Intranasal insulin suppresses endogenous glucose production in humans compared to placebo, in the presence of similar venous insulin concentration

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Abbreviated Title: INI and endogenous glucose production

Abbreviations: HGP (hepatic glucose production), EGP (endogenous glucose production), CNS (central nervous system), CSF (cerebrospinal fluid)

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Abstract

Intranasal insulin (INI) has been shown to modulate food intake and food related activity in the central nervous system in humans. As INI increases cerebrospinal fluid insulin concentration, these effects have been postulated to be mediated via insulin action in the brain, although peripheral effects of insulin cannot be excluded. INI has been shown to lower plasma glucose in some studies but it is not known whether it regulates endogenous glucose production (EGP).

To assess the role of INI in the regulation of EGP, 8 healthy men were studied in a single-blind, crossover study with 2 randomized visits (one with 40 IU INI and the other with intranasal placebo (INP) administration) 4 weeks apart. EGP was assessed under conditions of an arterial pancreatic clamp, with a primed, constant infusion of deuterated glucose and infusion of 20% dextrose as required to maintain euglycemia.

Between 180 and 360 minutes after administration, INI significantly suppressed EGP by 35.6% compared to INP, despite similar venous insulin concentrations.

In conclusion, INI lowers EGP in humans compared to INP, despite similar venous insulin, under experimental conditions of relative portal hypoinsulinemia. INI may therefore be of value in treating excess liver glucose production in diabetes.
Introduction

Dysregulation of insulin mediated suppression of hepatic glucose production (HGP) is a hallmark of type 2 diabetes (1). It is well established that activation of hepatic insulin receptors with ensuing activation of downstream insulin signaling pathways lowers hepatic glucose output by decreasing gluconeogenesis and increasing glycogen synthesis (1). In addition to the direct action of insulin on hepatocytes, insulin can indirectly affect hepatic glucose production by altering free fatty acid (FFA) flux and suppression of glucagon secretion (2-4). Animal have indicated that insulin may also indirectly regulate hepatic glucose output via effects on the central nervous system (CNS); the so called ‘brain-liver axis’ (1; 5; 6).

Injection of insulin into the 3rd cerebral ventricle in mice activates $K_{\text{ATP}}$ channels in the mediobasal hypothalamus (via activation of the insulin receptor-PI3 kinase pathway), which activates second order neurons in the brainstem. This in turn lowers expression of gluconeogenic enzymes and glucose production by the liver an effect that is abrogated with surgical resection of the hepatic branch of the vagus nerve (6). More recently CNS insulin action in the dorsal vagal complex has also been shown to regulate HGP via activation of the insulin-insulin receptor-ERK kinase pathway (7). These brain-liver axis effects are seen in the absence of changes in plasma insulin concentration (5-7). A brain-liver axis has also been demonstrated in dogs. Insulin delivery into the head arteries augments hepatic glycogen synthesis and reduces mRNA expression of gluconeogenic enzymes but with no acute change in HGP (8).

In recent human studies a single dose of intranasal insulin (INI), at a dose of 160 IU, has been shown to modulate food-related activity in the CNS (9), reduce overall food intake in
males (10), improve cognitive performance in females (10) and reduce intake of palatable food and increase satiety in females (11). These effects are likely mediated by direct CNS insulin action, although other non-CNS effects of nasally administered insulin cannot be definitively excluded. INI, at a lower dose of 40IU, has previously been shown to increase CSF insulin concentration with no significant change in measured serum insulin concentration sampled at 10 minute intervals for the 1st 40 minutes and 20 minute intervals for a further 40 minutes (12). Small lipophilic peptides such as insulin can potentially enter the CNS directly by diffusing across the olfactory epithelia and intercellular spaces into the subarachnoid space (12; 13). Intranasally administered peptides can also enter the CNS indirectly via uptake into the olfactory bulb and axonal transport (12; 13). Recent studies in humans have also shown that INI, administered at a higher dose of 160IU, can acutely lower plasma glucose and alter peripheral insulin sensitivity (11; 14; 15), an effect postulated to occur via insulin delivery to the CNS. However, the higher dose (160IU) of INI transiently increased peripheral insulin concentration (9; 14; 15) which may contribute to the acute effects of INI on peripheral glucose metabolism and insulin sensitivity (11; 14; 15).

In the present single blind, placebo controlled, crossover study, we aimed to investigate whether INI action regulates EGP in humans. We assessed EGP following the administration of 40 IU of INI or intranasal placebo (INP) with primed, constant infusion of d-[6,6'-2H2]glucose (D2-glucose) (Figure 1). Participants were studied under conditions of an arterial pancreatic clamp in which systemic venous insulin and glucagon concentrations are clamped at basal levels to prevent fluctuations in peripheral arterial insulin and glucagon concentrations. It is noted with this clamp technique that portal insulin and glucagon concentrations are lower than would be expected in the basal state i.e. this is a state of
hepatic insulin and glucagon deficiency. Under these conditions we have demonstrated for
the first time in humans that INI lowers EGP. This effect was seen in the presence of similar
venous insulin concentration.

Research Design and Methods

Study Participants

8 healthy men, with no medical illnesses and taking no medications, were recruited by
advertisements in the local press. Their demographic and biochemical parameters are
shown in Table 1. They underwent a 75 gram oral glucose tolerance test, routine screening
blood tests and urinalysis. Those with abnormal tests were excluded. Each participant was
studied on 2 occasions, 4 weeks apart, in a single-blind, placebo-controlled crossover trial.
Each participant received 40 IU of INI lispro (®Humalog, Eli Lilly, Toronto, ON, Canada)
during one visit and placebo during the other. The order of the visits was randomized.

Insulin dosing:

Pilot studies indicated that peripheral insulin spillover was inevitable, with doses ranging
from 80 IU to as low as 10 IU (Supplementary Figure S1). 40 IU of INI was chosen as this
dose has previously been shown to increase CSF insulin concentration in humans (12) and in
our pilot studies a higher dose of INI (80 IU) resulted in a greater spillover of insulin lispro
into the peripheral circulation (Supplementary Figure S1). In order to ensure similar venous
plasma insulin concentrations between treatments, 0.005 IU/kg insulin lispro was infused
intravenously over 30 min, starting at the time of administration of INP. The intravenous
insulin lispro dose was identified in a pilot study with varied doses (data not shown).
Study outline (Figure 1)

Participants were admitted to the Metabolic Test Centre of Toronto General Hospital the morning before the study. Volunteers had a standardized mixed meal at 5pm and were not permitted any food or drink orally except water until the conclusion of the study. The following day at 7am (t=-120 minutes), an arterial pancreatic clamp was started and continued for the remainder of the study (until 3pm, t=360 minutes) to neutralize any potential effects of arterial pancreatic hormone fluctuations on EGP. The clamp comprised the following infusions: somatostatin (Sandostatin, Novartis Pharmaceuticals Canada, Dorval, QC, Canada) 30 µg/h to inhibit pancreatic insulin and glucagon secretion with concomitant replacement at basal levels of insulin (Humulin R, Eli Lilly Canada, Toronto, ON, Canada) at 0.05 mU/kg/min, human recombinant growth hormone (Humatrope, Eli Lilly, Canada) at 3 ng/kg/min and glucagon (Eli Lilly, Canada) at 0.325 ng/kg/min. Autologous serum (3 mL), freshly prepared from the subject’s blood, was added to the saline as carrier prior to hormone dilution.

Also at 7am (t= -120 minutes), a primed, constant infusion (22.5 µmol/kg bolus followed by 0.25 µmol/kg/minute) of d-[6,6'-2H2]glucose (D2 glucose; Cambridge Isotope Laboratories, MA) was started and continued until the conclusion of the study at 3pm (t= 360 minutes). At 9am (t= 0 minutes), participants received either 40 IU of insulin lispro (100 IU/ml Humalog, Eli Lilly) or placebo (insulin diluent, Eli Lilly) via a metered nasal dispenser (Pharmasystems, Markham, ON, Canada). A single spray dispenses 0.1 ml (10 IU of insulin). A single spray was administered in each nostril while inhaling. 1 minute later a further spray was similarly administered in each nostril to give a total dose of 40 IU of insulin lispro. Due to spillover of 40 IU insulin lispro into the peripheral circulation, (Supplementary figure S1),
during the placebo visit, volunteers received an intravenous infusion of insulin lispro to ensure similar venous insulin concentration in both treatment groups, as described above.

Blood samples (10 mls) were drawn every 30 minutes for the first 120 minutes (t=-120 minutes to 0 minutes) after starting the arterial pancreatic clamp, every 5 minutes for 30 minutes after administration of INI/INP (t=0 minutes to 30 minutes) and every 10 minutes thereafter till the conclusion of the study (t=30 minutes to 360 minutes). 20% dextrose solution was administered to maintain euglycemia as necessary.

**Laboratory Methods**

Plasma was separated from blood samples in a refrigerated centrifuge at 3000 rpm for 15 min at 4º C. Sodium azide (70 mg/l blood) (Sigma Aldrich, Oakville, Canada) and aprotinin (1.94 mg/l blood) (Sigma Aldrich, Oakville, Canada) were added to the plasma to prevent hydrolysis and protein degradation. Plasma was dried, derivatized and stable isotope enrichments determined (16). Derivatized samples were analyzed with GC/MS (Agilent 5975/6890N, Agilent Technologies Canada Inc, Mississauga, ON, Canada) with electron impact ionization using helium as the carrier gas. Selective ion monitoring at m/z =242 and 244 was performed. Atom percent excess fraction (APE) was calculated for each sample as APE= tracer/(tracer + tracee).

Commercial kits were used to measure total insulin (Millipore, Billerica, MA, USA), growth hormone (Abcam Inc, Toronto, Canada), FFA (Wako Industrials, Osaka, Japan), TG (Roche Diagnostics) and glucagon (Millipore). An insulin lispro (Millipore) kit (specificity for lispro: 100%, specificity for human insulin ~0.05%) was used to measure lispro concentration in our pilot studies (Supplementary figure S1).
Analysis of endogenous glucose production

EGP was calculated as described previously (17). During steady state, rate of glucose appearance ($R_a$) = rate of glucose disappearance ($R_d$) where $R_a = \text{tracer infusion rate} / \text{APE fraction}$. Endogenous glucose production (EGP) rate = $R_a - \text{glucose infusion rate}$ (17).

Statistics

Results are presented as mean ± SEM. Paired t-test was used to compare plasma glucose, venous insulin, plasma glucagon, glucose infusion rates and endogenous glucose production. A p value < 0.05 was considered significant. Post hoc analysis revealed a power of 98% to detect a change in EGP as well as plasma insulin in the final 180 minutes of the study (t=180-360). The power to detect a change in insulin concentration during the entire study was 94%.

Study oversight

The study was carried out according to the principles of the Declaration of Helsinki and was approved by University Health Network Research Ethics Board, Toronto. All participants gave written informed consent.

Results

**Plasma glucose and insulin concentrations.** Mean glucose concentration over time during the study is depicted in Figure 2A. INI treatment transiently lowered plasma glucose concentrations, with mean nadir concentration at 180 minutes (INP 6.1±0.3 vs. INI 5.1±0.1 mmol/l, p=0.01) (Figure 2A). With infusion of 20% dextrose blood glucose concentrations were not significantly different in the final 120 minutes of the study. There was no
significant difference in mean venous plasma insulin concentration (Figure 2B) between treatments (INP 71±7 vs nasal insulin 77±11 pmol/l, p=0.6). As expected, based on pilot data, there was a transient increase in venous insulin after INI administration (Supplementary Figure S1). Administration of a 30 minute intravenous insulin lispro infusion along with INP administration ensured venous insulin concentrations were similar between the groups.

INI treatment increases intravenous glucose infusion requirements to maintain euglycemia.

Intravenous glucose in the form of 20% dextrose was infused, as required, to maintain euglycemia. The mean dextrose infusion rate over time is illustrated in Figure 2C. The mean dextrose infusion rate in the final 180 minutes of the study (t=180-360 minutes) was significantly higher with INI treatment (placebo 1.1±0.9 vs. INI 6.6±1.6 umol/min/kg, p=0.015) (Figure 2D) with the maximal difference seen at 250 minutes (placebo 1±1 vs. INI 8.4±1.1 umol/min/kg, p=0.006) (Figure 2C). These changes were seen despite similar venous insulin concentrations.

INI suppresses endogenous glucose production without affecting glucose disposal.

Mean EGP rate over time is shown in Figure 3A. INI lowered EGP, with the nadir value at 250 minutes (placebo 12.3±1.3 vs. INI 6±0.9 µmol/min/kg, p=0.02). Mean EGP in the final 180 minutes of the study (t=180-360 minutes) was significantly lower with INI treatment (placebo 11.7±1 vs. INI 7.6±0.6 µmol/min/kg, p=0.02) (Figure 3B). The correlation coefficient between peak insulin after INI or placebo administration and decline in EGP from baseline in the final 180 minutes of the study was 0.38.
The rate of glucose disposal (Rd) over time is depicted in Figure 3C. There was no significant difference in glucose disposal. Mean Rd in the final 180 minutes of the study was not different between the groups (placebo 12.9±1.2 vs INI 13.7±1 umol/min/kg, p=0.4) (Figure 3D). The specific activity of D2-glucose over time is shown in Supplementary Figure S3.

**Plasma free fatty acid (FFA) and triglyceride (TG) concentration**

Plasma FFA concentration are shown in Figure 4A. FFA concentration was significantly lower at 240 minutes with INI (placebo 0.2±0.05 vs INI 0.1±0.03 mmol/l, p=0.03). Mean FFA concentration in the final 180 minutes (t=180-360 minutes) was not significantly different between treatments (placebo 0.20±0.05 vs INI 0.13±0.03 mmol/l, p=0.12) (Figure 4B).

Plasma TG concentration is shown in Figure 4C. There was no significant difference in mean TG concentration from t=180-360 minutes (placebo 0.7±0.1 vs INI 0.6±0.1, p=0.23) (Figure 4D).

**Discussion**

Rodent studies have demonstrated that insulin action in the CNS can reduce HGP (5; 6). Previous human studies that have deployed INI, at a dose that increases CSF insulin concentration (12), have reported changes in peripheral glucose concentration and insulin sensitivity suggesting CNS insulin may regulate peripheral glucose metabolism (11; 14; 15). However, in these studies, there was a transient increase in venous insulin concentration after INI administration. The present study is the first human study to definitively demonstrate that INI (40 IU) suppresses EGP compared to INP. Importantly the experimental design of our study ensured that venous insulin concentrations were similar between treatments.
40 IU of INI has previously been shown to rapidly raise CSF insulin concentration (12) without increasing serum insulin. In our hands, under conditions of an arterial pancreatic clamp during which endogenous insulin secretion cannot be modulated, 40 IU of INI (insulin lispro, Eli Lilly, Canada) also transiently raised venous insulin concentration as measured by a specific lispro assay. We also detected peripheral spillover with a dose as low as 10 IU (Supplementary Figure S1). In this study, we administered the lowest dose of nasal insulin that has previously been shown to raise CSF insulin concentration (12), while minimizing systemic spillover by not using a higher dose. Subjects treated with INP were given an infusion of insulin lispro to try and mimic the increase in venous insulin concentrations after spillover of INI (Figure 1). This experimental approach ensured similar venous insulin concentrations between the treatment groups.

The peripheral spillover of INI, detected with frequent blood sampling (every 5-10 minutes) may have implications in the interpretation of certain findings from previous studies with higher doses of INI. Three studies have utilized higher doses of INI (160 IU) and reported relatively modest lowering of plasma glucose concentration within 30-45 minutes of administration (11; 14) as well as improved peripheral insulin sensitivity (15). These studies did not measure plasma insulin as frequently as in the current study. In one study (14), plasma insulin (measured every 30 minutes) was significantly higher at 30 minutes with a decline in C-peptide and subsequent insulin concentration, suggestive of peripheral spillover of insulin with decline in endogenous insulin secretion. Plasma insulin concentration (measured every 15 minutes) was transiently higher at 15 minutes in a recent study by the same group after administration of 160 IU of INI (15). Insulin concentration was not reported for the first 45 minutes in the study by Hallschmid et al (11). In these studies of INI,
it is not possible to exclude a contribution of peripheral insulin action to the rapid lowering of plasma glucose concentration. It is worth noting that other studies with 160 IU of INI have not reported changes in plasma glucose levels (9; 18). This may be due to the relative infrequency of blood sampling (every 30 minutes compared to every 5-10 minutes in the present study) (11). In addition in the absence of an arterial pancreatic clamp, endogenous insulin and glucagon secretion can be modulated to prevent major fluctuations in plasma glucose (9; 14). Additionally, with an arterial pancreatic clamp, the normal portal to peripheral insulin gradient is lost resulting in relative hepatic insulin deficiency which may have permitted INI to lower EGP. In the aforementioned studies (9; 14) the physiological portal peripheral gradients of insulin and glucagon are maintained which may have rendered the liver less sensitive to INI. Lastly, these studies were of a shorter duration (9; 11; 14; 18) (≤180 minutes) than the current study (360 minutes), in which EGP declined after 180 minutes and therefore a late glucose lowering effect would have gone undetected.

Unlike the relatively rapid lowering of plasma glucose seen with INI in some studies (11; 14; 15), it has previously been shown in humans that extra-pancreatic K\textsubscript{ATP} channel activation (likely CNS K\textsubscript{ATP} channel activation, a downstream target of insulin action in the CNS) lowers EGP over the course of ~6-7 hours (parallel studies in rats demonstrated that an equivalent dose of diazoxide is detectable in CSF after 1 hour and plateaus at ~4 hours) (16). We therefore speculate that the rapid lowering of plasma glucose seen with 160 IU in previously published studies (11; 14) was likely due to transient systemic insulin absorption from the INI administration that in turn induces peripheral insulin action and that any potential CNS regulation of EGP by insulin would be a slower process. Consistent with our hypothesis, despite similar venous insulin concentrations in both treatment groups throughout the
study, INI lowered EGP after ~180 minutes, with EGP production rates remaining lower at
the conclusion of the study at 360 minutes. This time scale of INI action is similar to that
reported with intracerebroventricular injection of insulin in rodents (5; 6) and suggests that
INI, potentially via CNS insulin action, does not rapidly regulate EGP in the acute setting. It is
not known whether prior exposure to INI as seen with longer term administration of INI
affects EGP, but 8 weeks of treatment with INI did not affect fasting insulin and glucose (19).

Although the exact mechanism by which INI lowers EGP remains to be determined, reduced
expression of hepatic gluconeogenic enzymes such as PEPCK and G6Pase via hepatic vagal
efferents secondary to CNS insulin action, is a plausible explanation. This is based on the
previous findings that 1) INI increases CSF insulin concentration (12) and 2) that in rodents
intracerebroventricular insulin injection reduces expression of gluconeogenic enzymes and
hepatic glucose production, an effect abrogated by resection of hepatic vagal efferents (5;
6). A previous study has demonstrated a reduction in FFA with 160IU of INI treatment in
humans with no reported change in plasma insulin concentration (18), although as
discussed above at this dose peripheral insulin spillover may have contributed. In the
current study, mean FFA between 180 and 360 minutes tended to be lower with INI, but did
not reach statistical significance. In view of the relatively small sample size of the current
study we cannot exclude a significant difference in FFA had we studied more individuals,
and therefore reduced FFA flux to the liver could potentially contribute to the lowering of
hepatic glucose production. There were no significant differences in the concentration of
growth hormone and glucagon between treatments. Previous studies in dogs have
demonstrated that pulmonary insulin delivery can regulate insulin sensitivity independent of
plasma insulin concentration (20). Nasally inhaled aerosols have been shown to be
deposited in the lungs (21). INI can potentially deliver insulin to the lungs with secondary effects on glucose metabolism. It is not possible to rule out as yet unidentified CNS and/or non-CNS mediated effects as contributors to EGP reduction.

Although the present study suggests that INI possibly acting via the CNS, can regulate EGP, the contribution of this pathway to glucose homeostasis in normal human physiology remains to be determined. Under our arterial pancreatic clamp conditions, the pancreas is unable to modulate endogenous insulin and glucagon secretion with changing glucose concentration. Additionally with an arterial pancreatic clamp, as was the case with previous rodent studies (5; 6), portal and peripheral insulin concentrations are likely to be identical, since the normal portal-peripheral insulin concentration gradient is abolished when insulin delivery does not occur via the portal circulation. In normal physiology, portal insulin concentration is ~3 times greater than its peripheral concentration (8; 22). Hence, during an arterial pancreatic clamp there is relative hepatic insulin deficiency (8; 22). Under these conditions, CNS-mediated effects of insulin are dominant and lower EGP. However with an arterial pancreatic clamp, the portal to peripheral glucagon gradient (23) is also lost which may have minimized the effects of relative hepatic insulin deficiency on EGP. Consistent with the hypothesis that only under conditions of relative hepatic insulin deficiency can CNS insulin lower EGP, in experiments carried out in dogs, when a pancreatic clamp is instituted with normal portal-peripheral insulin concentration gradient, CNS insulin delivery augments glycogen synthesis but has no effect on EGP four hours after administration (8). However, in this study, the CNS to non-CNS insulin gradient was not maintained. Blockade of hypothalamic insulin action in the setting of physiological hyperinsulinemia and portal-peripheral insulin concentration gradient, blunted induction of glucokinase gene
transcription and abrogated the inhibition of glycogen synthase 3β transcription but with no net change in EGP (24). This effect was seen with a similar physiological rise in CNS, liver and peripheral circulation. Although it is possible some differences might be ascribed to interspecies effects, the presence of a portal-peripheral insulin gradient likely abrogated the effect of CNS insulin action. Intriguingly, there was a significant reduction in mRNA levels without significant changes in protein expression of gluconeogenic enzymes (8). It remains unclear whether a study of longer duration, such as our 6 hour study, would have affected EGP.

It is currently not known whether INI would lower plasma glucose in a more physiological setting, in the absence of an arterial pancreatic clamp and presence of a normal portal-peripheral insulin concentration gradient, over the time course of the current study (between 180 and 360 minutes after administration). Previous studies with intranasal insulin, in the absence of a pancreatic clamp, have reported very modest (~5%) reduction in plasma glucose concentration within 2 hours of administration which may reflect insulin action due to peripheral spillover (11; 14). Notwithstanding interspecies differences and differing assays, based on hyperinsulinemic clamp studies in dogs with measurement of plasma and CSF insulin (25) as well as CSF insulin concentration after administration of 40 IU of INI in humans (12), it is likely that 40 IU of insulin causes a supra-physiological rise in CSF insulin concentration. In a previous study assessing the effects of activation of extra-pancreatic K\textsubscript{ATP} channels with an arterial pancreatic clamp, a physiological increase in insulin concentrations in the placebo group did not alter EGP, although pharmacological activation of K\textsubscript{ATP} channels did reduce EGP (16). Despite the non-physiological aspects of the study discussed above, we have demonstrated for the first time that nasally administered insulin
lowers EGP in humans compared to intranasal placebo in the presence of similar venous insulin concentration.

Resistance to direct insulin action in the liver and increased hepatic glucose production is a hallmark of type 2 diabetes (1). Current treatment modalities for type 2 diabetes have potential side effects, including weight gain with subcutaneous insulin and sulphonylureas which can potentially exacerbate hepatic insulin resistance (26). It remains to be determined whether INI can acutely lower EGP in individuals with insulin resistance and type 2 diabetes and whether chronic treatment with INI affects glycemic control. An additional potential advantage of INI is that unlike subcutaneous insulin, it is less likely to cause weight gain. Animal studies have suggested that CNS insulin action reduces appetite and results in weight loss (27). In human studies with INI, an acute reduction in appetite has been reported after a single dose in men (10) with a reduction in postprandial satiety and intake of palatable food in women (11). Additionally, 8 weeks of INI treatment in slim healthy volunteers, modestly reduced fat mass and body weight in men with modest weight gain seen in women which is thought to be due to a rise in extracellular water but importantly with no change in body fat (19). It must be noted however that in rodent models of insulin resistance induced by a high fat diet, CNS-mediated effects of insulin are blunted (28), suggestive of the presence of hypothalamic insulin resistance. It remains to be determined whether a similar phenomenon occurs in obese insulin resistant humans.

In conclusion, we have shown that INI, at a dose that is known to increase CSF insulin concentration, lowers EGP under conditions of experimental portal hypoinsulinemia compared to INP. This effect is seen despite similar venous insulin concentration between
treatments. Additional studies are needed to evaluate whether this pathway is amenable to therapeutic manipulation in insulin resistance and type 2 diabetes.

**Author Contributions:**

SD, CX, CM, and GFL designed the study and interpreted data. SD, CX, CM and KK acquired and analyzed data. SD and GFL wrote the manuscript and all authors edited the manuscript. GFL obtained funding and supervised the study. GFL is the guarantor and had final responsibility for the decision to submit for publication.

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**Conflict of interest:** This study was funded by Eli Lilly Canada (Grant recipient GFL). GFL has served on advisory boards to Eli Lilly Canada.
REFERENCES

Table 1. Baseline demographics & biochemistry

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BMI: body mass index
Figure Legends

Figure 1.

Outline of study. Participants were admitted to the Metabolic Test Centre the day before the study. They were given a mixed meal at 5pm. At 7am (T= -120 minutes) a primed, constant infusion of D2-glucose was started and continued for the duration of the study. At the same time an arterial pancreatic clamp was started with infusion of somatostatin along with replacement doses of insulin, glucagon and growth hormone, as described in the ‘Research design and methods’ section. At 9am (T= 0 minutes) 40 IU of INI or placebo was administered.

† In order to ensure similar venous insulin concentration between treatments, the placebo study subjects were given 0.005 IU/kg insulin lispro i.v. (intravenous) over 30 minutes starting at the same time as the intranasal placebo as there was spill over of intranasal insulin. NPO: nil per os (nothing by mouth)

Figure 2.

A. Mean plasma glucose concentrations over time during the course of the study (INP: white diamond and dotted line ---◊---; INI: black square and solid line —■—). INI treatment lowered plasma glucose concentrations with mean nadir concentration at 180 minutes. With infusion of 20% dextrose in order to maintain euglycemia (as shown in fig 2C), blood glucose concentrations were not significantly different between treatments in the final 120 minutes of the study.

B. Mean venous insulin concentrations over time during the course of the study. (INP: white diamond and dotted line ---◊---; INI: black square and solid line —■—). There were no significant differences in venous insulin concentration between treatments. Consistent with our pilot data (Supplementary Figure S1), there was a transient increase in venous insulin concentration with INI, which was mimicked in the INP group by administration of intravenous insulin lispro from 0 to 30 minutes.

C. Time course of mean glucose infusion requirements (20% dextrose in umol/min/kg), in order to maintain euglycemia, during the study (INP: white diamond and dotted line ---◊---; INI: black square and solid line —■—). Glucose infusion rates increased after ~160 to 180 minutes with INI treatment, with the maximal difference in infusion rate at 250 minutes (* p=0.006).
D. Mean glucose infusion rates over the final 180 minutes (T=180-360 minutes) of the study (INP: white bar, INI: black bar). Mean glucose infusion rates were significantly higher with INI treatment (p=0.015).

Figure 3.

A. Time course of mean endogenous glucose production (EGP) rates during the study (INP: white diamond and dotted line $\cdots\cdots$; INI: black square and solid line $\square$). EGP rates declined with INI treatment from $\sim$ 180 minutes with a nadir at 250 minutes ($\dagger p=0.01$)
B. Mean EGP in the final 180 minutes of the study (T=180-360 minutes) (INP: white bar; INI: black bar). Mean EGP was lower with INI treatment ($¶ p=0.02$).
C. Time course of mean glucose disposal (Rd) rates during the study (INP: white diamond and dotted line $\cdots\cdots$; INI: black square and solid line $\square$). No significant difference was seen between treatments.
D. Mean Rd in the final 180 minutes of the study (T=180-360 minutes) (INP: white bar; INI: black bar). No significant difference was seen between treatments.

Figure 4.

A. Time course of plasma free fatty acid (FFA) concentration during the study (INP: white diamond and dotted line $\cdots\cdots$; INI: black square and solid line $\square$). FFA concentration was significantly lower with INI at 240 minutes ($\# p=0.03$).
B. Mean plasma FFA concentration over the final 180 minutes (T=180-360 minutes) of the study (INP: white bar, INI: black bar). No significant difference was seen between treatments.
C. Time course of plasma triglyceride (TG) concentration during the study (INP: white diamond and dotted line $\cdots\cdots$; INI: black square and solid line $\square$). No significant difference was seen between treatments.
D. Mean plasma TG concentration over the final 180 minutes (T=180-360 minutes) of the study (INP: white bar, INI: black bar). No significant difference was seen between treatments.
**Supplementary Figure S1.**

Venous insulin lispro concentration over time from 3 separate subjects administered 3 distinct doses of intranasal insulin to assess whether INI increases plasma insulin concentration. Intranasal insulin was administered at T= 0 minutes. There was a transient increase in plasma lispro with all 3 doses.

A. 80 IU of INI (arrow) was administered at 0 minutes.
B. 40 IU of INI (arrow) was administered at 0 minutes.
C. 10 IU of INI (arrow) was administered at 0 minutes.

**Supplementary Figure S2.**

A. Mean plasma glucagon concentration over time during the study (INP: white diamond and dotted line ---○--; INI: black square and solid line −→). There were no significant differences between treatments.

B. Mean plasma growth hormone concentration over time during the study (INP: white diamond and dotted line ---○--; INI: black square and solid line −→). There were no significant differences between treatments.

**Supplementary Figure S3.**

D2-glucose specific activity during the study (INP: white diamond and dotted line ---○--; INI: black square and solid line −→).
Figure 1

Mixed meal
then NPO until conclusion of study

5pm

Pancreatic clamp
Primed constant infusion of D2 glucose

7am
(T=-120 minutes)

9am
(T=0 minutes)

20% dextrose
(as required to maintain euglycemia)

3pm
(T=360 minutes)

40 IU intranasal insulin lispro
OR
Intranasal placebo plus i.v lispro 0.005 IU/kg over 30 minutes†
Figure 3

A

![Graph showing EGP (umol/min/KG) over time (minutes). The graph illustrates two data sets with error bars, and a trend line with a P-value of 0.01 labeled with a † symbol.]

B

![Bar chart showing Mean EGP (180-360 minutes) with error bars. The bar chart compares two groups with a P-value of 0.02 labeled with a ‡ symbol.]

C

![Graph showing Rd (Umol/min/kg) over time (minutes). The graph illustrates two data sets with error bars, and a trend line with a P-value of 0.4 labeled with a ‡ symbol.]

D

![Bar chart showing Mean Rd (180-360 minutes) with error bars. The bar chart compares two groups with a P-value of 0.4 labeled with a ‡ symbol.]

Diabetes
Figure 4

A. Mean FFA (180-360 minutes) mmol/l

B. Mean plasma TG (180-360 minutes) mmol/l

C. Plasma TG mmol/l

D. Mean plasma TG (180-360 minutes) mmol/l

P-values:
- A: P = 0.03
- B: P = 0.12
- D: P = 0.12
Supplementary Figure S1

A

\[ \text{Lispro pmol/l} \]

\[ 0 \rightarrow 30 \rightarrow 60 \]

\[ 15.0 \rightarrow 20.0 \rightarrow 30.0 \rightarrow 40.0 \rightarrow 60.0 \]

\[ -60 \rightarrow -30 \rightarrow 0 \rightarrow 30 \rightarrow 60 \]

\[ \text{Time (minutes)} \]

\[ 80 \text{ IU} \]

B

\[ \text{Lispro pmol/l} \]

\[ 0 \rightarrow 30 \rightarrow 60 \]

\[ 15.0 \rightarrow 20.0 \rightarrow 25.0 \rightarrow 30.0 \rightarrow 35.0 \rightarrow 40.0 \rightarrow 45.0 \]

\[ -60 \rightarrow -30 \rightarrow 0 \rightarrow 30 \rightarrow 60 \]

\[ \text{Time (minutes)} \]

\[ 40 \text{ IU} \]

C

\[ \text{Lispro pmol/l} \]

\[ 0 \rightarrow 30 \rightarrow 60 \]

\[ 10.0 \rightarrow 20.0 \rightarrow 30.0 \rightarrow 40.0 \rightarrow 50.0 \rightarrow 60.0 \]

\[ -60 \rightarrow -30 \rightarrow 0 \rightarrow 30 \rightarrow 60 \]

\[ \text{Time (minutes)} \]

\[ 10 \text{ IU} \]
Supplementary Figure S3

D2-glucose specific activity

Time (minutes)