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Glucagon Increases Insulin Levels by Stimulating Insulin Secretion Without Effect on Insulin Clearance in Mice

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Abstract

Circulating insulin is dependent on a balance between insulin appearance through secretion and insulin clearance. However, to what extent changes in insulin clearance contribute to the increased insulin levels after glucagon administration is not known. This study therefore assessed and quantified any potential effect of glucagon on insulin kinetics in mice. Prehepatic insulin secretion in mice was first estimated following glucose (0.35 g/kg i.v.) and following glucose plus glucagon (10 µg/kg i.v.) using deconvolution of plasma C-peptide concentrations. Plasma concentrations of glucose, insulin, and glucagon were then measured simultaneously in individual mice following glucose alone or glucose plus glucagon (pre dose and at 1, 5, 10, 20 min post). Using the previously determined insulin secretion profiles and the insulin concentration-time measurements, a population modeling analysis was applied to estimate the one-compartment kinetics of insulin disposition with and without glucagon. Glucagon with glucose significantly enhanced prehepatic insulin secretion (C_{max} and AUC_{0-20}) compared to that with glucose alone ($p < 0.0001$). From the modeling analysis, the population mean and between-animal SD of insulin clearance was 6.4 ± 0.34 mL/min for glucose alone and 5.8 ± 1.5 mL/min for glucagon plus glucose, with no significant effect of glucagon on mean insulin clearance. Therefore, we conclude that the enhancement of circulating insulin after glucagon administration is solely due to stimulated insulin secretion.

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CONTRIBUTION

GS and DZD performed data analysis and modelling, and contributed to interpretation of results and writing the paper. BA designed the study, carried out the experiments and contributed to interpretation of results and revision of the manuscript. GP participated in the design of the study and model analysis, in the interpretation of the results and in the revision of the manuscript. All authors approved the final version of the manuscript to be published.

CONFLICTS OF INTEREST

The authors declare no conflict of interests related to this work.

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Keywords

Glucagon; Insulin secretion; Insulin clearance; Population analysis

1. Introduction

The chronically elevated blood glucose concentrations in type 2 diabetes (T2D) result mainly from insulin resistance in peripheral tissues in association with decreased β -cell function (1). In addition, elevated concentrations of blood glucagon are also associated with increased insulin resistance and elevated glucose concentrations both in normal subjects and in T2D (2, 3), and, therefore, glucagon also seems important for the pathophysiology of T2D.

A well-known effect of glucagon is to stimulate insulin secretion from the islet beta cells, which raises insulin concentrations (4). There is, however, also a suggestion that glucagon may alter insulin kinetics. Thus, in healthy human subjects who underwent hyperglycemic clamp (5), insulin clearance was lower during greater stimulation of insulin secretion at higher glucose-clamp levels, suggesting that hyperglucagonemia may have an indirect influence on insulin clearance by inducing hyperglycemia and hyperinsulinemia. Furthermore, studies using rat skeletal muscle homogenates have shown that glucagon inhibits insulin-degrading enzymes (IDE) (6, 7). These studies are suggestive of a possible role of glucagon in altering insulin clearance which may contribute to increased circulating insulin after glucagon administration.

However, whether glucagon actually affects insulin kinetics is not known. Therefore, the present study was designed to quantify any effects of glucagon on insulin's disposition in a mouse model. Toward this end, prehepatic insulin secretion rate following intravenous glucose tolerance test (IVGTT) was determined both with and without exogenous glucagon, via C-peptide deconvolution methods. Then, using glucose, insulin and glucagon measured simultaneously following IVGTT, a modeling analysis was applied to estimate the clearance of insulin with and without exogenous glucagon.

2. Materials and methods**2.1 Animals**

All experiments were approved by the regional ethics committee in Lund, Sweden and conducted in agreement with the policy. Female C57BL/6J mice, weighing 22.9 ± 1.2 g (range 19.7– 24.8 g), were purchased from Taconic (Skensved, Denmark). Eight to ten week old mice were used in the study. Mice were maintained in a temperature-controlled room (22°C) on a light-dark cycle of 12 h each. Mice were fed a standard pellet diet (Lactamin, Stockholm, Sweden) and tap water ad libitum.

2.2 Experiments

After 4 weeks of acclimatization, mice were divided into two groups: a control cohort (n=15, weight 22.2 ± 1.5 g): and a glucagon cohort (n=15, weight 21.9 ± 1.2 g). The mice were

anaesthetized with an intraperitoneal injection of midazolam (0.14 mg/mouse, Dormicum; Hoffman-La Roche, Basel, Switzerland) and a combination of fluanisone (0.9 mg/mouse) and fentanyl (0.02 mg/mouse; Hypnorm; Janssen, Beerse, Belgium). A basal blood sample was taken from the retrobulbar, intraorbital, capillary plexus in heparinised tubes, followed by rapid intravenous injection (volume 10 μ L) of D-glucose (0.35 g/kg) or D-glucose (0.35 g/kg) together with glucagon (10 μ g/kg) into a tail vein. Since our study was the first of its kind study to assess and quantify the effects of glucagon on insulin dynamics, we wanted to conduct experiments in a controlled setting. Our experiments were designed in an effort to mimic hyperglucagonemia observed in diabetic animal models or diabetic patients with glucagon injection. Plasma glucose, glucagon, and insulin were also measured in each animal at 1, 5, 10, and 20 min after the i.v. administration of glucose alone or glucagon plus glucose. In a separate set of experiments, human C-peptide (dissolved in saline; Sigma, St Louis, MO, USA) was given intravenously (i.v.) over 3 s in a tail vein at the dose of 3.0 nmol/kg in 15 mice (weight 20.8 ± 1.1 g). Blood samples were taken at 1, 5, 10, 20, 30 and 50 min after the i.v. administration for measurement of plasma human C-peptide. All plasma samples were separated by centrifugation immediately and stored at -20°C until analysis.

2.3 Assays

Plasma insulin and glucagon were analyzed with sandwich immunoassay techniques (ELISA; Mercodia, Uppsala, Sweden) using double monoclonal antibodies. Mouse C-peptide was determined with a double-antibody radioimmunoassay using guinea pig anti-rat C-peptide antibody (cross-reacts to 100% with mouse C-peptide), rat C-peptide standard and ^{125}I -labelled rat C-peptide as tracer (Mercodia). Human C-peptide was determined by a double-antibody radioimmunoassay using guinea-pig anti-human C-peptide antibodies, ^{125}I -labelled human C-peptide and, as standard, human C-peptide (Merck Millipore, Darmstadt, Germany). Glucose was measured by the glucose oxidase technique and glucagon was determined by RIA (Millipore, Billerica, USA).

2.4 Assessment of C-peptide kinetics

The disposition of human C-peptide in the mouse was described by a two-compartment model, where the two compartments represent rapidly and more slowly equilibrating spaces, with irreversible loss occurring from the former as described previously (8). In the model diagram presented in Figure 1A, the parameters V_1 and V_2 (L) are the distribution volumes of the two spaces, while CL_d and CL_t (L/min) represent the distributional and total clearance rates. The C-peptide concentration-time data from all mice studied (3.0 nmol/kg ($n=15$)) were analyzed simultaneously using a hierarchical, non-linear mixed effects modeling approach. This population analysis yields estimates of the mean and inter-animal variability for the four model parameters. The hierarchical analysis was performed using the MLEM algorithm in the ADAPT software (version 5) (9).

2.5 Assessment of insulin secretion

β -cell insulin secretion was reconstructed via model-based deconvolution using the measured plasma C-peptide concentration profiles following IVGTT with ($n=15$) and without ($n=15$) glucagon administration in a previously reported study (10). The kinetic parameters of the two-compartment used in the deconvolution were set at their mean values

obtained from the human C-peptide kinetic study above. The C-peptide concentration-time profiles were pooled and a single set of prehepatic insulin secretion rates was estimated by maximum likelihood estimation using ADAPT (9), including the endogenous pre-IVGTT secretion rate (population analysis was attempted but led to large uncertainties in estimated parameters). The overall insulin delivery profile ($I(t)$ pmol/min) was constructed as the piece-wise collection of the individual sample period delivery rates determined by deconvolution.

2.6 Non-compartmental analysis (NCA)

The area under the concentration versus time curve (AUC) from 0 to 20 min (AUC_{0-20}) and C_{max} of glucose, insulin, and glucagon time course measurements in each animal was calculated using the NCA application in the ADAPT software (9). Glucose elimination rate (λ , min^{-1}) was calculated based on the glucose concentration values from time 5 to 20 min (10).

2.7 Population analysis for insulin kinetics and statistical inference

For the insulin study, a one-compartment model (Figure 1B) was used to describe insulin kinetics following IVGTT as in other studies (11). The respective insulin secretion profile, $I(t)$, determined by deconvolution of plasma C-peptide data as described above, was used as input for all mice in the cohort. For each of the two groups, the data from all mice were pooled and analyzed simultaneously using a hierarchical modeling analysis (MLEM algorithm in the ADAPT software (version 5) (9)). The parameters of the insulin kinetic model (clearance –CL (mL/min) and distribution volume-V (mL)) were assumed to follow a log-normal distribution and the errors associated with the measured insulin were assumed to be normally distributed with standard deviation linearly related to insulin (proportional and additive terms). Model selection was based on the resulting values of the Akaike Information Criterion (AIC, ref. 9), goodness-of-fit residual analysis and on the plausibility of the results. Differences in plasma C-peptide or insulin concentrations and insulin model parameter estimates (*e.g.*, CL) between cohorts were assessed using a *t* test.

3. Results

3.1 C-peptide kinetics

Plasma human C-peptide concentration versus time profile after administration of human C-peptide at 3 nmol/kg is presented in Figure 2A. The estimated population mean total C-peptide clearance (CL_t) was 1.5 mL/min with an inter-animal SD of 0.39 mL/min. The population mean of distributional clearance (CL_d) was 1.3 mL/min with an inter-animal SD of 0.76 mL/min. The population estimates for V_c were 8.0 ± 2.7 mL and for V_p were 7.1 ± 2.2 mL.

3.2 Insulin secretion rates

The plasma C-peptide concentrations following IVGTT or IVGTT with glucagon (10 mg/kg) are summarized in Figure 2B. The addition of glucagon substantially increased C-peptide levels with the peak concentration observed at 1min (mean $\pm$ SD: 2.0 ± 0.6 nmol/L for glucose alone vs. 4.0 ± 1.1 nmol/L for glucagon plus glucose, $p < 0.0001$). Figures 3A and

3B present the estimated insulin secretion profiles, $I(t)$, following glucose alone or glucagon together with glucose in mice, respectively. Estimates of insulin secretion rate in both groups are summarized in Table 1. Glucose alone and glucose plus glucagon significantly increased insulin secretion above basal rate in the interval 0–1 min ($p<0.0001$). In addition, the peak insulin secretion rate between 0 and 1 min was significantly elevated after addition of glucagon compared with that with glucose alone ($p<0.0001$). Most insulin was shown to be released during the first minute and no substantial increase in insulin secretion was observed in the later time period. Model predictions of C-peptide concentrations based on estimated $I(t)$ and the two-compartment model of C-peptide kinetics were well-fitted to the measured data (Figure 3C and D).

3.3 NCA Analysis

Insulin, glucose, and glucagon were measured in individual mice after administration of glucose alone (n=15 for control cohort) or glucose with glucagon (n=15 for glucagon cohort). Figure 4 shows the concentration versus time profiles of glucose, insulin, and glucagon in these experiments. There were no significant differences in the concentration versus time profiles of glucose between groups (Figure 4A). Accordingly, the estimated glucose elimination rate constants from the two groups were not significantly different ($0.03 \pm 0.01 \text{ min}^{-1}$ for glucose alone vs. $0.03 \pm 0.01 \text{ min}^{-1}$ for glucagon plus glucose). However, glucagon administration significantly increased the insulin peak concentration at 1 min compared to that obtained with glucose alone ($0.9 \pm 0.2 \text{ nmol/L}$ vs. $2.6 \pm 0.7 \text{ pmol/L}$; $p<0.0001$) indicating that glucagon may influence the early insulin secretion (Figure 4B). Plasma glucagon was also measured and the concentration versus time profile of glucagon is presented in Figure 4C. As a response to glucagon administration, we assessed AUCs (AUC_{0-20}) for glucose production, insulin release, and glucagon release via non-compartment analysis. The AUC results are summarized in Table 2. Consistent with C_{max} data, there was no significant difference in glucose levels (AUC_{0-20}) between two groups. However, glucagon administration together with glucose significantly enhanced insulin (AUC_{0-20} of insulin) and glucagon level (AUC_{0-20} of glucagon) compared to those after glucose alone.

3.4 Insulin clearance

From the population analysis of insulin kinetics based on a one-compartment model, the resulting estimates of insulin distribution volume were 13 mL with the inter-animal variability (SD) 4.0 mL for the glucose only group and 11 mL with the inter-animal variability (SD) 3.2 mL with glucose plus glucagon. The respective population mean and inter-animal SD of insulin clearance was $6.4 \pm 0.34 \text{ mL/min}$ for glucose alone and $5.8 \pm 1.5 \text{ mL/min}$ for glucagon plus glucose (Table 3). These findings indicate glucagon administration did not significantly affect insulin volume and clearance compared to glucose alone ($p>0.05$). Model predictions of insulin concentrations based on estimated $I(t)$ and the one-compartment model of insulin kinetics was well-fitted to the measured data for each group (Figure 5).

4. Discussion

This work represents the first study on the effects of glucagon on insulin dynamics (secretion and disposition) following IVGTT in a mouse model. To assess and quantify any potential effects of glucagon on insulin disposition, prehepatic insulin secretion rate following IVGTT was first estimated from experiments both with and without exogenous glucagon, by applying C-peptide deconvolution methods. Then, using glucose, insulin and glucagon measured simultaneously following IVGTT, a hierarchical modeling analysis was applied to estimate the one-compartment kinetics of insulin disposition with and without exogenous glucagon. The main finding of the study was that glucagon does not alter the clearance nor the distribution volume of insulin during IVGTT. Thus, altered insulin clearance does not contribute to raised insulin levels after glucagon administration.

A number of assumptions underlie the results presented in this work, as well as their broader extrapolation. The results and conclusions were obtained using an anesthetized mouse preparation and were based on a modeling analysis that used a simple one-compartment model to represent insulin kinetics. Given that the same experimental preparation and modeling analysis was used in both the control and glucagon groups, however, it is expected that the main finding of lack of glucagon effect on insulin's kinetics applies in the awake animal using a more complete insulin disposition model (12). Of course, we cannot exclude the possibility that the results reported herein are species specific. In humans, there is also a more marked 2nd phase insulin secretion compared to mice. Therefore, whether glucagon also does not affect insulin clearance in other species remains to be studied. In addition, in estimating the insulin kinetic parameters in each animal under control and glucagon administration, the insulin secretion profile was fixed to estimated population values for each group instead of being individually estimated for each animal from the current experiments. Using the same population mean estimates for insulin delivery rate for all animals in the respective groups may lead to bias of our reported estimates for insulin kinetics. However, it would be unexpected that any such bias would preferentially alter the estimated values of insulin clearance in one group versus the other. Thus, it is likely that our overall conclusion is robust to such bias as might result from using population mean insulin release profiles.

Glucagon secreted from pancreatic α -cells is a key hormone regulating glucose by stimulating hepatic glycogenolysis and gluconeogenesis (13). Glucagon also activates specific G-protein coupled receptors on pancreatic β -cells leading to activation of adenylate cyclase and subsequent stimulation of insulin secretion (14). The importance of this effect for glucagon physiology is underscored by a recent study, showing that glucose-stimulated insulin secretion from isolated islets from mice with glucagon receptors genetically deleted is impaired (15). Thus, in our study, it is possible that the increased insulin release after glucagon administration may be attributable both to augmented glucagon-stimulated glucose production and a direct action of glucagon on insulin secretion from the β -cells. It is noteworthy that overall response of glucose in the control and glucagon groups was comparable. Given that plasma insulin half-life is short (4–6 minutes) (7), increased total insulin release may counteract glucagon-stimulated glucose production in the liver, leading to similar plasma glucose concentration profiles between two groups.

In conclusion, our work has therefore demonstrated that hyperglucagonemia yields an increased i.v. glucose-stimulated insulin secretion, but it has no effect on insulin clearance. Thus, the enhanced insulin levels after glucagon administration is mainly achieved by stimulated insulin secretion and not through changes in insulin clearance. Glucagon is therefore different from GLP-1, which has been shown to both stimulate insulin secretion and diminish insulin clearance (10) thereby increasing insulin levels through two mechanisms. Given the hyperglucagonemia in T2D, it is now important to assess any possible effects of glucagon on insulin disposition in humans, and to study whether this is of importance for insulin-based limitations of the study.

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Highlights

- Glucagon effects on insulin kinetics in mice were assessed using population modelling analysis approach
- Glucagon was shown to enhance prehepatic insulin secretion, but there was no effect on insulin clearance in mice
- Our findings therefore show that the increase in circulating insulin achieved by glucagon is due to increased insulin secretion without any further influence on insulin clearance

Figure 1.
(A) Two-compartment model of C-peptide kinetics and (B) one-compartment model of insulin kinetics.

Figure 2.

(A) Plasma human C-peptide concentration versus time profile after i.v. administration of 3 nmol/kg (open circle, n=15) of human C-peptide in mice. (B) Plasma mouse C-peptide concentration versus time profile after IVGTT (glucose 0.35 g/kg) (n=15) and IVGTT plus glucagon (10 μ g/kg) (n=15). Data are presented as mean $\pm$ SD.

Figure 3. Insulin secretion profile, $I(t)$, reconstructed by deconvolution method from the measured plasma C-peptide concentration profiles using C-peptide kinetic parameters after IVGTT (A) and IVGTT plus glucagon (B). Comparison between C-peptide measurements (closed circle) and model prediction (continuous line) based on $I(t)$ (shown in (A) and (B)) and the two-compartment model of C-peptide kinetics after IVGTT (C) and IVGTT plus glucagon (D).

Figure 4. Plasma concentration versus time profiles of (A) glucose, (B) insulin, and (C) glucagon after IVGTT (n=15) and IVGTT plus glucagon (n=15). Data are presented as mean $\pm$ SD.

Figure 5. Comparison of Insulin measurement (closed circle) and model prediction (continuous line) based on one-compartment model of insulin kinetics and reconstructed insulin secretion rate, $I(t)$, as input after IVGTT (A) and IVGTT plus glucagon (B). Data are presented as mean $\pm$ SD. Model prediction was constructed as the average of the individual mouse results.

Table 1

Insulin secretion rate (pmol/min) reconstructed by deconvolution methods using measured plasma C-peptide concentrations in mice.

Parameter	IVGTT (Mean $\pm$ SE)	IVGTT + Glucagon (Mean $\pm$ SE)
Rate at baseline	1.2 $\pm$ 0.06	0.99 $\pm$ 0.06
Rate between 0–1 min	13 $\pm$ 0.11	34 $\pm$ 0.07
Rate between 1–5 min	0.84 $\pm$ 0.38	2.8 $\pm$ 0.20
Rate between 5–10 min	0.52 $\pm$ 0.34	0.06 $\pm$ 4.1
Rate between 10–20 min	0.98 $\pm$ 0.11	0.70 $\pm$ 0.17
Rate between 20–30 min	1.3 $\pm$ 0.10	0.73 $\pm$ 0.13
Rate between 30–50 min	1.2 $\pm$ 0.08	1.00 $\pm$ 0.07

AUC for C-peptide, glucose and insulin concentrations for the IVGTT and IVGTT + Glucagon groups. Results are presented as mean $\pm$ SD.

Table 2

Group	n	Glucose		Insulin		Glucagon	
		Cmax (mmol/L)	AUC ₀₋₂₀ (mmol•min/L)	Cmax (pmol/L)	AUC ₀₋₂₀ (pmol•min/L)	Cmax (pmol/L)	AUC ₀₋₂₀ (pmol•min/L)
IVGTT	15	14.5 $\pm$ 1.78	209 $\pm$ 23.4	908 $\pm$ 228	4,780 $\pm$ 637	5.20 $\pm$ 3.60	40.5 $\pm$ 26.7
IVGTT + Glucagon	15	14.6 $\pm$ 1.41	234 $\pm$ 27.0	2,590 $\pm$ 701	11,800 $\pm$ 3,030	378 $\pm$ 164	1,910 $\pm$ 596
<i>P</i>		NS	NS	<0.0001	0.001	<0.0001	<0.0001

Table 3

Population parameter estimates for the linear, one-compartment insulin kinetic model

Parameter	IVGTT		IVGTT + Glucagon	
	Population mean	Inter-animal SD	Population mean	Inter-animal SD
CL (mL/min)	6.4	0.34	5.8	1.5
V (mL)	13	4.0	11	3.2