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Glucagon clearance is regulated by nutritional state: evidence from experimental studies in mice

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Abstract

Aims/hypothesis—Given the importance of glucagon in the development of type 2 diabetes and as a potential therapeutic agent, the aim of this study was to characterise glucagon kinetics in mice and its regulation by the nutritional state.

Methods—Anaesthetised C57BL/6 mice fed normal or high-fat diets, or fasted, were injected intravenously with glucagon (0.1, 0.3, 1.0, 10.0 or 20 µg/kg); blood samples were withdrawn before injection and 1, 3, 5, 10, 20 min thereafter for glucagon assay by RIA. Glucagon kinetics were described by two-compartment models using a population analysis.

Results—The population mean and between-animal SD of glucagon clearance in the fed mice was 6.03 ± 2.58 ml/min, with a rapid elimination half-life of 2.92 ± 1.21 min. Fasted mice showed a slower glucagon clearance. The kinetics of glucagon in the fed and fasted group was linear across this large dose range. The mice fed a high-fat diet, however, showed non-linear kinetics with a faster terminal clearance of 20.4 ± 5.45 ml/min ($p < 0.001$) and a shorter elimination half-life of 1.59 ± 0.606 ($p < 0.001$) min relative to normal mice.

Conclusions/interpretation—This first systematic dose-ranging study of glucagon kinetics produced several findings: (1) a linear two-compartment model describes glucagon in normal C57BL/6 mice; (2) fasting reduces the clearance of glucagon and (3) high-fat diet enhances the clearance of glucagon. These results may direct future studies on glucagon physiology and indicate that there are other mechanisms, not included in the current model, needed to fully explain glucagon's kinetics.

Keywords

Glucagon clearance; Glucagon kinetics; Population analysis

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Duality of interest

The authors declare that there is no duality of interest associated with this manuscript.

Contribution statement

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

Introduction

Type 2 diabetes mellitus is characterised by inadequate glucose homeostasis, which leads to elevated blood glucose levels. In addition to insulin resistance and insulin insufficiency, hyperglucagonaemia contributes significantly to hyperglycaemia through exaggerated hepatic glucose production [1, 2] due to an increase in glycogen turnover [3]. Glucagon is a 29 amino acid hormone secreted by pancreatic alpha cells; it opposes insulin by stimulating hepatic glycogenolysis and gluconeogenesis, resulting in elevated blood glucose levels [4]. The importance of glucagon for hepatic glucose production is evident from studies showing that rapid reduction in circulating glucagon, following an infusion of somatostatin, causes hepatic glucose output to be reduced by 75% in healthy individuals [5]. Hyperglucagonaemia in type 2 diabetes is also a potential target for treating the disease. Glucagon antagonists have, in fact, been suggested as novel glucose-lowering therapies, although they have not yet reached the clinic [6, 7]. Glucagon has also been shown to stimulate energy expenditure, lower lipids and induce satiety, which has prompted interest in the hormone as a therapeutic agent for the treatment of dyslipidaemia and obesity [8].

Hyperglucagonaemia in type 2 diabetes is mainly due to the inability of glucose and insulin to inhibit glucagon secretion. However, changes in glucagon kinetics may also contribute, but to what extent these mechanisms are involved is not known. In fact, only a few quantitative analyses of glucagon kinetics (distribution and elimination) have been reported to date, and it is not known whether altered glucagon kinetics may additionally contribute to hyperglucagonaemia in type 2 diabetes. Furthermore, detailed knowledge of glucagon kinetics is also of importance for the development of glucagon's therapeutic potential in obesity and dyslipidaemia.

While the process of glucagon elimination is not fully understood, the limited available studies indicate that both the kidneys and the liver may be the primary eliminating organs. Dobbins et al [9] found that significant elimination of glucagon occurred via both the liver and kidney of dogs fed a standard diet, and estimated the clearance of glucagon to be 4.2 ± 0.8 ml kg⁻¹ min⁻¹ in the liver and 2.9 ± 0.6 ml kg⁻¹ min⁻¹ in the kidneys using a one-compartment model. Deacon et al [10], however, in studies in pig concluded that the kidney is the most significant route for glucagon elimination, accounting for more than 40%, with metabolism in the liver responsible for only 1% of glucagon elimination. Renal elimination of glucagon is thought to occur via the enzyme dipeptidyl peptidase-4 (DPP-4) located in the renal tubular brush border [11]. Other studies report that liver plasma membranes can degrade glucagon, forming miniglucagon [glucagon-(18–29)], through receptors involving both saturable and non-saturable mechanisms [12].

Given the limited understanding of glucagon's distribution and elimination, we conducted dose-ranging biodistribution studies following exogenous glucagon administration in anaesthetised mice. Population compartment modelling analysis was used to quantify the processes of distribution and elimination. To study the potential dependence on nutritional state, we also examined the impact of high-fat diet and fasting on glucagon kinetics.

Methods

Animals and experimental protocol

Female C57BL/6 mice (Taconic, Skensved, Denmark) were 4 weeks old on arrival. After 1 week of acclimatisation, the mice were divided into two groups, one group receiving a standard rodent diet with 10% of energy from fat (D12450B, Research Diets, New Brunswick, NJ, USA) and another receiving a high-fat diet with 60% of energy from fat (D12492, Research Diets). After 8 weeks of feeding with the respective diets, non-fasted

mice (normal cohort, $n = 99$, body weight 21 ± 1.1 g), mice fasted for 16 h (fasted cohort, $n = 26$, 21 ± 1.4 g) and mice fed the high-fat diet (high-fat cohort, $n = 24$, body weight 36 ± 3.9 g) were intravenously injected with glucagon. The mice were anaesthetised with an intraperitoneal injection of midazolam (0.4 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 containing the protease inhibitor aprotinin (Trasylol, 500 KIE/ml; Bayer, Leverkusen, Germany), followed by rapid intravenous injection of glucagon into a tail vein at the following five doses ($\mu\text{g/kg}$): 0.1 ($n = 17$), 0.3 ($n = 39$), 1.0 ($n = 39$), 10 ($n = 45$) and 20 ($n = 8$) (see Table 1 for details). Additional samples were taken at 1, 3, 5, 10 and 20 min after the intravenous administration of glucagon. Serial blood samples were taken from the retrobulbar plexus. Plasma samples were separated by centrifugation immediately and stored at 20 °C until analysis. The animal studies were approved by the regional ethics committee in Lund, Sweden.

Sample analysis

Plasma glucagon was measured by RIA (Millipore, Billerica, USA). The intra-assay CV of the method is 7% at both low and high levels, while the interassay CV is 8% at both low and high levels. The lower limit of quantification of the assay is 4 pg/ml. Selected plasma samples in some dietary cohorts and for some glucagon dose levels were also analysed for either insulin or glucose. Plasma insulin was measured by ELISA (Mercodia, Uppsala, Sweden). The intra-assay CV of the method is 4% at both low and high levels, while the interassay CV is 5% at both low and high levels. The lower limit of quantification of the assay is 6 pmol/l. Plasma glucose concentrations were determined using the glucose oxidase method.

Glucagon kinetic modelling

Mathematical models describing plasma glucagon kinetic were developed for each of the three dietary cohorts separately. Both one- and two-compartment linear models, with and without endogenous glucagon production, were tested for each of the three different cohorts. In cases where dose-dependent kinetics were observed, one- and two-compartment models with saturable (Michaelis–Menten) elimination were evaluated. The general model structure is shown in Fig. 1 (the equations describing the models can be found in the electronic supplementary material [ESM] Methods along with the definition for other derived parameters, including glucagon total clearance CL (ml/min), terminal elimination half-life $t_{1/2}$ (min) and distribution clearance CL_d (ml/min). The endogenous glucagon production rate term shown in Fig. 1 was assumed to be zero in the normal and high-fat cohorts and a non-zero constant value (to be estimated) in the fasted cohort (see below).

Population analysis and statistical inference

For each cohort, the data from all mice were pooled and analysed simultaneously using a hierarchical, non-linear mixed effects modelling approach. In hierarchical modelling, data from all mice are analysed jointly, thus allowing information from the separate experiments to collectively inform the model estimation [13]. This approach, therefore, is especially well-suited to situations where only limited data are available from any one individual or animal but where multiple experiments are conducted over a range of doses. The hierarchical modelling analysis yields estimates of the mean and inter-animal variability for parameters of the tested models of glucagon kinetics. The hierarchical analysis was accomplished via maximum likelihood estimation using the MLEM algorithm in the ADAPT 5 software for pharmacokinetic/pharmacodynamic system analysis [14]. The parameters of the glucagon kinetic model were assumed to follow a multivariate normal

distribution and the error associated with the measured glucagon was assumed to be normally distributed with SD linearly related to glucagon (proportional and additive terms). Model selection was based on the resulting values of the Akaike Information Criterion (AIC, see [14]), goodness-of-fit residual analysis and on the plausibility of the results. Differences in glucagon model parameter estimates (e.g. clearance) between cohorts were assessed using a *t* test. Multiple comparisons involving plasma glucose or insulin levels over time were performed via ANOVA with Holm test pairwise comparison against pre-dose baseline.

Results

Figure 2 displays the mean and SD of the observed glucagon concentrations (pg/ml) at the sampled times (min) for the normal (Fig. 2a), high-fat (Fig. 2c) and fasted (Fig. 2e) mice. Also shown (Fig. 2 b, d, f) are the corresponding dose-normalised glucagon concentrations (pg/ml per μg dose of glucagon). Inspection of the data displayed in Fig. 2 led to several considerations in the modelling analysis. The lack of dose proportionality evident in the dose-normalised data for the high-fat cohort suggested the use of models with saturable Michaelis–Menten elimination kinetics. Since the fasted cohort showed significant levels of basal plasma glucagon, models with a non-zero endogenous glucagon production rate were tested.

Normal cohort

A linear, two-compartment model better described the glucagon time course data in normal mice when compared with a one-compartment model based on the goodness of fit and the model selection criterion. In this cohort, the endogenous glucagon production was minimal, as indicated by the numerous basal glucagon concentrations below the quantification limit and an average detectable basal glucagon concentration of 7.5 pg/ml. Also, plasma glucose was elevated during the course of the study, from 7.3 ± 1.4 mmol/l at baseline to 10.3 ± 2.8 mmol/l at 20 min post dose, which would act to suppress the endogenous release of glucagon even further from its low basal value. Thus in the modelling analysis, the endogenous glucagon production rate was set to zero. Figure 3a shows the resulting model fits for the 0.3, 1.0 and 10 $\mu\text{g/kg}$ dose groups, and a model diagnostic plot comparing the measured glucagon to the predicted concentrations for all dose groups is shown in Fig. 3b. Table 2 lists the two-compartment model parameter estimation results, both population mean and inter-animal SD, showing an estimate for the mean clearance of 6.03 ml/min with an interindividual SD of 2.58 ml/min. The resulting estimates for other secondary model parameters (see ESM Methods) include the terminal elimination half-life, 2.92 ± 1.21 min, and the inter-compartmental or distribution clearance, 6.61 ± 3.18 ml/min. The value estimated for the distribution clearance was significantly different from 0 ($p < 0.001$), further supporting the two-compartment model assumption. We noted that insulin values at baseline, 5 and 20 min were, respectively, 102 ± 37 pmol/l, 154 ± 74 pmol/l and 173 ± 100 pmol/l, but this increase over time was not found to be statistically significant (see Discussion).

High-fat cohort

The dose dependency of the glucagon kinetics in high-fat-fed mice showed characteristics of saturable elimination and therefore a two-compartment model with Michaelis–Menten elimination was considered. This non-linear kinetic model resulted in an estimate for the population mean K_m of 3,320 pg/ml (Table 3), which is well within the range of measured glucagon plasma concentrations, thus suggesting that the non-linear glucagon dose–response observed in the dose-normalised plot in Fig. 2d can be explained by a saturable glucagon elimination process. The basal glucagon concentrations in the high-fat-fed mice were all below the level of quantification (and therefore endogenous glucagon) suggesting negligible

endogenous glucagon production. During the study, moreover, plasma glucose was elevated from 9.0 ± 0.4 mmol/l at baseline to 13.2 ± 1.2 mmol/l at 20 min post dose, which would further act to reinforce the suppression of glucagon release. Therefore, endogenous release was again assumed to be zero. Figure 3c shows the resulting model fits for all three dose groups, while a model diagnostic plot comparing the measured glucagon to the predicted concentrations is shown in Fig. 3d. Table 3 shows the model parameter estimation results, including the population mean and inter-animal SD. Visual inspection of the model fits for the corresponding dose groups between normal and high-fat cohorts (Fig. 3 a, c) shows an overall faster elimination of glucagon predicted for the high-fat-fed mice. While glucagon elimination clearance is concentration dependent in the non-linear model, the estimated mean terminal glucagon clearance elimination and half-life (defined for glucagon concentration $\ll K_m$) in the high-fat cohort (20.4 ± 5.45 ml/min and 1.59 ± 0.606 min, respectively) were significantly different ($p < 0.001$ and $p < 0.001$, respectively) from the corresponding values (6.03 ± 2.58 ml/min and 2.92 ± 1.21 min, respectively) in the normal cohort. No insulin levels were measured in this cohort.

Fasted cohort

Two models were considered when analysing the data from the fasted cohort: a linear two-compartment model with zero endogenous glucagon production, and one in which endogenous glucagon production is assumed to be constant (non-zero) before and during the experiment. In this cohort, glucose was elevated only modestly from 6.8 ± 1.4 mmol/l at baseline to 7.7 ± 2.9 mmol/l at 20 min post dose. Table 4 shows the resulting parameter estimates obtained using both models. The estimated clearance was 3.42 ± 1.05 ml/min for the model with zero glucagon production and 3.74 ± 0.898 ml/min for the model with constant glucagon production. The corresponding estimate of the terminal elimination half-life was 4.39 ± 1.40 min and 6.87 ± 5.25 min, respectively. This expected larger value of glucagon clearance and smaller half-life for the latter model is associated with an estimated endogenous glucagon production rate of 289 ± 120 pg/min. Figure 3e shows the resulting model fits for all three dose groups, while the model diagnostic plot comparing the measured glucagon to the predicted concentrations for the model with endogenous glucagon production is shown in Fig. 3f. Finally, we noted that insulin values at baseline, 5 and 20 min were, respectively, 114 ± 60 pmol/l, 389 ± 262 pmol/l, 80 ± 48 pmol/l. Only the 5 min sample was found to be different from the basal value (see Discussion).

Discussion

This study aimed to quantify glucagon kinetics in mice under normal and fasting dietary conditions, as well as in mice fed a high-fat diet, to provide insights for further investigation of the role of glucagon kinetics in the hyperglucagonaemia of type 2 diabetes. To the best of our knowledge, this is the first systematic dose-ranging study of glucagon kinetics investigating the role of nutritional state.

An understanding of glucagon kinetics is important given the impact of glucagon on fasting and prandial glycaemia in individuals with impaired glucose tolerance and diabetes [1, 2]. Of particular note is our finding that nutritional state affects glucagon kinetics, since this may yield insights into the relative contribution of hyperglucagonaemia in diabetes, which may differ in fed or fasting conditions or after nutritional challenges. Moreover, the results reported in this work are especially relevant given recent reports proposing glucagon receptor activation as a novel therapy to stimulate energy expenditure in obesity, in particular when used in combination with glucagon-like peptide 1 [15]. The inhibition of glucagon levels after incretin therapy, known to contribute to its improvement of glycaemia [16], may also be influenced by nutritional factors. Therefore, the work reported herein not

only contributes to the basic understanding of glucagon kinetics, but also provides a basis for further preclinical and clinical studies focusing on glucagon in obesity and type 2 diabetes.

Using a hierarchical population modelling approach, we found that glucagon kinetics were best described in all three dietary cohorts by a two-compartment model, vs one-compartment model, indicating significant distribution of glucagon to tissues not in equilibrium with the plasma. In the normal cohort, glucagon kinetics were linear with an estimated clearance of approximately 6.0 ml/min. We also found that glucagon kinetics were regulated by the nutritional state. Thus, mice fed a high-fat diet showed characteristics of saturable elimination and in this cohort glucagon kinetics were better described using a model with Michaelis–Menten elimination. These mice eliminated glucagon at a faster rate than did mice fed a standard diet over the glucagon concentration range observed in the present study, with a terminal clearance of approximately 20 ml/min, more than three times as fast as that in mice in the normal cohort. The glucagon concentration range was similar between the normal and high-fat cohorts for the corresponding dose, thus the underlying elimination process appears to be enhanced in the high-fat cohort, which may suggest an attempt to compensate for hyperglucagonaemia in mice fed a high-fat diet. In contrast, fasting reduced glucagon clearance by about one-half to 3.4–3.8 ml/min depending on the endogenous production assumptions. This slower clearance and longer half-life in fasted mice may physiologically contribute to preventing hypoglycaemia. It should be noted that the study examined female mice only and, therefore, we cannot exclude the possibility that glucagon kinetics may differ due to sex of the animal. Also, the studies were conducted under anaesthesia, which is known to influence glucagon secretion. While the same anaesthesia protocol was used for the mice in all three cohorts, we cannot rule out the possibility that anaesthesia might have had a differential effect on glucagon kinetics in the three dietary cohorts. Our quantification of the effects of injecting exogenous glucagon on its clearance and half-life in different dietary conditions is important not only for the understanding of hyperglucagonaemia in diabetes, but also for targeting glucagon in the treatment of diabetes. Our study cannot, however, determine the mechanistic basis for the differences in glucagon kinetics observed under these different dietary conditions. The most widely accepted route for glucagon metabolism is through the renal bed with high concentrations of DPP-4 [11]. It can be hypothesised that either there is a difference in the concentration of DPP-4 between the mice in the fasted and high-fat cohorts, vs those fed a standard diet, or there is a secondary mechanism that controls the activity of these enzymes, thereby slowing down or speeding up the clearance rate to maintain appropriate levels of glucagon for glucose homeostasis. Other clearance routes are also possible, including target-mediated degradation in the liver, which may be different under various dietary conditions. Also, other studies have reported an influence of feeding patterns on daily rhythms in basal plasma glucagon concentrations [17]. Further studies are required to elucidate these potential mechanisms.

Another issue that could potentially confound the interpretation of the results of the current study is that of endogenous glucagon production. This is difficult to assess fully without the ability to distinguish endogenous from exogenous glucagon in the blood samples. In the normal and high-fat cohorts it is reasonable to assume that the production of endogenous glucagon is shut off following exogenous glucagon administration, based on both the initially low values of basal glucagon (below the limit of quantification of the assay in the fasted cohort) and the elevated values of glucose measured during the experiment. In the fasted cohort, however, this does not appear to be the case. The analysis in this study, therefore, investigated the two limiting cases: glucagon production throughout the experiment is assumed to be either constant at its basal value, or to shut off completely. The most likely scenario is that the production varies during the experiment, dropping initially and rising back up to steady-state levels. Previous studies in healthy rats and dogs fed a

standard diet found that the half-life of exogenous glucagon is 1.9 ± 0.1 min and 5.5 ± 0.5 min, respectively [18, 19], somewhat comparable with the value of 2.92 ± 1.21 min found in the present study in the normal cohort.

We also observed that there were some changes in plasma insulin in the normal and fasted cohorts, although for the former the change did not reach the level of significance. Through its action on glucose, insulin can affect endogenous glucagon release, which we have accounted for as described above. However, we cannot exclude the possibility that observed elevation in plasma insulin could act directly on glucagon turnover or glucagon tissue uptake, or both, thus biasing our reported estimates for glucagon kinetics.

The current study explored the effects of fasting and high-fat conditions on glucagon clearance kinetics; this is crucial in understanding the action of glucagon in a diabetic patient. The finding that dietary conditions do have an impact on clearance rate will influence the use of glucagon as a potential therapeutic agent for the treatment of dyslipidaemia and obesity, as has recently been explored [8]. The respective longer and shorter half-lives in fasted and high-fat-fed animals will alter the impact of a dose of glucagon depending on the animal's nutritional state. While these findings need to be confirmed in humans, they suggest that close regulation of dose amounts and time of injection (postprandial or preprandial) may be necessary in any future studies that aim to examine glucagon as a component of the regimen in the treatment of diabetes.

This first systematic dose-ranging study of glucagon kinetics produced the following findings: (1) a linear two-compartment model describes glucagon kinetics in C57BL/6 mice fed a standard diet; (2) fasting reduces the clearance of glucagon and (3) a high-fat diet enhances the clearance of glucagon. These results form the basis for future studies on glucagon physiology and indicate that other mechanisms, not included in the current model, may be needed to fully explain glucagon's plasma kinetics.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

DPP-4 Dipeptidyl peptidase-4

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Fig. 1.

Diagram of the general two-compartment model structure used in the population analysis of glucose kinetics. IV Glucagon, intravenously injected glucagon dose of 0.1, 0.3, 1.0, 10 and 20 $\mu\text{g/kg}$; V_c , volume of the central compartment (ml); GN_{prod} , endogenous glucagon production rate (pg/min); k_{cp} and k_{pc} , linear rate constants (min^{-1}) between the central (c) and peripheral (p) compartments; k_e , elimination rate constant (min^{-1}); K_m , glucagon concentration (pg/ml) at half of V_{max} ; V_{max} , maximum clearance rate (pg/min)

Fig. 2.

Glucagon concentration means and SD for each of the three cohorts: normal (**a**); high-fat (**c**); fasted (**e**). Dose-normalised glucagon concentrations (measured concentration/dose of administered glucagon) for each of the three cohorts: normal (**b**); high-fat (**d**); fasted (**f**). Parts (**a**), (**c**) and (**e**) show mean and SD, while parts (**b**), (**d**) and (**f**) show mean only for clarity. Triangles, 0.1 $\mu\text{g/kg}$ dose (only available in the normal cohort); squares, 0.3 $\mu\text{g/kg}$ dose; circles, 1.0 $\mu\text{g/kg}$ dose; diamonds, 10 $\mu\text{g/kg}$ dose; inverted triangles, 20 $\mu\text{g/kg}$ dose (only available in the normal cohort). For the high-fat cohort, the basal values of glucagon were below the lower limit of quantification of the assay (4 pg/ml) in all mice

Fig. 3.

(a, c, e) Average model predictions of the individual mice in each dose group (solid lines), together with the mean and SD of the measured glucagon levels (symbols). (b, d, f) Plot of all measured glucagon levels vs the model predictions for all dose groups (line of identity also shown). Normal cohort (a, b); high-fat (c, d); fasted (e, f). Squares, 0.3 $\mu\text{g/kg}$ dose; circles, 1.0 $\mu\text{g/kg}$ dose; diamonds, 10 $\mu\text{g/kg}$ dose

Table 1

Number of mice in each glucagon dose group for each of the three cohorts

Cohort	Dose group					Total No.	Body weight (g)
	0.1 μg/kg	0.3 μg/kg	1.0 μg/kg	10 μg/kg	20 μg/kg		
Normal	17	25	23	26	8	99	21 ± 1.1
Fasted	0	7	9	9	0	26	21 ± 1.4
High-fat	0	7	7	10	0	24	35 ± 3.9

Body weight is shown as mean ± SD

Table 2

Population parameter estimates for the linear, two-compartment glucagon model for the normal cohort

Parameter	Population mean	Inter-animal SD
K_e (min^{-1})	0.505	0.166
V_e (ml)	11.9	3.34
k_{cp} (min^{-1})	0.556	0.217
k_{pc} (min^{-1})	0.730	0.372

Table 3

Population parameter estimates for the non-linear, two-compartment glucagon model for high-fat-fed mice

Parameter	Population mean	Inter-animal SD
K_m (pg/ml)	3,320	491
V_{max} (pg/min)	67,700	15,000
V_c (ml)	15.6	4.24
K_{cp} (min ⁻¹)	0.474	0.168
K_{pc} (min ⁻¹)	0.673	0.388

Table 4

Population parameter estimates for the linear, two-compartment glucagon model for fasted mice

Parameter	Model a		Model b	
	Population mean	Inter-animal SD	Population mean	Inter-animal SD
K_e (min^{-1})	0.282	0.0807	0.284	0.0420
V_c (ml)	12.1	4.37	13.2	3.97
k_{cp} (min^{-1})	0.237	0.0808	0.102	0.0683
k_{pc} (min^{-1})	0.455	0.218	0.101	0.0502
GN_{prod} (pg/min)	0	0	289	120

Model a, GN_{prod} fixed at 0; Model b, GN_{prod} estimated