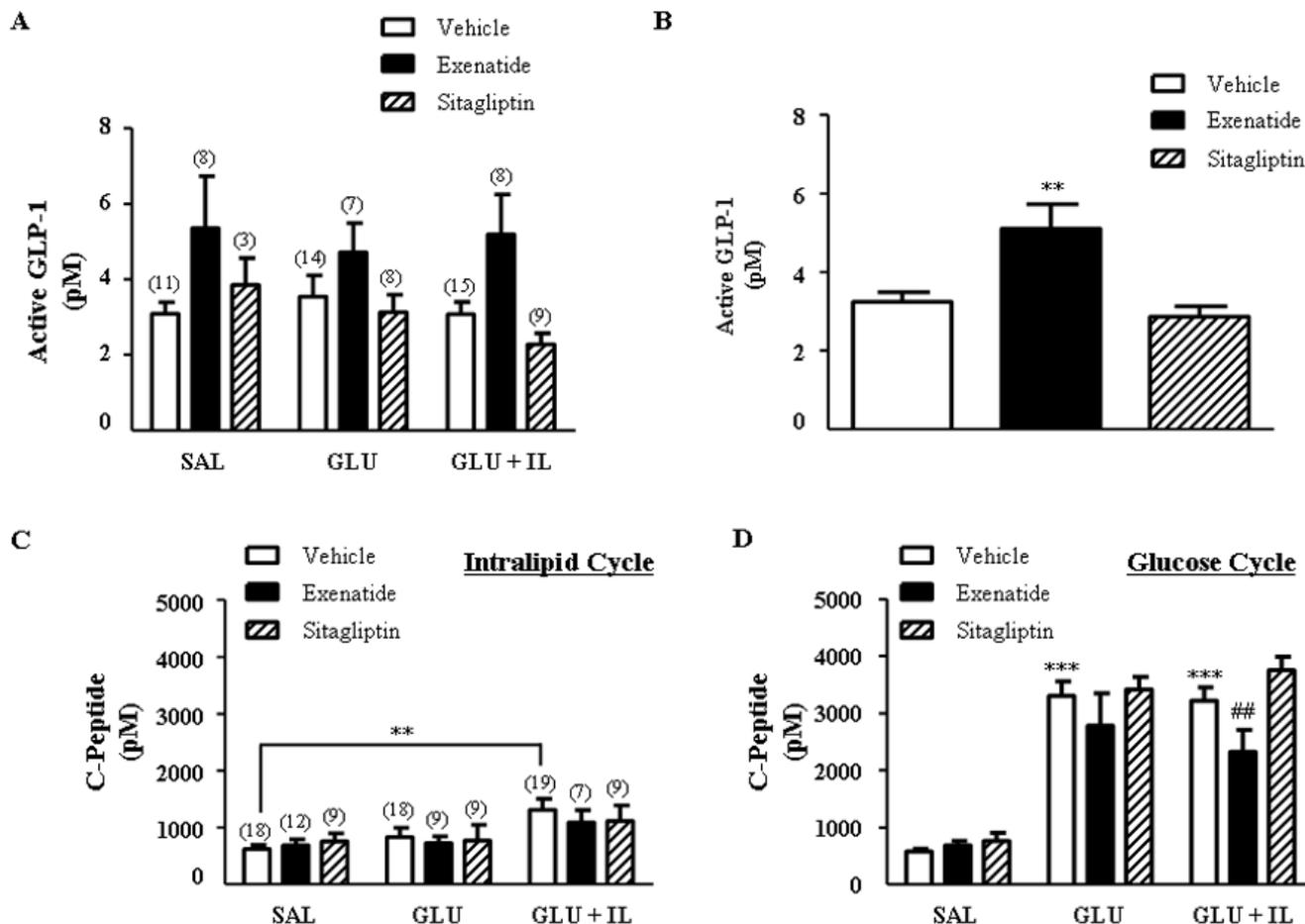


Figure 3. Circulating hormones levels after exenatide or sitagliptin treatment in 72-h infused rats Circulating levels of active GLP-1 at the end of the 72-h infusion period in each individual group (A) or pooled within each infusion regimen (B). C, D- Circulating C-peptide levels during the Intralipid (C) and glucose (D) cycles of the 72-h infusion. Results are expressed as the average C-peptide levels during the corresponding cycles for the entire 72-h infusion period, and are mean \pm SEM of (n) animals in each group as indicated on panel A. **: P<0.01 vs. corresponding SAL control.***, ###: P<0.001 vs. corresponding SAL control.

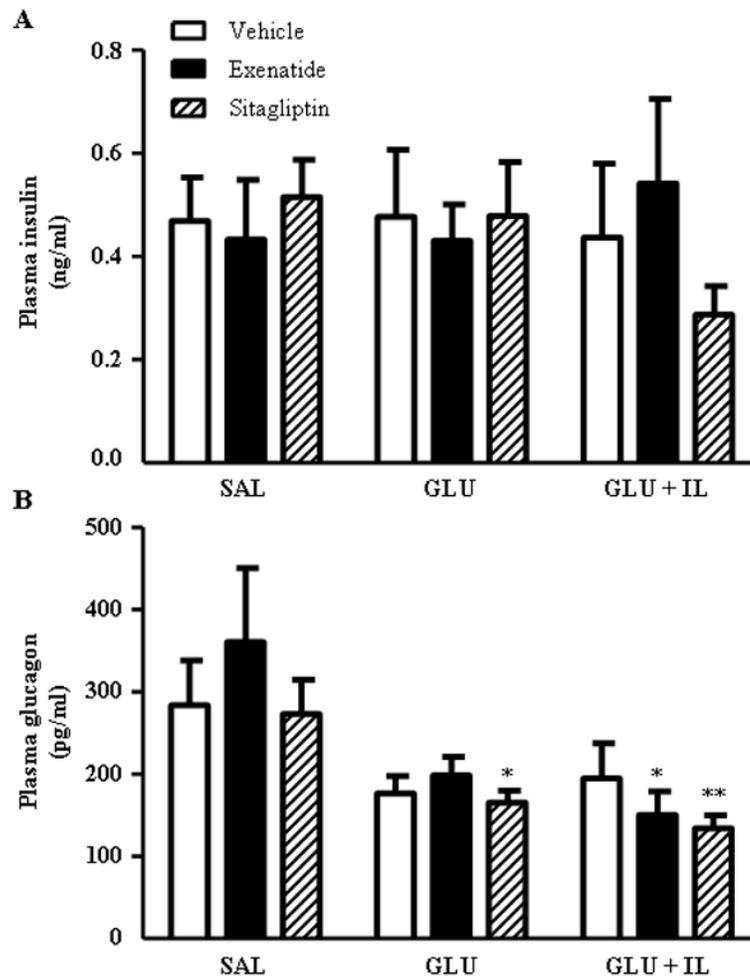


Figure 4. Plasma insulin (A) and glucagon (B) levels at the end of the 72-h infusion
 Insulin and glucagon levels were measured at the end of the infusion period, after the last cycle of either Intralipid or saline. Results are mean \pm SEM of 5–9 animals per group. *: $P < 0.05$, **: $P < 0.01$ vs. SAL control.

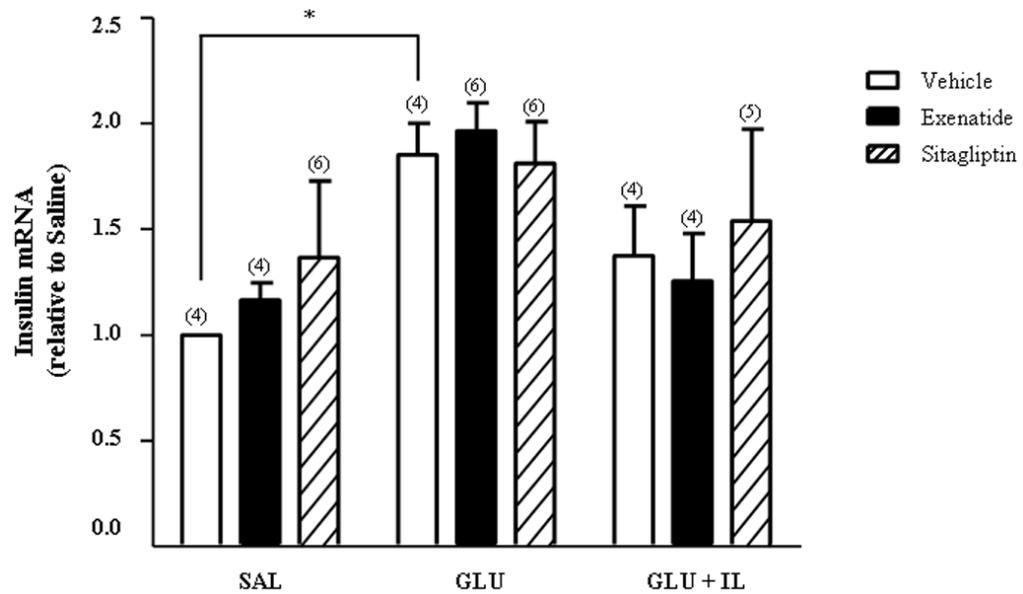


Figure 5. Insulin gene expression after exenatide or sitagliptin treatment in islets isolated from 72-h infused rats

Insulin mRNA levels in isolated islets following the 72-h infusion. Results are expressed as fold increase of the ratio of insulin/ β -actin mRNA over the control (SAL) value, and are mean \pm SEM of (n) animals in each group. *: $P < 0.05$ vs. SAL control.