

**Acute DPP-4 Inhibition rapidly Enhances Insulin-mediated Suppression of Endogenous Glucose
Production in Mice**

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ABSTRACT

Pharmacological approaches that enhance incretin action for the treatment of Type 2 diabetes mellitus have recently been developed, i.e. injectable GLP-1R agonists with prolonged plasma half-lives and orally available inhibitors of dipeptidyl peptidase (DPP)-4, the main enzyme responsible for the rapid degradation of circulating GLP-1 and GIP. The mechanism(s) underlying the glucose-lowering effect of these two pharmacotherapies differs and is not yet fully understood. Here we investigated whether acute GLP-1R activation (exendin-4) or DPP-4 inhibition (des-F-sitagliptin) modulates insulin action in mice using a hyperinsulinemic euglycemic clamp. A single iv bolus of des-F-sitagliptin (11mg/kg) was administered to mice 15 minutes after the start of the clamp and its effect was compared to a 50 ng bolus of exendin-4 or the same volume of saline. Despite matched levels of plasma glucose and insulin, within 15 minutes the glucose infusion rate required to maintain euglycemia was significantly greater after des-F-sitagliptin compared to saline or exendin-4. This difference was entirely due to enhancement of insulin-mediated suppression of endogenous glucose production by des-F-sitagliptin, with no difference in glucose disposal rate. These findings illustrate that DPP-4 inhibition modulates glucose homeostasis through pathways distinct from those utilized by GLP-1r agonists in mice.

ABBREVIATIONS

EGP, endogenous glucose production; GIP, glucose-dependent insulinotropic peptide; GIR, glucose infusion rate; GLP-1, glucagon-like peptide-1; GSIS, glucose-stimulated insulin secretion; DPP-4, dipeptidyl peptidase-4; Ra, rate of appearance (EGP); Rd, rate of disposal (glucose utilization)

INTRODUCTION

Diabetes affects an increasing number of individuals and has now assumed “epidemic” proportions. Promising pharmacological approaches recently approved for the treatment of type 2 diabetes include two incretin-based therapies, the glucagon-like peptide-1 receptor (GLP-1R) agonists or molecules that inhibit the breakdown of incretin hormones (i.e. dipeptidyl peptidase [DPP]-4 inhibitors) (1). Incretin-based therapies exert their insulinotropic effect in a glucose-dependent fashion, thereby limiting the occurrence of hypoglycemia. These agents may also either induce weight loss (GLP-1R agonists) or are not associated with weight gain (DPP-4 inhibitors), in contrast to findings with other presently available treatments for type 2 diabetes (1).

GLP-1 and glucose-dependent insulinotropic peptide (GIP) are the two principal hormones acting as “incretins”, which are released by the intestine and act on pancreatic islets to potentiate glucose-stimulated insulin secretion (GSIS) (2;3). Exogenous infusion of GLP-1 or a potent GLP-1R agonist such as exendin-4 enhances GSIS and improves glycemic control in diabetic animals and humans, whereas administration of a GLP-1 receptor (GLP-1R) antagonist (exendin 9-39amide) or genetic disruption of the GLP-1R gene in mice impairs glucose control (4-7). Intriguingly, although GIP receptor (GIPR) agonists potentiate GSIS, GIP antagonists or genetic ablation of the GIPR gene produces a complex metabolic phenotype reflecting the integrated importance of GIP for the function of both β -cells and adipose tissue (8;9).

As GLP-1 and GIP are degraded by the protease DPP-4 (10;11), several strategies have been developed to prolong their half-life in the circulation in order to enhance their biological effect. Inhibition of DPP-4 activity produces improvements in glucose control in the setting of experimental and clinical diabetes mellitus (1;12;13). Conversely, rats with an inactivating DPP-4 mutation exhibit improved glucose tolerance (14) and mice with deletion of the DPP-4 gene exhibit improved glucose tolerance, increased plasma levels of GLP-1 levels, improved GSIS, and resistance to diet-induced obesity (15;16).

The dominant mechanism whereby DPP-4 inhibition improves glycemic control is assumed to be the prolongation of the half-life of GLP-1 in the circulation, thereby enhancing its incretin effect (stimulation of glucose-dependent insulin secretion). However, the increment in plasma levels of the intact incretin hormones with DPP-4 inhibitor treatment is modest, and anti-diabetic therapy with DPP-4 inhibitors has little or no effect on insulin secretion (17). This has led to the speculation that the therapeutic benefit of DPP-4 inhibition cannot be fully explained only by prolongation of the GLP-1 half-life in the systemic circulation and that additional mechanism(s) may contribute to the therapeutic effect of DPP-4 inhibitors (18).

In contrast to the well-established effect of incretins on GSIS, their putative effect on insulin action remains incompletely understood. While several studies failed to demonstrate a significant effect of GLP-1 on insulin action in humans (19-21), Prigeon *et al.* found enhanced suppression of endogenous glucose production by GLP-1 in men under fasting conditions (22). Similarly, GLP-1 infusion in type 2 diabetic patients subjected to hyperglycemic clamp enhanced glucose disposal, although a trend toward lower glucagon levels may have explained part of this effect (23). Moreover, preclinical studies of the effects of GLP-1 or DPP-4 inhibition on insulin sensitivity have yielded variable results, often confounded by changes in levels of insulin or glucagon, although some studies have shown that GLP-1 increases insulin sensitivity in depancreatized models or in the presence of somatostatin infusion, which suppresses insulin and glucagon secretion (13;24-29).

The aim of the present study, therefore, was to examine the acute effect of the DPP-4 inhibitor des-F-sitagliptin on hepatic and extrahepatic insulin action in awake mice using the hyperinsulinemic euglycemic clamp method, and to compare this effect with that of the synthetic DPP-4-resistant GLP-1R agonist exendin-4. We found that acute DPP-4 inhibition, but not GLP-1R agonist administration, induces a rapid enhancement of insulin-mediated suppression of endogenous glucose production, independently of its effects on pancreatic function. Because this

experiment was performed in fasted and somatostatin-infused mice with very low incretin levels, this effect may be independent of any enhancement of incretin levels and action.

MATERIAL AND METHODS

Experimental animals

Male wild-type mice on a C57BL/6 genetic background were purchased from Charles River Laboratories (Charles River, Quebec, Canada) at the age of 6-7 weeks and were housed in the animal facility of the MaRS Centre/Toronto General Research Institute with a 12-hour light/dark cycle for at least one week before the experiment. They received a regular chow diet and water *ad libitum*. All animal experimentation described here was conducted in accord with accepted standards of humane animal care. All procedures were approved by the Animal Care Committee of the University Health Network, University of Toronto.

Assessment of plasma DPP-4 activity.

After a 4h fasting period, groups of 9 week-old C57BL/6 male mice were dosed with saline or a bolus of des-F-sitagliptin (Merck Research Laboratories, Rahway, New Jersey, USA) administered by gavage (25 mg/kg body weight of the salt form) or intravenously (10 mg/kg body weight) via a catheter inserted into the jugular vein 5 days prior to the experiment, as described below. The DPP-4 inhibitor des-F-sitagliptin is a chemically-related analogue of sitagliptin that Merck was willing to provide for our studies prior to sitagliptin receiving regulatory approval. Its use in preclinical studies has previously been described (30;31). Blood was collected from the tail vein at regular time intervals and DPP-4 activity in plasma was determined as previously described (12). Briefly, a 40 μ l plasma sample was incubated at 37°C in the presence of Gly-Pro *p*-nitroanilide (=substrate) (Sigma-Aldrich), and the rate of its conversion into *p*-nitroaniline by DPP-4-mediated enzymatic action was monitored at 0, 15, 30, 45, and 60 min. Yellow color development was monitored at the 405-nm wavelength. Concentration of *p*-nitroaniline produced by DPP-4 action on the substrate was calculated from a standard curve obtained with known concentrations of *p*-

nitroaniline. The kinetics of the enzymatic reaction are directly proportional to the slope of the curve calculated by linear regression. DPP-4 activity is expressed as μ mol/l units of activity per minute of incubation with the substrate.

Hyperinsulinemic euglycemic clamps

Surgical procedure. Three to 5 days before the experiment, mice were anesthetized with a ketamine (100 mg/kg)/xylazine (7.5 mg/kg) cocktail administered intraperitoneally, and a catheter (Microrenathane, MRE 0.25, Braintree Scientific Inc., Braintree, Massachusetts, USA) was inserted into the right jugular vein and tunneled subcutaneously to the back (32). Mice were allowed a 3 to 5 day recovery period and only mice that lost less than 10% of their preoperative weight were used for subsequent analyses.

Hyperinsulinemic euglycemic clamp. After a 5-hour fasting period, a hyperinsulinemic, euglycemic clamp was conducted in awake, tail-restrained mice, as previously described (32) and as depicted in Figure 1. The infusion studies lasted a total of 230 minutes. After a baseline blood sample was taken from the tail vein, an intravenous infusion of [3 - 3 H]-glucose tracer (Amersham) was started (10 μ Ci bolus, 0.05 μ Ci/min) for a 100 min equilibration (Basal) period. During the last 20 minutes of the basal period, 3 sequential blood samples were taken at 10 minute intervals from the tip of the tail for determination of basal glucose specific activity, blood glucose concentration and plasma hormones. At time 0, a primed, continuous (60 mU/kg bolus, 4 mU/kg/min) intravenous infusion of insulin (Humulin R, Eli Lilly, Indianapolis prepared in BSA 0.5%) was initiated. Somatostatin (3 μ g/kg/min) (Sigma-Aldrich) was started simultaneously as previously described (33) to suppress pancreatic hormone secretion and avoid any confounding effect of increased insulin secretion or suppression of glucagon secretion on the clamp outcome. The [3 - 3 H]-glucose tracer infusion was continued at the same rate (0.05 μ Ci/min). Concentration of the tracer and insulin infusates was calculated for an infusion rate of 2 μ l/min. Blood glucose was measured every 10 min throughout the experiment using a glucometer (in 1 μ l blood) and 25% dextrose (containing adequate amount of [3 - 3 H]-glucose to maintain glucose specific activity) was infused at a variable rate to maintain plasma

glucose at approximately 7 mM. Steady state (achieved within 100-130 min) was when a fixed glucose infusion rate to maintain blood glucose was constant for 30 min. Blood samples were collected at 10 minute intervals during steady state (last 30 minutes of the clamp) for glucose specific activity and hormone measurement.

Treatment: At time 15 min (i.e. 15 minutes after the start of the insulin and somatostatin infusion), a bolus of saline or des-F-sitagliptin (Merck Research Laboratories, Rahway, New Jersey, USA, 15 mg/kg body weight of the salt form =11 mg/kg body weight of des-F-sitagliptin) or exendin-4 (50 ng/25g body weight) was administered iv. A blood sample was taken 15 minutes later for hormone measurement.

Oral Glucose Tolerance Test (OGGT)

C57BL/6 male mice were chronically cannulated as described above (these mice were different from those undertaking the hyperinsulinemic euglycemic clamp and OGTT were performed as separate experiments). After five days recovery, mice were fasted for 4 hours. A baseline blood sample was collected before the intravenous (through the iv catheter) administration of a 25 μ l bolus of either saline (n=5) or exendin-4, 50 ng/25g BW (n=5). Immediately following the iv administration of saline or exendin-4, mice received a bolus of glucose (1.5 g/kg) by oral gavage (Time 0). Blood samples were collected at frequent time intervals from the tail tip for glucose measurement (Beckton-Dickinson BD glucometer). For plasma insulin determinations, a blood sample (50 μ l) was removed from the tail vein at 5 minute after glucose administration.

Assays

Insulin, C-peptide and glucagon concentrations were determined by RIA (Linco Research Inc., St. Charles, Missouri, USA) following manufacturer's guidelines. For the determination of plasma [3 -H] glucose, plasma was deproteinized with ZnSO₄ and Ba(OH)₂, dried to remove 3 H₂O, resuspended, and counted in scintillation fluid (Beckman Coulter). GLP-1 plasma levels were measured by with the mouse Lincoplex kit (Linco #MENDO-75K) according to manufacturer's instructions.

Calculations

Rates of basal glucose turnover and whole-body glucose uptake at the end of the basal period and during the final 30 minutes of the hyperinsulinemic euglycemic clamp were calculated by use of a modified form of Steele's equation, which takes into account the extra tracer infused with the glucose infusate (34). Endogenous glucose production rate (EGP) during clamps was determined by subtracting the glucose infusion rate from the total glucose rate of appearance.

Statistics

All values are presented as mean \pm SEM. Differences were considered statistically significant at $p < 0.05$. Comparisons among groups were made using ANOVA followed by pair-wise Scheffe post-hoc analysis.

RESULTS

Determination of optimal dosing of des-F-sitagliptin and Exendin-4.

As doses of up to 576 mg/kg of des-F-sitagliptin (1.1% w/w in food) have been reported by others to result in no toxicity when administered to mice on a daily basis for up to 11 weeks (24), we carried out preliminary studies to assess the effects of acute des-F-sitagliptin administration on plasma DPP-4 activity. 10 mg/kg of des-F-sitagliptin administered iv was almost as effective as 25 mg/kg administered orally, and both administration regimens resulted in >80% inhibition of DPP-4 activity for the duration of the 130 minute clamp study (Figure 2).

Exendin-4 has been used at concentrations ranging from 50 to 500 ng/25g BW in mice to study glucose homeostasis (9;35). Initially we performed hyperinsulinemic, euglycemic clamp studies using a dose of 200 ng exendin-4 (per 25g body weight) administered as an iv bolus 15 min after starting the insulin infusion. At that concentration of exendin-4 (200ng/25g BW) the GIR was significantly increased (not shown), but interpretation of this effect was confounded by the simultaneous breakthrough stimulation of insulin secretion as demonstrated by elevated clamp C-peptide plasma levels, despite concurrent somatostatin infusion (Figure 3A). In contrast, a lower dose of exendin-4, 50 ng/25g BW did not result in breakthrough insulin secretion during the clamp study (Figure 3A). Moreover, exendin-4 at a concentration of 50 ng/25g BW significantly reduced glucose excursion and increased plasma insulin during an OGTT (Figure 3B and 3C respectively), demonstrating robust biological activity of this dose of exendin 4.

Acute DPP-4 inhibition but not exendin-4 administration potentiates the effect of insulin *in vivo*.

Hyperinsulinemic, euglycemic clamps were performed to assess whether acute DPP-4 inhibition or administration of exendin-4 affects insulin sensitivity *in vivo* using the protocol described above as depicted in Figure 1. Blood glucose was effectively clamped around 7 mmol/l and was not significantly different between the two groups (saline, 6.9 ± 0.2 mmol/l vs. exendin-4, 7.2 ± 0.4 mmol/l vs DPP-4 inhibitor, 7.04 ± 0.2 mmol/l, p>0.05) (Figure 4A). Despite matched plasma glucose

concentrations, the glucose infusion rate (GIR) required to maintain euglycemia was significantly higher (within 15 min) in mice treated with des-F-sitagliptin compared to saline or exendin-4, and remained higher during the remaining clamp period (mean GIR time 100-130 min: des-F-sitagliptin: 60.5 ± 3.3 mg/kg/min vs saline: 42.0 ± 2.7 mg/kg/min, p< 0.001 or exendin-4: 46.5 ± 2.9 mg/kg/min, p< 0.001; exendin-4 vs saline, p=ns) (Figure 4B). This effect was not due to an increase in insulin secretion during DPP-4 inhibition, as demonstrated by the absence of differences in plasma insulin levels in saline vs. des-F-sitagliptin treated animals (Figure 4C), permitting a valid comparison of the relative insulin-potentiating effects of des-F-sitagliptin vs saline. Specific activity during the clamp is shown in supplemental Figure S1. In addition, plasma C-peptide levels were appropriately suppressed during the clamp in both experimental groups, and were not significantly different between the two groups at t+15 or t+30 min, or at the end of the clamp (T+130 min) (Figure 4D), providing further confirmation that there was no breakthrough of endogenous insulin secretion throughout the clamp period. GLP-1 plasma levels were below the detection limit and therefore can not be accurately reported. These data indicate that des-F-sitagliptin induced a rapid insulin potentiation independent of any effect on insulin secretion.

Acute DPP-4 inhibition rapidly enhances insulin-mediated suppression of endogenous glucose production.

The effect of acute inhibition of DPP-4 on the glucose infusion rate may reflect either an increased rate of glucose utilization, greater suppression of endogenous (mainly hepatic) glucose production (EGP) or both. [³H]-glucose tracer analysis demonstrated that this profound difference in insulin action was entirely due to enhancement of insulin-mediated suppression of EGP by des-F-sitagliptin (% suppression of EGP in des-F-sitagliptin: 121±13 %, saline: 38±15 %, exendin-4: 19± 14 %, p< 0.001 for des-F-sitagliptin vs saline and exendin-4, p=ns for exendin-4 vs. saline) (Figure 5A). In contrast, insulin-stimulated glucose utilization (Rd) was not different between the groups (Figure 5B). In addition, the decrease in EGP after DPP-4 inhibition was not related to changes in

glucagon secretion as glucagon levels were not significantly different between saline and DPP-4 treated mice (Basal: saline, 24.76 ± 1.79 vs. DPP-4 inhibitor, 35.78 ± 4.67 , $p=0.07$; Clamp: saline, 11.85 ± 2.5 , vs DPP-4 inhibitor, 8.9 ± 0.78 , $p=0.344$).

DISCUSSION

In the present report we examined the effect of acute des-F-sitagliptin administration on whole body glucose metabolism in wild-type, chow-fed mice, independent of its effect on insulin secretion, and compared this effect to that of a GLP-1R agonist. We clamped plasma glucose levels in the high euglycemic range (7 mmol/l) and used a rather low insulin infusion rate (4mU/kg/min) in order to compare the various treatments on suppression of EGP. Our data clearly show that acute inhibition of DPP-4 activity following des-F-sitagliptin administration completely suppresses endogenous (mainly hepatic) glucose production, with no significant effect on the rate of glucose disposal. Moreover, we used a simultaneous infusion of somatostatin to ensure that insulin and glucagon concentrations did not differ between study groups. The greater suppression of EGP with des-F-sitagliptin, therefore, was not simply due to greater suppression of glucagon secretion nor was it attributable to greater stimulation of endogenous insulin secretion.

A single dose of the DPP-4 inhibitor vildagliptin in diabetic humans effectively suppressed hepatic glucose output with no change in glucose utilization in a meal tolerance test (36), an effect likely related to increased insulin and decreased glucagon levels. In contrast, vildagliptin administration has also been shown to induce insulin sensitivity with no effect on EGP through increased glucose utilization (Rd) (37). It is important to note that our study examined the effect of acute DPP-4 inhibition, whereas chronic DPP-4 inhibition in diabetic rats improved hepatic and peripheral insulin sensitivity as shown by increased GIR, reduced basal and clamp glucose output and increased Rd at matched insulin concentrations (13). The concomitant reduction in glucolipotoxicity may also contribute to enhanced insulin action in chronic studies, a confounding variable not present in our acute administration paradigm.

Elucidation of the mechanisms important for the glucoregulatory actions of DPP-4 inhibitors have focused on the two dominant incretins, GLP-1 and GIP. Interestingly, GLP-1 infusion in depancreatized dogs was shown to increase GIR and glucose utilization (Rd), with no net effect on EGP, in clamp experiments at high insulin concentrations, although the effect of GLP-1 on glucose utilization was lost at lower insulin doses (25). Studies in mice have demonstrated that the acute or chronic glucoregulatory actions of DPP-4 inhibitors are mediated through the GLP-1 and GIP receptors (9;12). Unexpectedly however, recent studies have demonstrated that *Glp1r*^{-/-}, *Gipr*^{-/-} and double incretin receptor knockout mice exhibit complex phenotypes characterized by increased locomotor activity, enhanced energy expenditure, and in some experimental circumstances, enhanced insulin sensitivity (38;39). In the present study, des-F-sitagliptin administration had a profound effect on insulin-mediated suppression of EGP, whereas administration of a bolus of exendin-4 did not induce any change to either Rd or EGP. These results indicate that, in our experimental setting, the effect of des-F-sitagliptin on insulin-mediated EGP suppression is unlikely to be mediated by GLP-1. In support of this hypothesis, GLP-1 levels were very low in our experimental conditions, probably as a result both of the somatostatin infusion (somatostatin has been previously shown to suppress GLP-1 as well as GIP both in vivo and in vitro (40-43) and of the 5 hour fasting period before the start of the clamp (a total of almost 7 hours without food ingestion since the clamp was preceded by a 100 min tracer equilibration period), and DPP-4 inhibition in the fasted state is not expected to lead to a relevant increase of GLP-1 levels. This dissociation between the effect of DPP-4 inhibition by des-F-sitagliptin and GLP-1R activation is consistent with the absence of effect of exendin-4 administration on EGP in the same experimental setting. Another possible explanation for our observation might involve the action of another incretin, GIP. However, as mentioned above, somatostatin also suppresses GIP secretion, making it unlikely that GIP is involved in the effect of des-F-sitagliptin on EGP. The underlying mechanism may be related in some way to the DPP-4 enzyme's ability to cleave other substrates such as neuropeptides and chemokines (44), resulting

in elevated plasma and/or tissue levels of these factors. Hence, future studies are needed to explore how DPP-4 inhibitors rapidly enhance insulin-mediated glucose production suppression and will need to carefully consider the optimal experimental model in light of these observations.

In conclusion, we have demonstrated that acute administration of the DPP-4 inhibitor des-F-sitagliptin induces insulin-mediated suppression of endogenous glucose production. This finding adds to the growing body of knowledge regarding the complex mechanism of action of these antidiabetic agents and serves to emphasize that mechanisms other than incretin-mediated enhancement of GSIS may be important in mediating the glucose-lowering effect of these therapeutic agents.

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FIGURE LEGENDS

Figure 1. Schematic representation of the pancreatic and hyperinsulinemic euglycemic clamp procedure.

During the first 100 min (equilibration period), only [3-³H]-glucose tracer was infused and continued throughout the entire study. Blood samples were collected at time -100 and between times -20min and 0min for basal plasma hormone and glucose specific activity measurements, respectively. At time 0, a primed, continuous (60mU/kg, 4mU/kg/min) insulin infusion was started and continued for 130 mins. Somatostatin (SRIF) infusion was started at time 0 and was continued for the remainder of the experiment to suppress pancreatic hormone secretion. At time 15 (i.e. 15min after the start of the insulin and somatostatin infusions, saline or exendin-4 (50ng/25g BW) or DPP-4 inhibitor (11 mg/kg BW) were administered as a single 25 μ L bolus. 25% dextrose (containing adequate amount of [3-³H]-glucose to maintain glucose specific activity constant) was infused at a variable rate to maintain plasma glucose at approximately 7 mmol/l. Steady state (achieved within 100-130 min) was when a fixed glucose infusion rate to maintain blood glucose was constant for 30min. During that half an hour period, blood samples were collected for the clamp period glucose specific activity and hormones.

Figure 2. Des-F-sitagliptin effectively inhibits DPP-4 activity.

These mice were different from those undertaking the hyperinsulinemic euglycemic clamp. Des-F-sitagliptin (DPP-4 inhibitor) was given either by oral gavage (25 mpk in 0.5% CMC, n=3) or iv (10 mpk in saline, jugular vein catheter, n=3) to 12-13 week C57Bl/6 male mice after a 3 to 4hr fasting period. A control group received a iv bolus of saline (n=3). Blood samples were collected for a 2h period and DPP-4 activity was measured as described under "Methods".

Figure 3. An iv bolus of 50ng/25g BW exendin-4 has an incretin effect *in vivo* and does not cause induction of insulin secretion during the clamp experiments

These mice were different from those undertaking the hyperinsulinemic euglycemic clamp experiments reported in the manuscript and OGTT were performed as separate experiments. (A) Plasma C-peptide

levels at baseline and at the end of the hyperinsulinemic euglycemic clamp. 50 or 200 ng/25 g BW exendin-4 was given as a single bolus at time +15. (B) Blood glucose during an oral glucose tolerance test performed using mice administered 50ng/25g BW exendin-4 (n=3) or saline (n=3), performed as described under “Methods” and (C) insulin levels in plasma 10 min after injection of exendin-4 (50ng/25g BW , n=3) or saline (n=3)

*p<0.05 by unpaired t-test

Figure 4. A rapid increase in glucose infusion (GIR) is required to maintain euglycemia after DPP-IV inhibition vs Exendin-4 and saline.

Blood glucose (A), Glucose infusion rate (B) and plasma insulin (C) and C-peptide (D) during the hyperinsulinemic euglycemic clamp in mice receiving a bolus of either saline (n=5), exendin-4 (50ng/25gBW) (n=6) or des-F-sitagliptin (11 mpk) (n=6) iv.

Figure 5. The profound difference in insulin sensitivity by des-F-sitagliptin is due to enhancement of insulin-mediated suppression of EGP, with no effect on glucose disposal rate.

Endogenous glucose production (EGP) (A) and Glucose disposal rate (Rd) (B) during (pancreatic) hyperinsulinemic euglycemic clamp in mice receiving a bolus of either saline (n=5), exendin-4 (50ng) (n=6) or des-F-sitagliptin (11 mpk) (n=6) iv. §§§ indicates a p value <0.001

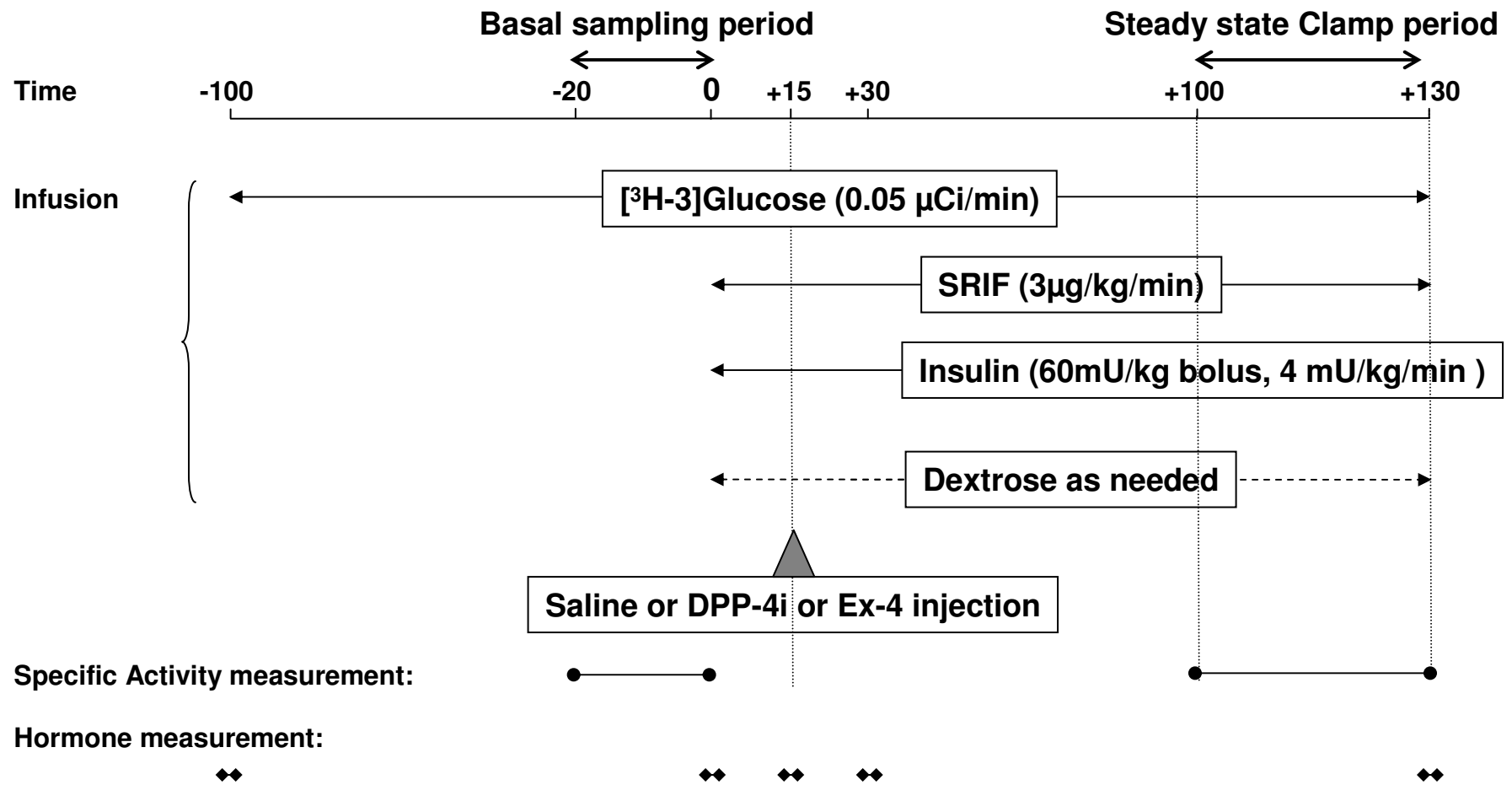


Figure 1. Duez et al.

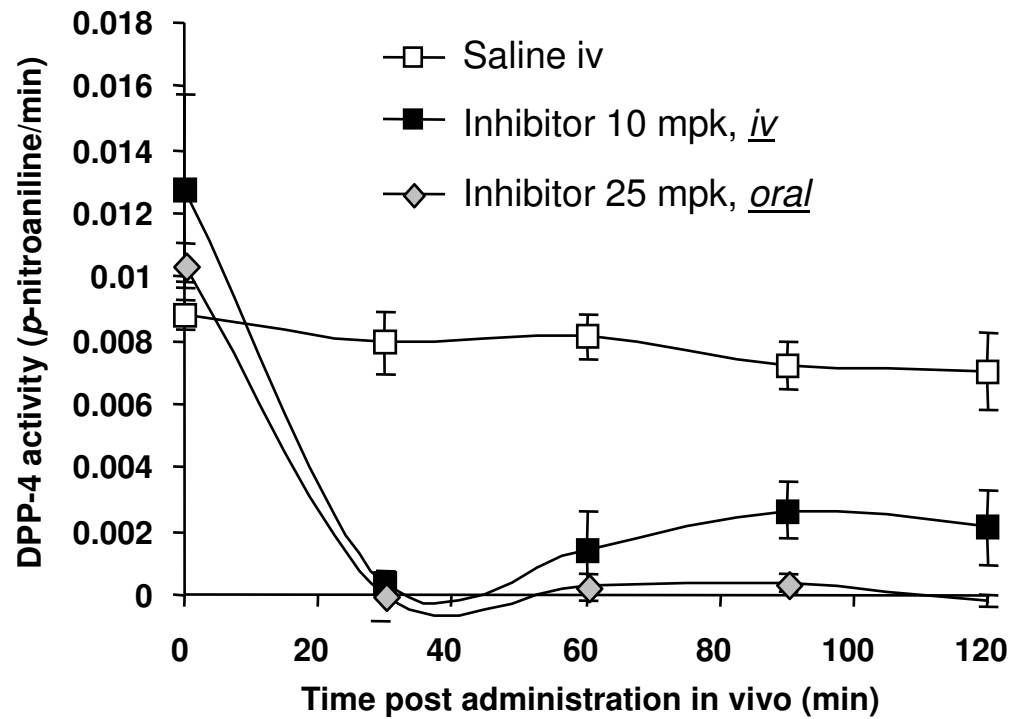


Figure 2. Duez et al.

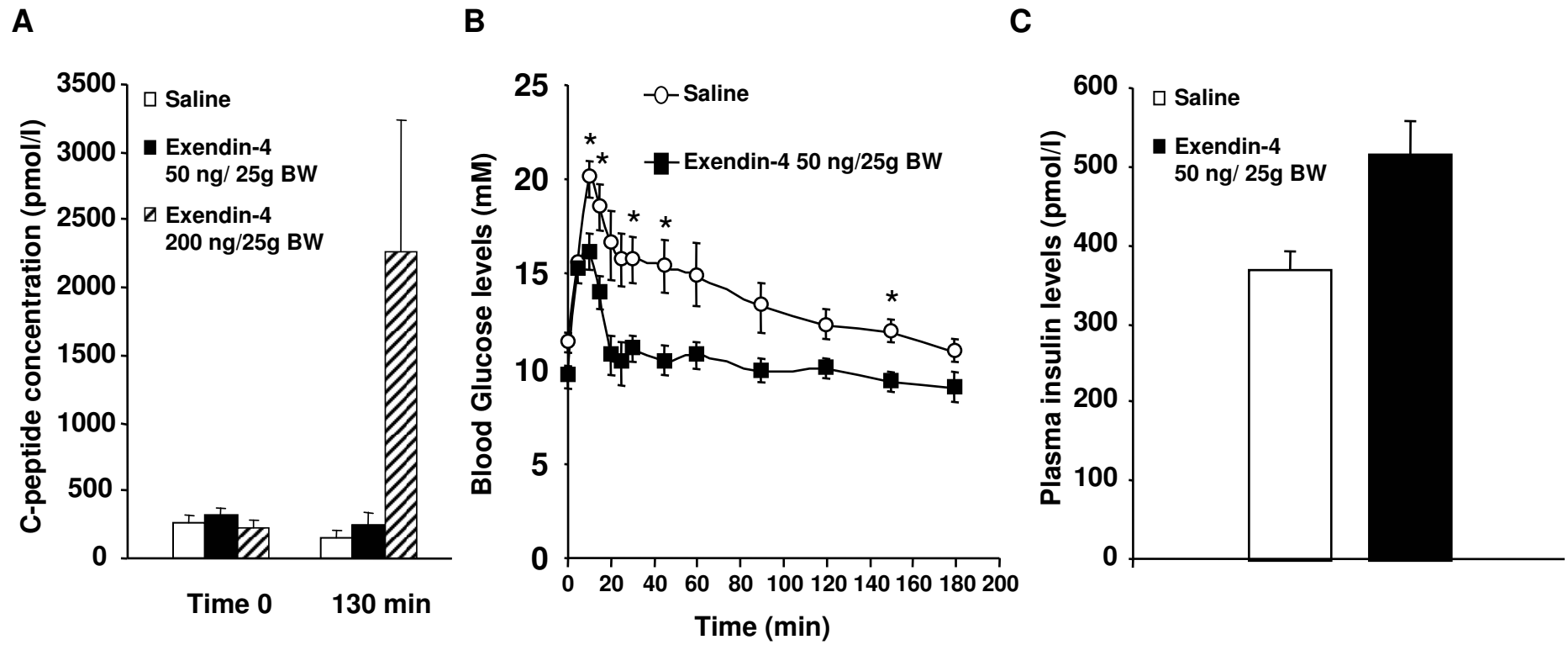


Figure 3. Duez et al.

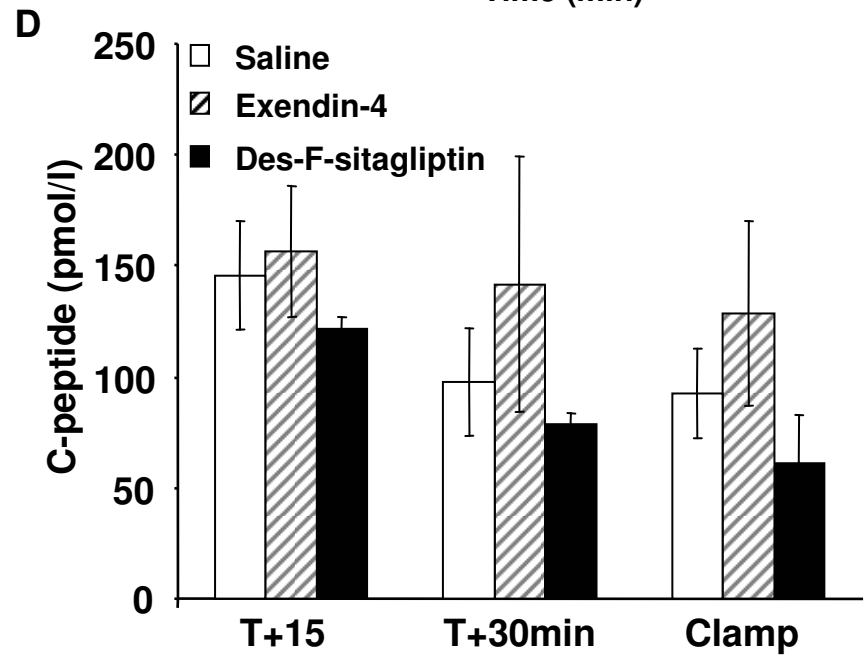
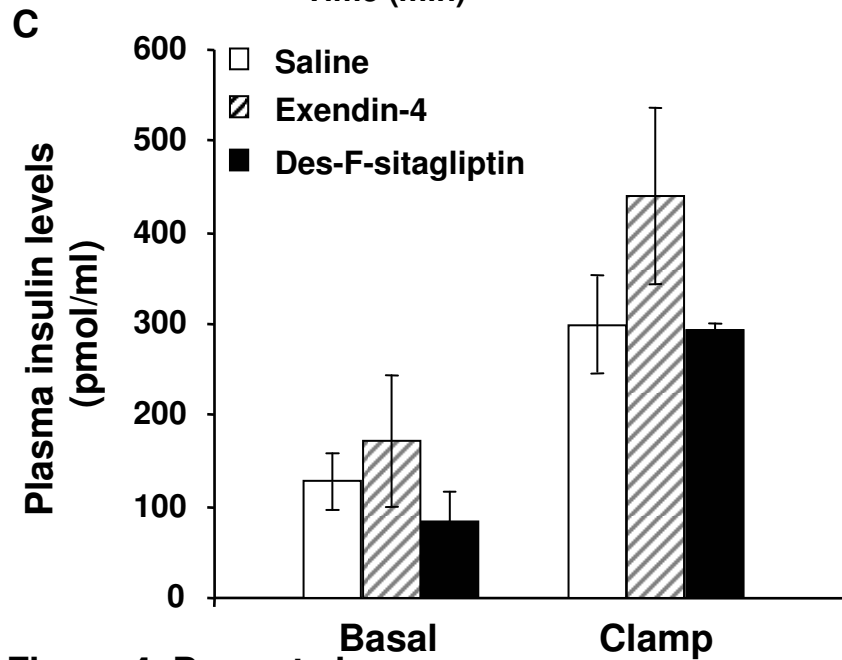
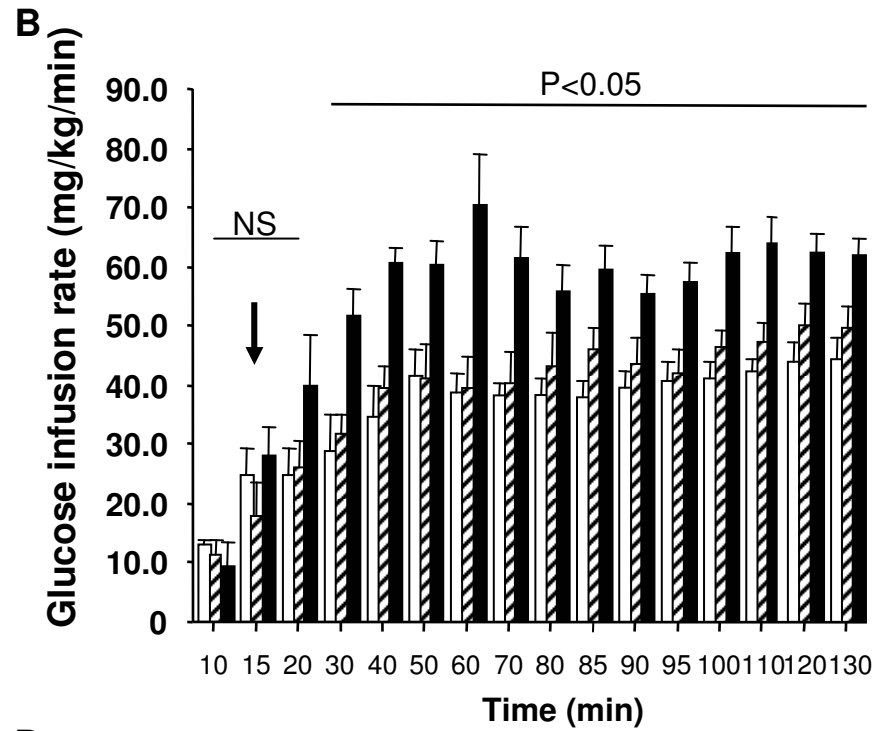
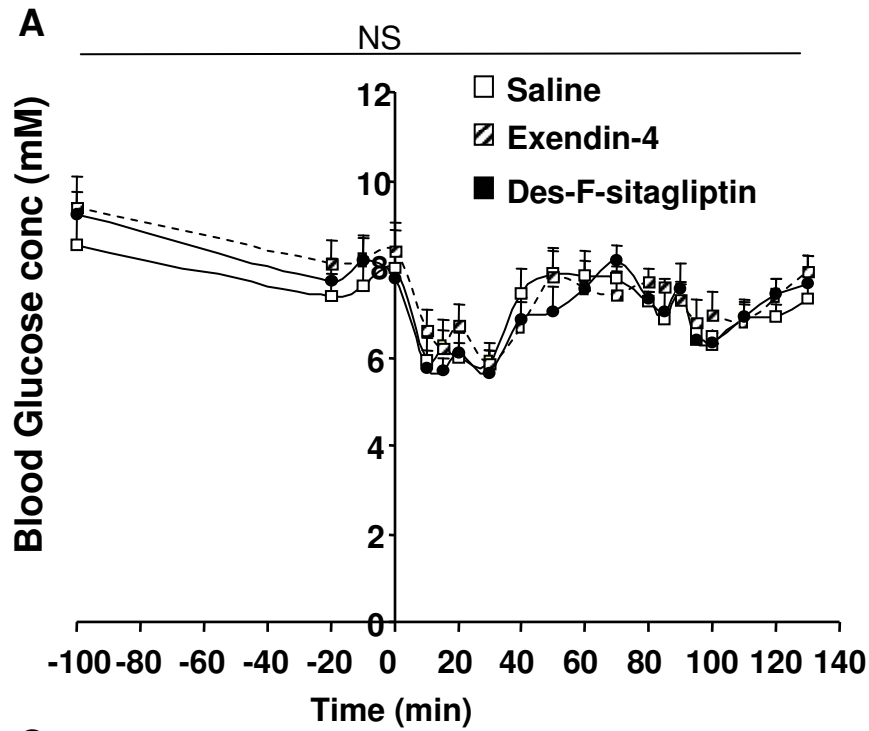


Figure 4. Duez et al.

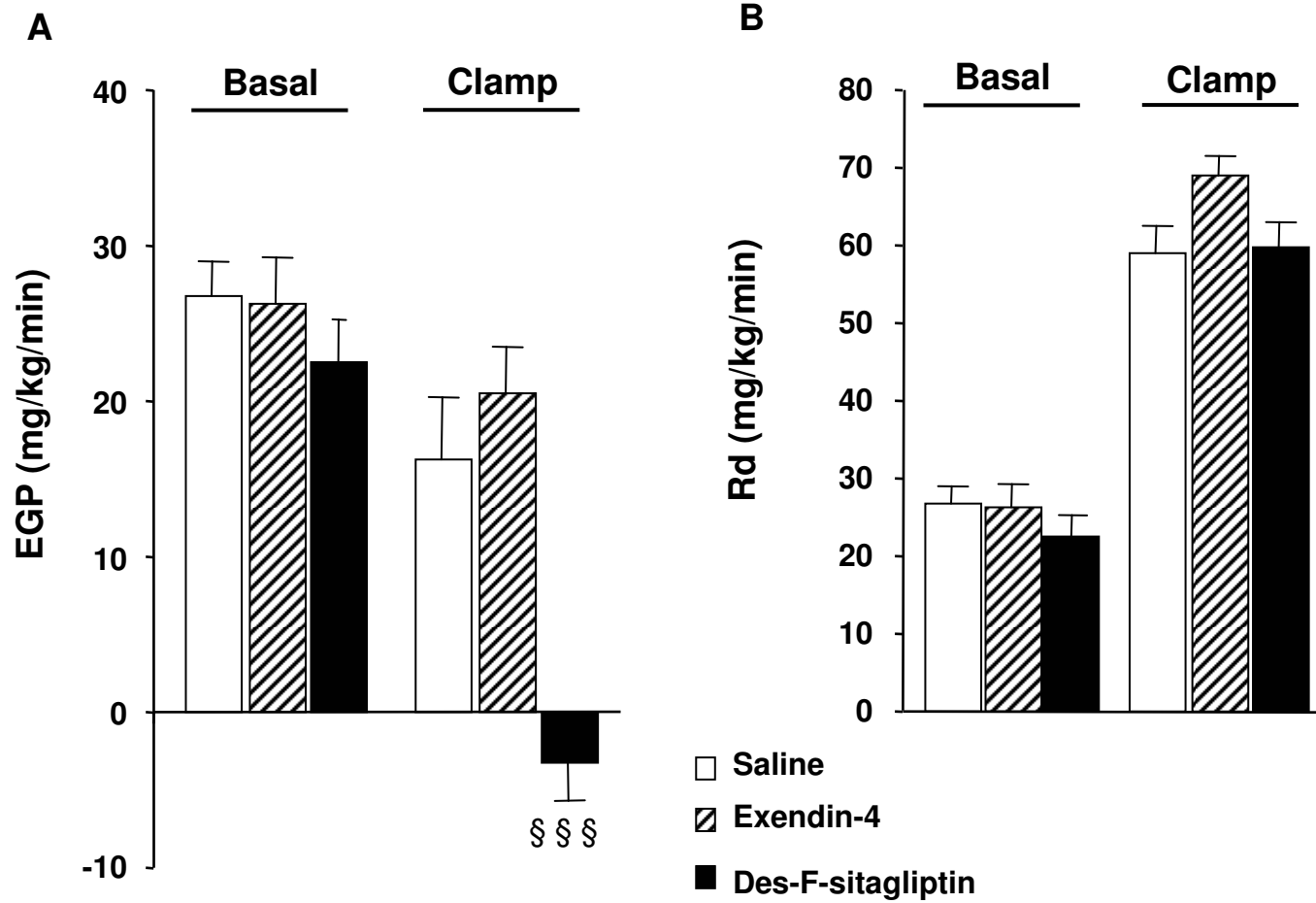


Figure 5. Duez et al.