Glucagon Clearance Is Preserved in Type 2 Diabetes

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Hyperglucagonemia is a common observation in both obesity and type 2 diabetes, and the etiology is primarily thought to be hypersecretion of glucagon. We investigated whether altered elimination kinetics of glucagon could contribute to hyperglucagonemia in type 2 diabetes and obesity. Individuals with type 2 diabetes and preserved kidney function (eight with and eight without obesity) and matched control individuals (eight with and eight without obesity) were recruited. Each participant underwent a 1-h glucagon infusion (4 ng/kg/min), achieving steady-state plasma glucagon concentrations, followed by a 1-h washout period. Plasma levels, metabolic clearance rate (MCR), half-life ($T_{1/2}$), and volume of distribution of glucagon were evaluated, and a pharmacokinetic model was constructed. Glucagon MCR and volume of distribution were significantly higher in the type 2 diabetes group compared with the control group, while no significant differences between the groups were found in glucagon $T_{1/2}$. Individuals with obesity had neither a significantly decreased MCR, $T_{1/2}$, nor volume of distribution of glucagon. In our pharmacokinetic model, glucagon MCR associated positivelywith fasting plasma glucose and negatively with body weight. In conclusion, our results suggest that impaired glucagon clearance is not a fundamental part of the hyperglucagonemia observed in obesity and type 2 diabetes.

Glucagon stimulates hepatic glucose production and is, together with insulin, a key factor for the maintenance of stable plasma glucose concentrations (1). Glucagon is released from pancreatic α-cells when glucose mobilization is needed (e.g., during fasting and low plasma glucose levels), while glucagon secretion is suppressed when plasma glucose levels are high. Many, but not all, patients with type 2 diabetes display elevated glucagon levels in the fasting state and inadequate suppression of glucagon secretion in the initial period after carbohydrate ingestion (2–4). Hyperglucagonemia, both absolute and relative to prevailing plasma glucose concentrations, increases hepatic glucose production (5), contributing to the hyperglycemic state of the disease (6,7). This pathophysiological trait has gained substantial clinical interest, and several drugs targeting glucagon secretion or signaling have been or are being developed for the treatment of type 2 diabetes (8,9). Despite intense research within the field, the mechanisms behind hyperglucagonemia in type 2 diabetes are still not clear (10). Most studies have focused on a potential hypersecretion of glucagon from the pancreas, often suggested to be due to α-cell resistance to the glucagon-suppressive effects of glucose and insulin in patients with type 2 diabetes (11,12). However, several lines of evidence suggest that α-cells in type 2 diabetes can respond normally to both glucose and insulin stimuli (2), warranting other explanations for hyperglucagonemia in type 2 diabetes. These might include gut hormones affecting glucagon secretion (13), extrapancreatic glucagon

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secretion (14), and amino acid–induced secretion as a result of hepatic glucagon resistance and obesity-induced steatosis (15). However, glucagon secretion is not the only factor determining circulating glucagon concentrations; the circulating concentration of any given hormone is determined by its secretion balanced by its rate of elimination. Thus, hyperglucagonemia in patients with type 2 diabetes could, in theory, be the result of altered glucagon elimination kinetics. The mechanisms underlying the elimination of glucagon in humans and the specific organs responsible for the elimination are not fully established. A previous study in patients with end-stage renal disease demonstrated a decreased metabolic clearance rate (MCR) of glucagon, pointing to the kidneys as an important site for glucagon elimination (16). This is in line with results from several animal experiments (17,18) and a contemporary study demonstrating an inverse relationship between kidney function and fasting glucagon levels (19). To our knowledge, a thorough investigation of glucagon kinetics has not been carried out in patients with type 2 diabetes and/or obesity. In 1976, Alford et al. (20) reported a prolonged half-life ($T_{1/2}$) of glucagon in type 1 diabetes, while Matsuda et al. (21) found no difference in the MCR of glucagon between patients with type 2 diabetes and healthy controls (but did not report measurements of elimination $T_{1/2}$ or volume of distribution [V_d]). Here, we studied individuals with normal kidney function, with and without type 2 diabetes, and with and without obesity to investigate whether the kinetics of glucagon are affected in either of these conditions.

RESEARCH DESIGN AND METHODS

The study was approved by the Scientific-Ethical Committee of the Capital Region of Denmark (registration no. H-1-2014-066) and registered as a clinical trial. The study was performed in accordance with the principles of the 7th Revision of the Declaration of Helsinki.

Study Participants

Enrolled participants comprised individuals with type 2 diabetes and matched healthy individuals. The eligibility of participants was evaluated at a screening visit. Inclusion criteria for the individuals in the type 2 diabetes group were a diagnosis of type 2 diabetes according to World Health Organization criteria, diabetes treatment with metformin and/or diet, preserved kidney function (estimated glomerular filtration rate [eGFR] >60 mL/min/1.73 m^2 and no albuminuria), and preserved liver function. Exclusion criteria were treatment with antidiabetic drugs other than metformin, known liver disease and/or liver enzymes greater than two times the normal value, known kidney disease and/or eGFR <60 mL/min/1.73 m^2, significant cardiovascular disease, recent illness or surgical procedures, and pregnancy or breastfeeding. Inclusion criteria for individuals in the control group were a normal glycated hemoglobin A_{1c} (HbA_{1c}) level, preserved kidney and liver function, and no first-degree relatives with diabetes, and exclusion criteria were impaired fasting plasma glucose (FPG), prediabetes, diabetes, known liver disease and/or liver enzymes greater than two times the normal value, known kidney disease and/or eGFR <60 mL/min/1.73 m^2, recent illness or surgical procedures, and pregnancy or breastfeeding. The individuals in the control group were matched 1:1 with the type 2 diabetes group on the basis of BMI, age, sex, and eGFR. Both the type 2 diabetes group and the control group consisted of two intentionally designed subgroups with eight individuals each: one subgroup where the individuals all had a BMI <27 kg/m^2 (the lean subgroup) and one subgroup where the individuals all had a BMI >33 kg/m^2 (the obese subgroup).

Experimental Design

Following screening and inclusion in the study, the 32 participants all underwent an identical experimental day at our research facility at Gentofte Hospital, University of Copenhagen. Participants were studied while resting in a bed after an overnight (10-h) fast, including abstinence from food, tobacco, liquids, and medications (any metformin treatment was paused for 7 days before the test day). Two cannulas were inserted into cubital veins: one for the infusion of glucagon and one in the contralateral arm for the collection of venous blood samples. The latter arm was kept warm (~50°C) to ensure arterialization of the venous blood. At time 0 min, after a 30-min bed rest baseline period, a 60-min glucagon infusion (4 ng/kg/min = 1.15 pmol/kg/min) was initiated. Once the infusion was terminated, the participants stayed in the bed for a 60-min washout period, after which they delivered a urine sample. Blood samples were obtained at time −30, −15, 0, 5, 10, 20, 50, 55, 60, 62, 64, 66, 68, 70, 75, 80, 85, 90, and 120 min. For the analysis of glucagon, blood was added to chilled tubes containing EDTA, aprotinin (500 kIU/mL blood, Trasylol; Bayer, Leverkusen, Germany), and a specific dipeptidyl peptidase 4 inhibitor (valine-pyrrolidine, final concentration 0.01 mmol/L; a gift from Novo Nordisk, Bagsværd, Denmark). For the analyses of insulin and C-peptide, blood was collected in plain tubes and left to coagulate (20 min at room temperature). All samples were centrifuged for 20 min at 1,200*g* and 4°C, and serum/plasma was transferred to ice-chilled storage tubes. Plasma samples for glucagon analyses were stored at −20°C, and serum samples for insulin and C-peptide analyses were stored at −80°C until batch analysis. For bedside measurement of plasma glucose, blood was collected in fluoride-coated tubes and centrifuged immediately at 7,400*g* for 2 min at room temperature.

Analyses

Plasma concentrations of glucagon were measured using a radioimmunoassay (RIA) directed against the C-terminal end of the glucagon molecule (antibody code 4305). The

glucagon 4305 RIA does not cross-react with glicentin or oxyntomodulin but does cross-react with proglucagon 1-61, which is present in small amounts in the peripheral circulation (22). The measurements from the RIA were validated by remeasuring and comparing a smaller number of the samples, scattered across the protocol, using a sandwich ELISA (Mercodia AB, Uppsala, Sweden) not cross-reacting with proglucagon 1-61 (23). Insulin and C-peptide were analyzed with two-site sandwich immunoassays using direct chemiluminescent technology (ADVIA Centaur XP, Siemens Healthcare A/S, Ballerup, Denmark). Plasma glucose concentrations were measured at bedside using the glucose oxidase method (YSI 2300 STAT PLUS analyzer; Yellow Springs Instruments, Yellow Springs, OH).

Calculations and Statistics

The study's main end point was the MCR of glucagon, defined as the amount of plasma cleared of glucagon per unit time adjusted for total body weight (expressed as mL/kg/min). We assumed that elimination of exogenous glucagon follows first-order kinetics. During steady-state conditions, the amount of glucagon added to the circulation per minute (i.e., the rate of exogenous glucagon infusion) will be equal to the amount of glucagon removed from the circulation. The MCR can be calculated from the infusion rate and the plasma concentration during steady-state using the following equation:

$$\mathrm{MCR}\ (\mathrm{mL} \times \mathrm{kg}^{-1} \times \mathrm{min}^{-1}) = \frac{\text{Glucagon infusion rate (pmol} \times \mathrm{kg}^{-1} \times \mathrm{min}^{-1})}{[\mathrm{Glucagon}]_{\text{steady-state}} - [\mathrm{Glucagon}]_{\text{endogenous}}\ (\mathrm{pmol} \times \mathrm{mL}^{-1})}$$

We defined $[\mathrm{Glucagon}]_{\text{steady-state}}$ as the mean glucagon concentration at time 50, 55, and 60 min and $[\mathrm{Glucagon}]_{\text{endogenous}}$ as the mean glucagon concentration at time −30, −15, 0, and 120 min. The MCR of glucagon was further evaluated in relation to the participants' body surface area (BSA) (expressed as $\mathrm{mL/m^2/min}$) using the equation $\mathrm{BSA} = \text{body weight}^{0.425} \times \text{height}^{0.725} \times 0.007184$. We calculated the elimination $T_{1/2}$ of glucagon as $T_{1/2} = \ln(2)/k$, where k is the first-order elimination constant. The glucagon concentrations during the decay phase were plotted on a logarithmic scale, and k was found using linear regression analysis on the individual decay curves (i.e., the drop in glucagon concentration) following termination of the glucagon infusion. The elimination constant k was further used to calculate the apparent V_d of glucagon as $V_d = \mathrm{MCR}/k$. For comparison of the increases in plasma concentrations between the groups, the area under the curve (AUC) was calculated using the trapezoidal rule and presented either as AUC or baseline-subtracted AUC (bsAUC) for the entire 120-min period. ANOVA was used to compare all four groups, while Student t test analysis was used to compare the two main groups or any two subgroups. A two-sided $P < 0.05$ was chosen to indicate significant differences. When applicable, Tukey post hoc test was used to minimize the risk of type I errors. All data are presented as mean ± SEM unless otherwise stated. In addition, we constructed and applied a pharmacokinetic model to the data using Monolix version 2019R1 software (Lixoft SAS, Antony, France). The parameters were estimated using the stochastic approximation expectation-maximization algorithm. We used a one-compartment model (Supplementary Figs. 1 and 2) based on the assumption of a constant endogenous glucagon secretion. A simple additive model, using a standard population-based pharmacokinetic modeling technique (24), was used to test the influence of several parameters on glucagon clearance rate, apparent V_d, and endogenous glucagon secretion. The parameters were body weight, BMI, type 2 diabetes, sex, age, baseline glucose (at time 0 min), C-peptide, HOMA of insulin resistance (HOMA-IR) (based on the University of Oxford's HOMA2 calculator [25]), HbA_{1c}, insulin, and eGFR. A log-normal distribution was assumed for the interindividual variability (26); for example, for V_d:

$$V_{d,i} = V_{d,pop} \times \left(\frac{BWi}{70\ \mathrm{kg}}\right)^{\beta_{Bw,V_d}} \times \left(\frac{Glucose_i}{5.4\ \mathrm{mmol/L}}\right)^{\beta_{glucose,V_d}} \times e^{\eta_i},$$

where η_i denotes the random effect in participant i, Vd_i the individual central volume of distribution, and $V_{d,pop}$ the population volume of distribution. $Glucose_i$ and BW_i are the plasma glucose value and body weight of participant i. $\beta_{BW,Vd}$ and $\beta_{glucose,Vd}$ are the coefficients of the effects of body weight and plasma glucose, respectively, on the V_d. The error term for the coefficient was assumed to be normally distributed. The ηs are zero mean random variables with variance ω^2. We assumed no covariance between the ηs. The βs were step-wise eliminated from the model, depending of the Wald statistics using a significance level of 0.05. The error model for the concentration consisted of a constant term and a term proportional to the estimated concentration.

Data and Resource Availability

The data sets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

RESULTS

Study Participants

Sixteen individuals with type 2 diabetes and 16 individuals without diabetes were included. Thirteen individuals with type 2 diabetes were treated with metformin as monotherapy, whereas three were managed by lifestyle modifications only. Clinical characteristics of the participants are displayed in Table 1. As expected, the type 2 diabetes group as a whole was characterized by higher HbA_{1c} and HOMA-IR but were otherwise carefully matched and, thus, did not differ significantly from the

Table 1—Participant demographic characteristics presented on both whole-group and subgroup levels

	Type 2 diabetes group			Control group		
	Lean	Obese	Total	Lean	Obese	Total
Participants (M/F)	8 (5/3)	8 (4/4)	16 (9/7)	8 (5/3)	8 (4/4)	16 (9/7)
Age (years)	60.1 (7.8)	57.8 (8.2)	58.8 (9.2)	62.1 (8.8)	55.5 (8.8)	58.9 (7.9)
Duration of type 2 diabetes (years)	8.2 (5.8)	6.8 (4.9)	7.5 (5.2)	—	—	—
Weight (kg)	74.6 (10.7)††	106 (9.0)††	90.3 (18.1)	80.6 (7.8)**	115.6 (10.7)**	98.1 (19.6)
BMI (kg/m^2)	25.3 (1.7)††	35.9 (1.8)††	30.6 (5.7)	26 (1.4)**	36.4 (3.2)**	31.2 (5.9)
BSA (m^2)	1.9 (0.2)	2.2 (0.2)	2.0 (0.2)	2.0 (0.1)	2.3 (0.2)	2.1 (0.2)
HbA_{1c} (%)	6.7 (3.9)	7.0 (3.3)	6.9 (3.6)##	5.4 (0.4)	5.2 (0.3)	5.3 (0.3)##
HbA_{1c} (mmol/mol)	49.9 (19.3)	53.4 (12.3)	51.6 (15.8)##	35.1 (4.1)	33.6 (3.0)	34.4 (3.5)##
eGFR (mL/min/1.73 m^2)	86.3 (13.7)	91.5 (10.5)	88.9 (12.1)	83.5 (15.6)	83.2 (10.8)	83.3 (13)
Waist-to-hip ratio	0.9 (0.1)	1.0 (0.1)	1.0 (0.1)	1.0 (0.1)	1.0 (0.1)	1.0 (0.1)
SBP (mmHg)	136 (20.9)	145.1 (7.2)	140.3 (16.2)	110.9 (47.2)	138.5 (20)	122.7 (39.4)
DBP (mmHg)	78 (10.7)	89.9 (2.7)	83.5 (9.9)	71.4 (31.2)	86.2 (14.7)	77.7 (25.8)
HOMA-IR	1.9 (1.1)	2.6 (0.8)	2.2 (1.0)#	1.3 (0.5)*	1.9 (0.3)*	1.6 (0.5)#

Data are mean (SD) unless otherwise indicated. DBP, diastolic blood pressure; F, female; M, male; SBP, systolic blood pressure. #$P < 0.05$, ##$P < 0.01$ between the type 2 diabetes and control groups. *$P < 0.05$, **$P < 0.01$ between the lean and obese control subgroups. ††$P < 0.01$ between the lean and obese type 2 diabetes subgroups.

control group in terms of age, BMI, waist-to-hip ratio, systolic and diastolic blood pressure, or eGFR.

Glucagon

Excursions of plasma glucagon concentrations are presented in Fig. 1, and data are provided in Table 2 (individual plots are depicted in Supplementary Fig. 3). Fasting levels of glucagon did not differ between the type 2 diabetes group and the control group, which were carefully matched for body weight, whereas fasting glucagon levels were significantly elevated in the obese control subgroup compared with the lean control subgroup (8.2 ± 2.2 vs. 2.4 ± 0.4 pmol/L, $P = 0.02$). Glucagon levels quickly rose after initiation of the glucagon infusion, reaching steady-state concentrations after ∼20 min. Steady-state concentrations were significantly higher in the obese control subgroup (92.4 ± 4.7 pmol/L) compared with the other three subgroups (lean 69.5 ± 4.8 pmol/L, obese 62.5 ± 4.1 pmol/L, participants with type 2 diabetes and lean participants 69.9 ± 2.5 pmol/L, $P < 0.001$). Comparison of the two whole groups revealed an overall significant difference between the type 2 diabetes group and the control group

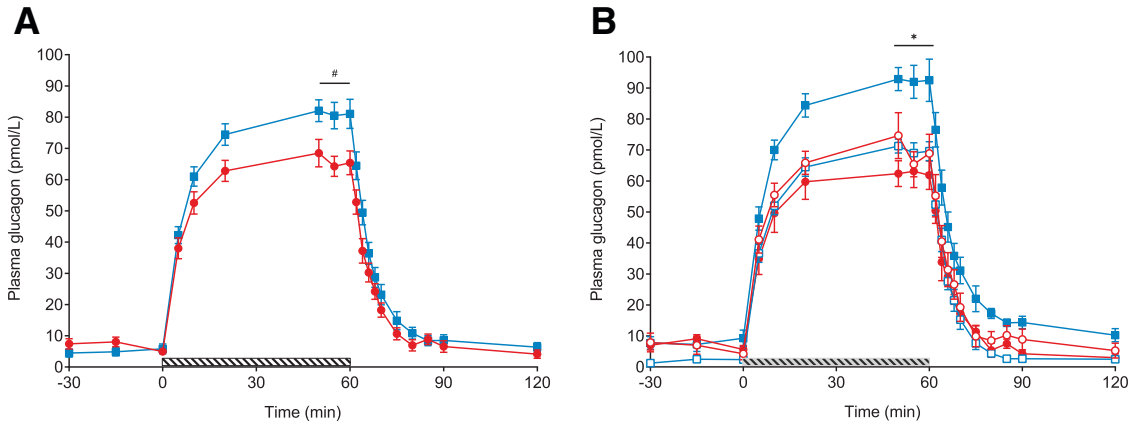

Figure 1—*A*: Glucagon excursions before, during, and after exogenous glucagon infusion in the type 2 diabetes group (red line, solid circles) and control group (blue line, solid squares). *B*: Glucagon excursions in the lean type 2 diabetes subgroup (red line, open circles), obese type 2 diabetes subgroup (red line, solid circles), lean control subgroup (blue line, open squares), and obese control subgroup (blue line, solid squares). The striped bar indicates timing of glucagon infusion. #$P < 0.05$ between the type 2 diabetes and control groups; *$P < 0.05$ between the lean and obese control subgroups.

Table 2—Glucagon concentrations and pharmacokinetic parameters presented on both whole-group and subgroup levels

	Type 2 diabetes group			Control group		
	Lean	Obese	Total	Lean	Obese	Total
Baseline (pmol/L)	6.5 (1.9; 11.1)	7.3 (5.3; 9.3)	6.9 (4.5; 9.3)	2.4 (1.6; 3.2)*	8.2 (4; 12.4)*	5.3 (2.8; 7.8)
Steady-state (pmol/L)	69.5 (59.3; 79.7)	62.5 (53.9; 71.1)	66.0 (59.3; 72.7)#	69.9 (64.6; 75.2)*	92.4 (83.2; 101.6)*	81.2 (73.5; 88.9)#
MCR (mL/kg/min)	19.2 (16.6; 21.8)	21.7 (18.4; 25)	20.4 (18.2; 22.6)##	17.6 (16.2; 19)	14.2 (13; 15.4)	15.9 (14.7; 17.1)##
MCR (mL/m^2/min)	747 (644; 850)††	1,035 (877; 1,192)††	891 (778; 1,004)##	702 (645; 760)	691 (640; 743)	697 (660; 733)##
$T_{1/2}$ (min)	4.3 (3.3; 5.3)	4.7 (3.7; 5.7)	4.5 (3.8; 5.2)	3.8 (2.8; 4.8)	5.2 (3.7; 6.7)	4.5 (3.6; 5.4)
V_d (mL/kg)	119 (87; 151)	152 (101; 203)	136 (105; 166)#	96 (71; 121)	105 (75; 136)	101 (81; 120)#

Data are mean (95% CI). #$P < 0.05$, ##$P < 0.01$ between the type 2 diabetes and control groups. *$P < 0.05$ between the lean and obese control subgroups. ††$P < 0.01$ between the lean and obese type 2 diabetes subgroups.

(66.0 ± 3.4 vs. 81.2 ± 3.9 pmol/L, $P = 0.03$). Following termination of the glucagon infusion, plasma glucagon levels dropped rapidly, reaching and remaining at baseline values ~25 min after infusion termination. Reanalysis of glucagon in four individuals with the Mercodia kit yielded slightly higher glucagon values (Supplementary Fig. 4). No significant differences in glucagon excursions (AUC) were found between the two measuring techniques.

Glucagon Clearance

The type 2 diabetes group exhibited a significantly higher MCR of glucagon than the control group when adjusted for both body weight (20.4 ± 1.1 vs. 15.9 ± 0.6 mL/min/kg, $P = 0.006$) and BSA (891 ± 58 vs. 697 ± 19 mL/m^2/min, $P = 0.004$). Within the groups, BSA-adjusted glucagon MCR was significantly higher in the obese type 2 diabetes subgroup, while no significant differences were observed between the lean and the obese control subgroups. In our pharmacokinetic model, glucagon MCR associated positively with FPG and negatively with body weight (Fig. 2*C* and *D*).

Glucagon $T_{1/2}$

No significant differences in glucagon $T_{1/2}$ were observed between the type 2 diabetes group and the control group (4.5 ± 0.4 vs. 4.5 ± 0.5 min, $P = 0.85$). Within the two groups, no significant differences between the lean and obese subgroups were observed.

Glucagon Apparent V_d

A significantly larger V_d was observed in the type 2 diabetes group than in the control group (137 ± 15 vs. 101 ± 9.7 mL/kg, $P = 0.03$). No significant differences were observed between the lean and obese subgroups. In our pharmacokinetic model, V_d was positively associated with FPG as well as with body weight (Fig. 2*A* and *B*).

Glucagon Secretion Rate

In our pharmacokinetic model, basal endogenous glucagon secretion rates were positively correlated with HOMA-IR in a linear fashion (Fig. 2*E*).

Glucose

Plasma glucose excursions are presented in Fig. 3*A* and *B*, and data are provided in Table 3. The type 2 diabetes group had significantly higher FPG levels than the control group (9.8 ± 0.7 vs. 5.5 ± 0.1 mmol/L, $P < 0.0001$). When analyzing change from baseline, the type 2 diabetes group exhibited a significantly greater rise in plasma glucose in response to the glucagon infusion compared with the control group (bsAUC 126.5 ± 26.2 vs. 55.6 ± 12.7 mmol/L × min, $P = 0.02$). Within the two groups, no significant differences in FPG, AUC, or bsAUC were found.

Insulin and C-Peptide

Insulin and C-peptide excursions are presented in Fig. 3*C–F*, and data are provided in Table 3. The type 2 diabetes group had significantly higher fasting levels of both C-peptide (826 ± 97 vs. 566 ± 51 pmol/L, $P = 0.02$) and insulin (114 ± 19 vs. 69 ± 8 pmol/L, $P = 0.03$) than the control group. Furthermore, fasting levels of insulin and C-peptide were significantly elevated in the obese control subgroup compared with the lean control subgroup ($P = 0.02$ and $P = 0.008$, respectively). Excursions of insulin and C-peptide throughout the test day differed significantly, as the control group exhibited a larger increase in insulin and C-peptide levels than the type 2 diabetes group ($bsAUC_{insulin}$ 3,350 ± 600 vs. 1,126 ± 758 pmol/L × min, $P = 0.03$; $bsAUC_{C\text{-}peptide}$ 1.6 ± 2.8 vs. 15.5 ± 7.5 nmol/L × min, $P < 0.001$).

DISCUSSION

In the current study, we investigated the elimination of glucagon by determining the key pharmacokinetic parameters MCR, V_d, and $T_{1/2}$ of glucagon in individuals with

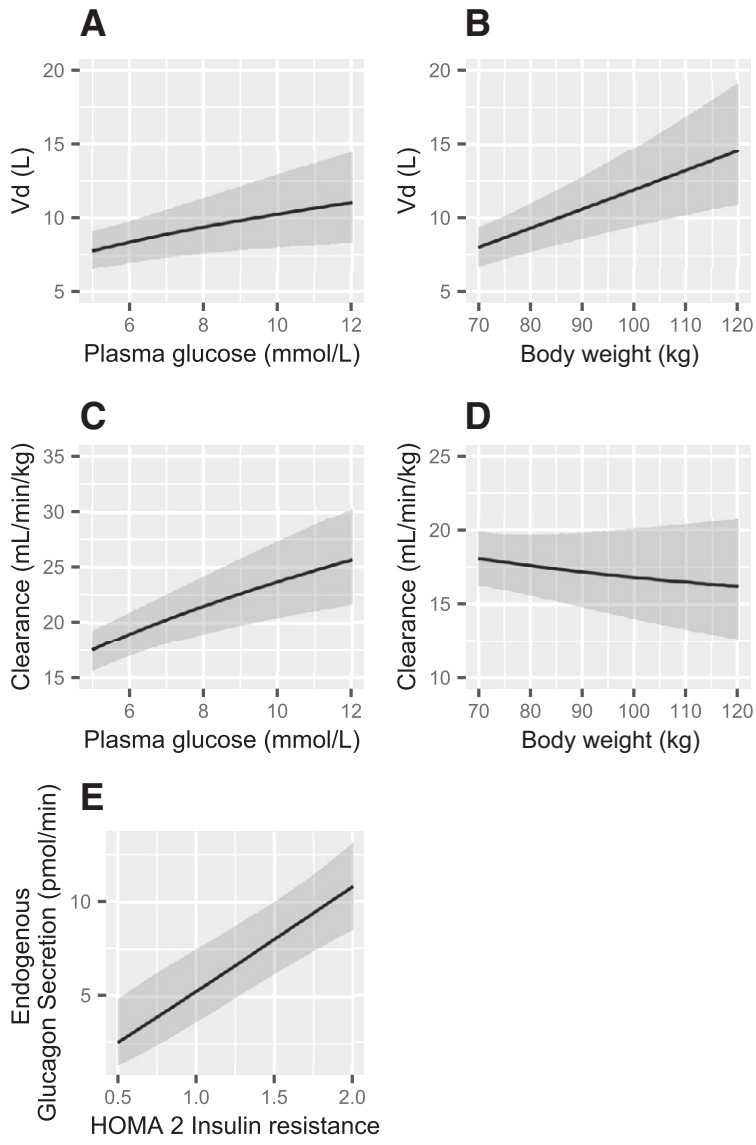

Figure 2—Visualization of the pharmacokinetic model. Effect of glucose (*A* and *C*), body weight (*B* and *D*), and HOMA2-IR (*E*) on V_d (*A* and *B*), glucagon clearance (*C* and *D*), and endogenous glucagon secretion (*E*). Body weight is standardized to 70 kg in *A* and *C*, and plasma glucose is standardized to 5.4 mmol/L in *B* and *D*.

type 2 diabetes and healthy control participants. We hypothesized that the inappropriate hyperglucagonemia observed in patients with type 2 diabetes could be caused, in part, by an impaired ability to eliminate glucagon from the bloodstream. We show that the ability to eliminate glucagon is preserved in individuals with type 2 diabetes. In fact, these individuals were characterized by a significantly higher MCR compared with those without diabetes. Thus, this study corroborates the notion that diabetic hyperglucagonemia results from inappropriate glucagon secretion and not from decreased removal of glucagon from the circulation.

The difference in glucagon MCR between the two main groups was observed regardless of adjustment for total body weight or BSA. As our participants were carefully matched by BMI, age, and kidney function, the observed differences in MCR are likely to be mediated by the metabolic differences between the healthy control group and the type 2 diabetes group. Data from our pharmacokinetic model further elaborate on the higher MCR in the type 2 diabetes group, as the clearance rate was seen to be positively correlated to baseline plasma glucose. Furthermore, the clearance rate of glucagon associated negatively with total body weight, which is in slight contrast to the direct comparison between the obese and lean subgroups, showing no significant difference. The current study does not provide additional insight into the mechanisms underlying the effect of body weight on the MCR of glucagon, but in general, clearance of a substance does not increase linearly with body weight (27). Similarly, the mechanisms

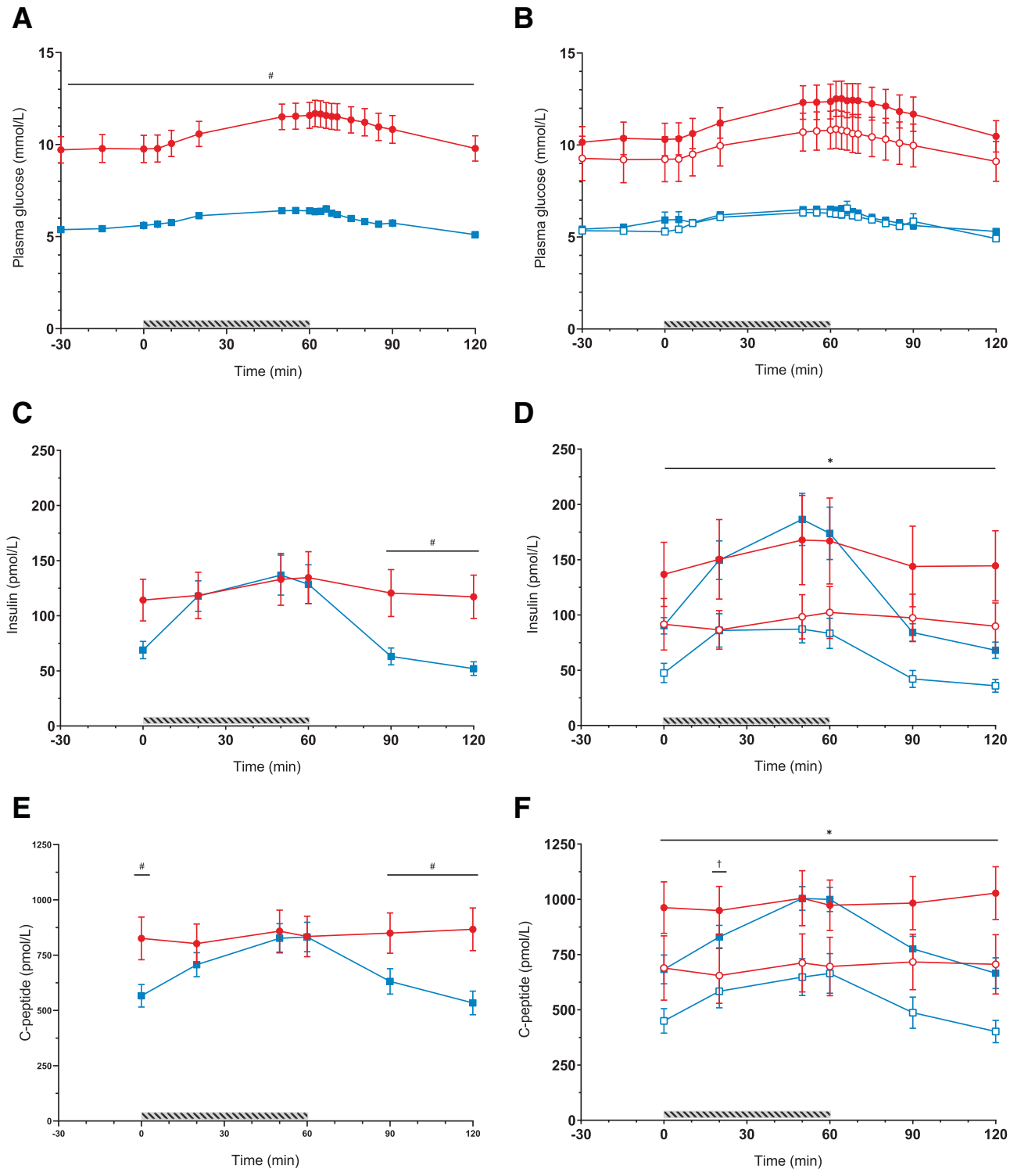

Figure 3—Excursions of plasma glucose, insulin, and C-peptide throughout the test day. *A*, *C*, and *E*: The type 2 diabetes group (red line, solid circles) and control group (blue line, solid squares) are presented as whole-group data. *B*, *D*, and *F*: Subgroup data are presented as the lean type 2 diabetes subgroup (red line, open circles), obese type 2 diabetes subgroup (red line, solid circles), lean control subgroup (blue line, open squares), and obese control subgroup (blue line, solid squares), respectively. The striped bar indicates timing of glucagon infusion. #$P < 0.05$ between the type 2 diabetes and control groups; *$P < 0.05$ between the lean and obese control subgroups; †$P < 0.05$ between the lean and obese type 2 diabetes subgroups.

Table 3—Baseline values and excursions of glucose, insulin, and C-peptide

	Type 2 diabetes			Control group		
	Lean	Obese	Total	Lean	Obese	Total
Plasma glucose						
Baseline (mmol/L)	9.2 (6.8; 11.6)	10.3 (8.6; 12)	9.8 (8.3; 11.3)##	5.3 (5.1; 5.5)	5.6 (5.3; 5.9)	5.5 (5.3; 5.7)##
AUC (mmol/L × min)	1,207 (949; 1,465)	1,387 (1,179; 1,595)	1,297 (1,130; 1,464)##	703 (668; 738)	720 (691; 749)	711 (688; 734)##
bsAUC (mmol/L × min)	98 (28; 168)	155 (81; 229)	127 (76; 178)##	65 (38; 92)	45 (3; 87)	55 (30; 80)##
Insulin						
Baseline (pmol/L)	92 (46; 138)	137 (80; 194)	114 (77; 151)#	48 (31; 65)*	90 (75; 105)*	69 (54; 84)#
bsAUC (pmol/L × min)	374 (−692; 1,440)	1,877 (−897; 4,651)	1,126 (−359; 2,611)#	2,127 (1,080; 3,174)	4,572 (2,784; 6,360)	3,350 (2,173; 4,527)#
C-peptide						
Baseline (pmol/L)	689 (404; 974)	962 (733; 1,191)	826 (636; 1,016)	450 (341; 559)*	683 (555; 811)*	566 (466; 666)
bsAUC (nmol/L × min)	0.82 (−3.9; 5.5)	2.4 (−8.0; 12.8)	1.6 (−3.9; 7.1)##	12.1 (5.2; 18.9)	19.0 (15.0; 22.9)	15.5 (11.3; 19.7)##

Data are mean (95% CI). #$P < 0.05$, ##$P < 0.01$ between the type 2 diabetes group and the control group. *$P < 0.05$ between the lean and obese control subgroups.

behind the increased MCR of glucagon in the type 2 diabetes group as well as the effect of plasma glucose on glucagon kinetics cannot be derived from the current study. Theoretically, an element of glomerular hyperfiltration in the type 2 diabetes group could be part of the explanation. As stated earlier, the mechanisms controlling glucagon clearance are not fully understood, but tissue extraction as well as renal clearance are thought to contribute (16), while any hepatic extraction seemingly is relatively small (17,28).

The MCR of glucagon has previously been investigated in patients with type 2 diabetes during pancreatic clamping with somatostatin, demonstrating preserved MCR compared with healthy controls subjects (21), but to our knowledge, a detailed investigation of the kinetics of glucagon in patients with type 2 diabetes has not been conducted. However, data from other studies investigating patients with type 1 diabetes (20,29), cirrhosis (28), and uremia (16) are available. MCR data from the control subjects in these studies are comparable to ours. In studies of patients with type 1 diabetes, a nonsignificant trend of higher glucagon MCR compared with healthy control subjects was found (i.e., a preserved ability to eliminate glucagon), which is in line with the results from our type 2 diabetes group. Furthermore, in those studies, a significantly prolonged $T_{1/2}$ and increased V_d were observed in patients with type 1 diabetes, leading the authors to conclude that some disturbances in glucagon kinetics were apparent in type 1 diabetes. In the current study, glucagon $T_{1/2}$ was similar (4.5 min) in both groups, roughly the same level as that of the control subjects described in the aforementioned type 1 diabetes studies. The fact that $T_{1/2}$ is similar between our groups despite a significant difference in MCR can be attributed to the significant differences in the V_d found between the groups. Regarding the V_d, our pharmacokinetic model showed it to be correlated positively with body weight. While this was expected, V_d was also positively correlated with FPG in our model. This finding was unexpected in light of multiple studies demonstrating decreased plasma volume in individuals with type 2 diabetes (30,31). It is important to note that several explanations for this potential association could exist and that the correlations derived from the pharmacodynamic model should be seen in the context of the whole-group analyses.

Fasting levels of glucagon were not different overall between the two groups, but because the individuals with type 2 diabetes had significantly higher plasma glucose levels, a state of relative hyperglucagonemia was evident. The absence of an absolute difference between the two groups was probably due to the careful matching of the two groups in terms of BMI, as obesity has been shown to constitute an important driver of increased fasting glucagon levels independently of the diabetic state and glycemic control (32). Thus, only a few of the lean participants with type 2 diabetes had overt fasting hyperglucagonemia (corroborating previous findings [33]), and several individuals in the obese control group displayed fasting hyperglucagonemia (also corroborating previous findings [33,34]). Conversely, the lean control subgroup had significantly lower fasting levels of glucagon compared with the obese control subgroup. When analyzing the MCR of glucagon only in the individuals characterized by fasting hyperglucagonemia, no differences in MCR were found compared with the individuals with normal glucagonemia (data not shown). Thus, our findings support the

hypothesis that obesity may constitute an independent and important contributor to fasting hyperglucagonemia, likely through steatosis-induced hepatic glucagon resistance at the level of ureagenesis and amino acid turnover, leading to elevated levels of glucagonotropic amino acids, which in turn stimulate glucagon secretion from the α-cells (32,34,35). In the pharmacokinetic model, a positive correlation between insulin resistance and increased endogenous glucagon secretion in the fasting state was seen, which is in line with this hypothesis.

As expected, the type 2 diabetes group was characterized by higher FPG values compared with the control group. The glucose responses to glucagon were also larger in the type 2 diabetes group. This finding highlights that the effect of glucagon on endogenous glucose production, even at supraphysiological levels, is not blunted but, rather, enhanced in patients with type 2 diabetes. The mechanism behind the larger effect of glucagon in patients with type 2 diabetes may be related to their insulin resistance and β-cell defect. The type 2 diabetes group was characterized by fasting hyperinsulinemia (illustrating these participants' insulin resistance) and much smaller insulin responses to the glucagon infusion compared with the control group (reflecting these participants' β-cell defect). On this background, it is not surprising that the normal, insulin-induced inhibition of hepatic glucose production appears blunted. Several reports (36,37) in recent years have documented insulinotropic paracrine effects of intraislet glucagon secretion, posing the interesting question of whether the smaller insulin response could in part be due to glucagon-resistant β-cells in type 2 diabetes. The answer, however, cannot be deduced from the current study.

The argument could be made that the differing insulin and glucose excursions in the two main groups, as well as a potentially different response to the glucagon infusion, could influence the basal glucagon secretion and thus influence our calculations of glucagon kinetics. However, if different endogenous secretion rates of glucagon were present at steady-state conditions, we would expect to see differences in the terminal part of the glucagon decay curve following termination of the exogenous glucagon infusion, which we did not. Furthermore, because we subtracted the basal glucagon concentration values in our MCR equation and achieved steady-state concentrations considerably above the basal level, any such influence would likely have been of little importance. In addition, since fasting glucagon levels would only, if at all, be expected to decrease during infusions, any error because of changes in endogenous glucagon levels would be small.

Our study has limitations. First, it comprised four groups of eight participants and, thus, had limited power in terms of subgroup analyses. Second, we chose to describe the glucagon kinetics on the basis of a single-compartment, single-exponential model of glucagon handling. This model probably represents a simplification of the actual physiological glucagon elimination kinetics. However, the data fit well into a single-compartment, first-order kinetic model, as demonstrated by our visual predictive check (Supplementary Figs. 2 and 3), validating our choice of model. This model was also recently validated in healthy individuals (38). The fact that our estimates of MCR, $T_{1/2}$, and V_d are all similar to those measured in other studies of glucagon clearance in humans further endorses this technique. We did not measure the participants' lean body mass, which could have provided insight into the relationship among body weight, obesity, and glucagon kinetics. Instead, we chose to adjust the glucagon MCR to BSA as well as to total body weight; both of these normalization factors are frequently used in pharmacokinetic analyses. The two normalization methods yielded similar results, except that the obese type 2 diabetes subgroup displayed a significantly elevated BSA-adjusted, but not body weight–adjusted, glucagon MCR.

Taken together, our findings suggest that a decreased ability to eliminate glucagon is not a fundamental part of type 2 diabetes or obesity.

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Author Contributions. M.F.G.G. and A.B.L. planned and executed the study, conducted the clinical experiments, researched data, performed statistical analyses, and wrote the manuscript. J.I.B. and M.B.C. planned the study and performed statistical and pharmacokinetic analyses. T.S.P. performed the pharmacokinetic analyses. N.J.W.A. and J.J.H. provided the analyses of glucagon. T.V. planned the study. F.K.K. conceptualized the study, wrote applications for funding, planned the study, and wrote the manuscript. All authors contributed to the discussion and critically reviewed the manuscript. M.F.G.G., A.B.L., and F.K.K. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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