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Insulin Resistance Is Accompanied by Increased Fasting Glucagon and Delayed Glucagon Suppression in Individuals With Normal and Impaired Glucose Regulation

Diabetes 2016;65:3473–3481 | DOI: 10.2337/db16-0240

Hyperinsulinemia is an adaptive mechanism that enables the maintenance of normoglycemia in the presence of insulin resistance. We assessed whether glucagon is also involved in the adaptation to insulin resistance. A total of 1,437 individuals underwent an oral glucose tolerance test with measurements of circulating glucose, insulin, and glucagon concentrations at 0, 30 and 120 min. Early glucagon suppression was defined as suppression in the period from 0 to 30 min, and late glucagon suppression as 30 to 120 min after glucose intake. Insulin sensitivity was estimated by the validated insulin sensitivity index. Individuals with screen-detected diabetes had 30% higher fasting glucagon levels and diminished early glucagon suppression, but greater late glucagon suppression when compared with individuals with normal glucose tolerance ($P \leq 0.014$). Higher insulin resistance was associated with higher fasting glucagon levels, less early glucagon suppression, and greater late glucagon suppression ($P < 0.001$). The relationship between insulin sensitivity and fasting glucagon concentrations was nonlinear ($P < 0.001$). In conclusion, increased fasting glucagon levels and delayed glucagon suppression, together with increased circulating insulin levels, develop in parallel with insulin resistance. Therefore, glucose maintenance during insulin resistance may depend not only on hyperinsulinemia but also on the ability to suppress glucagon early after glucose intake.

Glucagon acts as a counter regulatory hormone to insulin and is therefore essential for glucose regulation both in the fasting state and during glucose intake (1). In type 2 diabetes, fasting blood glucagon levels are elevated, but the reason for this dysregulation is not clearly established (2–5). Glucagon suppression in response to oral glucose is also diminished in overweight and obese individuals with impaired glucose tolerance (IGT) (6–8), and the suppression appears related to insulin sensitivity in individuals with normal glucose tolerance (NGT) (9,10). However, studies involving isoglycemic oral and intravenous glucose challenges in patients with type 2 diabetes (11) and first-degree relatives (12) have indicated that the failure to suppress glucagon secretion is mainly observed during the first 30–120 min after oral glucose intake, whereas normal suppression is seen after 2–4 h. These findings could indicate that the timing of glucagon suppression may play an important role in glucose regulation.

The secretion of glucagon is complex, involving a combination of paracrine, autocrine, hormonal, as well as

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Received 20 February 2016 and accepted 4 August 2016.

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autonomic neural mechanisms (13). Defective suppression of glucagon by glucose and insulin as well as insulin resistance in the α-cells have been suggested as potential mechanisms for the hyperglucagonemia in type 2 diabetes (1). However, in addition to glucose and insulin, glucagon secretion is regulated by gut incretin hormones. The most important ones are glucagon-like peptide 1 (GLP-1), which inhibits glucagon secretion (14), and glucose-dependent insulinotropic polypeptide (GIP), which stimulates glucagon secretion (15). Therefore, the interplay among glucose, insulin, GLP-1, and GIP is important to consider when studying glucagon secretion in vivo.

We hypothesized the existence of a mechanism linking α-cell hypersecretion/hyposuppressibility to a reduction in insulin sensitivity, as has been established for β-cell secretion (16). As a consequence, hyperglucagonemia and a failure to adequately suppress glucagon as insulin resistance increases could contribute to the impaired glucose regulation in prediabetes and type 2 diabetes. We examined in a large cohort of individuals at low to high diabetes risk *1*) whether plasma glucagon concentrations differed between individuals with NGT, prediabetes, and screen-detected type 2 diabetes; *2*) whether fasting glucagon concentrations are related to insulin sensitivity; and *3*) whether the timing of glucagon suppression after oral glucose intake was linked to insulin sensitivity and glucose tolerance status.

RESEARCH DESIGN AND METHODS

Study Population

The study was based on data from the Danish ADDITION-PRO Study (17), a risk-stratified cohort study of individuals at low to high risk for the development of type 2 diabetes, nested in the ADDITION-Denmark Study (18). Individuals with impaired glucose regulation at the ADDITION-Denmark screening and individuals from a random subsample of individuals at lower diabetes risk were invited to a follow-up health examination (2009–2011), and 2,082 participants (50% of those invited) attended (17). The study was approved by the Ethics Committee of the Central Denmark Region (reference no. 20080229) and was conducted in accordance with the Declaration of Helsinki. All participants provided oral and written informed consent before participating in the study.

Examination and Measurements

At the examination in 2009–2011, participants without known diabetes received a standard 75-g oral glucose tolerance test (OGTT) after an overnight fast of ≥8 h. Blood samples were drawn at 0, 30, and 120 min for the assessment of serum concentrations of insulin and plasma concentrations of glucose, glucagon, GLP-1, and GIP. Body weight was measured with participants wearing light indoor clothing without shoes to the nearest 0.1 kg with a Tanita (Tokyo, Japan) Body Composition Analyzer, and height was measured to the nearest millimeter using a fixed rigid stadiometer (Seca, Hamburg, Germany). The ADDITION-PRO Study is described in detail elsewhere (17).

Plasma glucose concentration was determined using the Hitachi 912 system (Roche Diagnostics, Mannheim, Germany) or the Vitros 5600 system (Ortho Clinical Diagnostics, Illkirch, France). Values measured by the Vitros 5600 system were converted to correspond to values from the Hitachi 912 system using a validated regression equation (17,19). Serum insulin concentrations were measured by immunoassay (AutoDELFIA; PerkinElmer, Waltham, MA). Blood samples for the measurement of glucagon, GLP-1, and GIP were obtained in tubes containing EDTA and put on ice immediately, centrifuged for plasma content, and stored at −80°C. Radioimmunological determinations of glucagon were performed as previously described (2,4,5) using a COOH terminus, which reliably measures intact glucagon as validated by sandwich ELISA and mass spectrometry (20,21). The analytical detection limit was 1 pmol/L, and the intra-assay and interassay coefficients of variation were <6% and <15%, respectively. Radioimmunological determinations of total plasma GLP-1 concentration (intact GLP-1 plus the metabolite GLP-1 9–36amide) were performed as described previously (22–24). The analytical detection limit was 1 pmol/L, and the intra-assay and interassay coefficients of variation were 6.0% and 1.5%, respectively at GLP-1 plasma concentrations of 20 pmol/L. Total plasma GIP concentrations (the sum of intact GIP plus the metabolite GIP 3–42) were measured by radioimmunoassay, as previously described (24,25). The assays for glucagon, GLP-1, and GIP measure the sum of the intact, active hormones and the metabolites generated by dipeptidylpeptidase-4 (GIP 3–42 and GLP-1 9–36-amide). The results therefore reflect the secretion of the hormones. The samples for determination of glucagon, GLP-1, and GIP were analyzed consecutively over 2 months using identical quality controls and identical batches for all reagents.

Calculations and Classification of Participants

Study participants were classified according to the World Health Organization 2006 criteria as having NGT, prediabetes (impaired fasting glucose [IFG] and/or IGT), or screen-detected type 2 diabetes. Participants with known diabetes (n = 336), those fasting <8 h prior to the health examination (n = 20), those who could not be classified because of missing information on fasting or 2-h plasma glucose concentrations (n = 12), and those with no glucagon measurements (n = 289) were excluded, leaving 1,437 individuals for analysis.

The relative glucagon suppression during the first 30 min after oral glucose administration was calculated as follows: $(1 - [\text{glucagon}_{30\ \text{min}}/\text{glucagon}_{0\ \text{min}}]) \times 100\%$. Similarly, the suppression of glucagon from 30 to 120 min was calculated as $(1 - [\text{glucagon}_{120\ \text{min}}/\text{glucagon}_{30\ \text{min}}]) \times 100\%$, and glucagon suppression during the entire OGTT was calculated as $(1 - [\text{glucagon}_{120\ \text{min}}/\text{glucagon}_{0\ \text{min}}]) \times 100\%$. Accordingly, this resulted in positive values for

those who did suppress glucagon and negative values for those who did not suppress glucagon during the OGTT.

As a measure of insulin sensitivity, we calculated the insulin sensitivity index (ISI_{0-120}), reflecting whole-body insulin sensitivity, as follows: 75,000 mg + ($glucose_{0\ min}$ − $glucose_{120\ min}$) × 0.19 × weight)/($glucose_{0min}$ + $glucose_{120min}$)/2)/log ($insulin_{0min}$ + $insulin_{120min}$)/2) (26).

Statistical Analyses

Characteristics of the study population are shown by glucose tolerance status. Data are presented as the means with SDs for normally distributed variables and as geometric means with corresponding 95% CIs for variables with a skewed distribution. For proportions, exact 95% CIs were calculated. An overall ANOVA was used to assess differences in characteristics between groups, and, if significant, post hoc *t* tests were used to study pairwise differences.

Absolute glucagon levels as well as relative glucagon suppression during the OGTT were studied by glucose tolerance status and by tertiles of insulin sensitivity. As an initial step, data were investigated graphically. This indicated a nonlinear association between fasting glucagon and insulin sensitivity. Therefore, we fitted a hyperbolic relationship between fasting glucagon levels and insulin sensitivity, and tested for a modifying effect of glucose tolerance status on the association.

In multivariate linear regression analyses, we modeled the association of early and late glucagon suppression with insulin sensitivity in separate models, using a stepwise approach. First, the data were adjusted for age, sex, and initial glucagon level (i.e., fasting glucagon level for early suppression and 30 min glucagon level for late suppression) (Model 1). Second, data were further adjusted for fasting and 2-h plasma glucose concentration (Model 2). Last, further adjustment for the relative change in GIP and GLP-1 was performed (from 0 to 30 min for early glucagon suppression as outcome, and from 30 to 120 min for late glucagon suppression as outcome) (Model 3).

Statistical analyses were performed in R, version 3.2.1 (The R Foundation for Statistical Computing, Vienna, Austria) and SAS version 9.2 (SAS Institute, Cary, NC). A two-sided 5% level of significance was used for all analyses.

RESULTS

Glucagon Levels in Relation to Glucose Tolerance Status

Table 1 shows characteristics of the study population stratified by NGT, prediabetes, and screen-detected type 2 diabetes. Absolute glucagon levels and the percentage of glucagon suppression during the OGTT in these groups are shown in Fig. 1*A* and *B*. Individuals with screen-detected diabetes had on average 12–59% higher glucagon levels at all measured time points (0, 30, and 120 min) than the other groups ($P < 0.05$). Furthermore, individuals with screen-detected diabetes did not suppress glucagon levels from 0 to 30 min during the OGTT ($P = 0.776$). Persons with prediabetes had on average 12–22% higher glucagon levels at 0 and 30 min compared with the NGT group, and their level of early glucagon suppression was lower than that in the NGT group ($P < 0.001$). Noteworthy, individuals with screen-detected diabetes suppressed glucagon more than individuals with NGT from 30 to 120 min after oral glucose intake ($P < 0.001$).

When exploring glucagon levels in subgroups of prediabetes and type 2 diabetes, we found differences between

Table 1—Characteristics of the study population by glucose tolerance status

	NGT (*N* = 763)	Prediabetes (*N* = 514)	Type 2 diabetes (*N* = 160)	*P* value
Age (years)	65.7 (7.5)	66.8 (6.6)[a]	66.1 (6.5)	0.020
Women, % (95% CI)	52.2 (48.6; 55.8)	43.2 (38.9; 47.6)[a]	33.8 (26.5; 41.6)[a,b]	<0.001
BMI (kg/m^2)	26.0 (4.0)	27.9 (4.4)[a]	29.8 (5.6)[a,b]	<0.001
Fasting glucose (mmol/L)	5.6 (0.4)	6.3 (0.4)[a]	7.3 (1.0)[a,b]	<0.001
30-min glucose (mmol/L)	8.3 (1.4)	9.7 (1.2)[a]	11.4 (1.9)[a,b]	<0.001
120-min glucose (mmol/L)	5.6 (1.1)	7.4 (1.7)[a]	10.6 (3.1)[a,b]	<0.001
Fasting insulin (pmol/L)	30.4 (29.2; 31.7)	43.3 (41.2; 45.6)[a]	58.9 (53.0;65.6)[a,b]	<0.001
30-min insulin (pmol/L)	205.6 (196.6; 215.1)	223.0 (210.0; 236.8)[a]	229.0 (207.5; 252.6)[a]	0.038
120 min insulin (pmol/L)	134.8 (128.3; 141.5)	247.4 (231.5; 264.3)[a]	357.8 (317.1; 403.8)[a,b]	<0.001
Glucagon suppression, 0–30 min (%)*	16.5 (13.9; 19.1)	9.5 (5.7; 13.2)[a]	−3.8 (−12.2; 3.9)[a,b]	<0.001
Glucagon suppression, 30–120 min (%)‡	27.9 (25.3; 30.5)	39.6 (37.1; 42.0)[a]	48.3 (44.4; 52.0)[a,b]	<0.001
Glucagon suppression, 0–120 min (%)§	39.7 (37.3; 42)	45.2 (42.6; 47.7)[a]	46.2 (41.7; 50.5)[a]	0.002

Data are the mean (SD) or geometric mean (95% CI), unless otherwise indicated. *Calculated as: (1 − [$glucagon_{30\ min}$/$glucagon_{0\ min}$]) × 100. ‡Calculated as: (1 − [$glucagon_{120\ min}$/$glucagon_{30\ min}$]) × 100. §Calculated as: (1 − [$glucagon_{120\ min}$/$glucagon_{0\ min}$]) × 100. [a]$P < 0.05$ vs. NGT. [b]$P < 0.05$ vs. prediabetes.

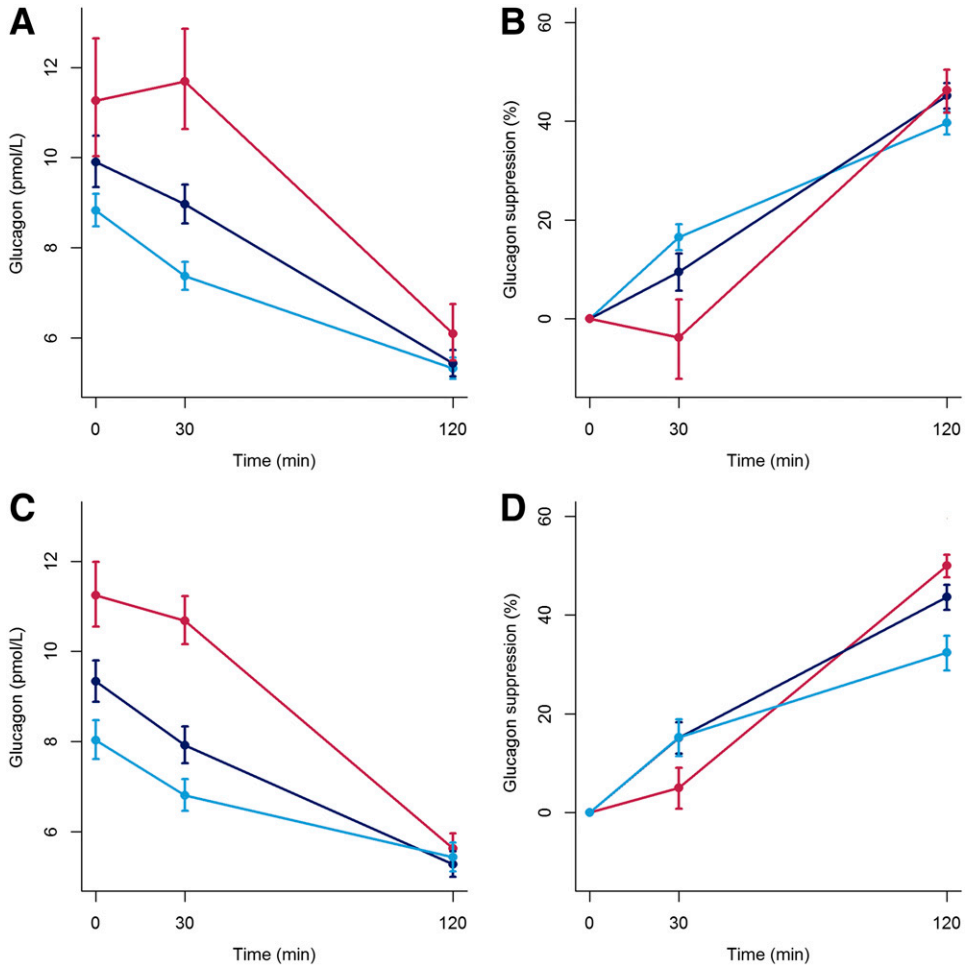

Figure 1—Plasma glucagon (*A* and *C*) and glucagon suppression (*B* and *D*) during the OGTT. Top row (*A* and *B*) by glucose tolerance status: NGT (light blue), prediabetes (dark blue), and type 2 diabetes (red). Bottom row (*C* and *D*) by tertiles of insulin sensitivity (ISI_{0-120}): high insulin sensitivity (light blue), median insulin sensitivity (dark blue), and low insulin sensitivity (red). Data are geometric means with 95% CI. *A*: Overall $P_{0\ min} < 0.001$ (prediabetes [Pre-D] vs. NGT $P = 0.001$; type 2 diabetes [T2D] vs. NGT $P < 0.001$; T2D vs. Pre-D $P = 0.024$); overall $P_{30\ min} < 0.001$ (Pre-D vs. NGT $P < 0.001$; T2D vs. NGT $P < 0.001$; T2D vs. Pre-D $P < 0.001$); overall $P_{120\ min} = 0.048$ (Pre-D vs. NGT $P = 0.561$; T2D vs. NGT $P = 0.014$; T2D vs. Pre-D $P = 0.046$). *C*: Overall $P_{0\ min} < 0.001$ (median vs. high: $P < 0.001$; low vs. high $P < 0.001$; low vs. median $P < 0.001$); overall $P_{30\ min} < 0.001$ (median vs. high $P < 0.001$; low vs. high $P < 0.001$; low vs. median $P < 0.001$); overall $P_{120\ min} = 0.278$ (median vs. high $P = 0.468$; low vs. high $P = 0.383$; low vs. median $P = 0.110$).

individuals in whom diagnosis was made by fasting glucose levels versus the 2-h glucose levels. Individuals with isolated IFG had 24–31% less late and overall glucagon suppression than individuals with IGT (and IFG plus IGT), but the groups did not differ in terms of early glucagon suppression (Table 2). Individuals in whom screen-detected diabetes was diagnosed by elevated 2-h glucose levels had 50–71% higher fasting and 30-min glucagon levels, and greater glucagon suppression from 30 to 120 min than those in whom it was diagnosed by elevated fasting glucose only (Table 3).

Glucagon Levels in Relation to Insulin Sensitivity

Absolute glucagon levels and the percentage of glucagon suppression during the OGTT stratified by tertiles of insulin sensitivity are shown in Fig. 1*C* and *D*. Fasting glucagon levels increased with decreasing levels of insulin sensitivity, and those with the poorest insulin sensitivity had impaired glucagon suppression at 30 min, but glucagon levels were equal in all three groups at 120 min. Thus, glucagon suppression from 30 to 120 min was most pronounced in those with the poorest insulin sensitivity ($P < 0.001$).

The relationship of fasting glucagon concentration with insulin sensitivity stratified by glucose tolerance status is shown on a normal axis in Fig. 2*A* and on a logarithmic axis in Fig. 2*B*. For all glucose tolerance groups, nonlinear (inverse function) relationships were present (i.e., fasting glucagon increased exponentially with lower levels of insulin sensitivity). Interestingly, for a given level of glucagon, the insulin sensitivity differed between the glucose tolerance groups with lowest levels for individuals with screen-detected diabetes, intermediate levels for individuals with prediabetes, and the highest levels for those with NGT ($P < 0.001$). Figure 3*A–C* shows fasting plasma

Table 2—Glucagon characteristics in individuals with different subtypes of prediabetes

	i-IFG (*N* = 281)	i-IGT (*N* = 110)	IFG+IGT (*N* = 123)	*P* value
Fasting glucagon (pmol/L)	9.7 (9; 10.5)	9.8 (8.6; 11.1)	10.6 (9.3; 11.9)	0.190
30-min glucagon (pmol/L)	8.7 (8.1; 9.3)	8.9 (8.2; 9.8)	9.7 (8.8; 10.7)	0.144
120-min glucagon (pmol/L)	5.8 (5.4; 6.3)	4.9 (4.4; 5.4)[a]	5.1 (4.5; 5.6)[a]	0.002
Glucagon suppression, 0–30 min (%)*	10.4 (5.2; 15.4)	8.5 (−0.1; 16.3)	8.2 (0.1; 15.7)	0.864
Glucagon suppression, 30–120 min (%)‡	33.1 (29.6; 36.5)	45.2 (40.8; 49.3)[a]	47.8 (43.0; 52.3)[a]	<0.001
Glucagon suppression, 0–120 min (%)§	39.8 (35.8; 43.6)	49.7 (45.2; 53.9)[a]	52.1 (47.5; 56.3)[a]	<0.001

Data are geometric mean (95% CI), unless otherwise indicated. i-IFG, isolated IFG; i-IGT, isolated IGT; IFG+IGT, combined IFG and IGT. [a]$P <$ 0.05 vs. NGT. *Calculated as: $(1 - [\text{glucagon}_{30\ min}/\text{glucagon}_{0\ min}]) \times 100$. ‡Calculated as: $(1 - [\text{glucagon}_{120\ min}/\text{glucagon}_{30\ min}]) \times 100$. §Calculated as: $(1 - [\text{glucagon}_{120\ min}/\text{glucagon}_{0\ min}]) \times 100$.

glucagon concentrations as functions of insulin sensitivity stratified by the glucose tolerance groups.

Results From Linear Regression Analysis

From the linear regression analysis, it was found that a doubling in insulin sensitivity was associated with 28.0% points greater early suppression and 12.4% points less late suppression of glucagon (Table 4) ($P < 0.001$). Adjustment for fasting and 2-h plasma glucose levels attenuated the association by ~30%, although it remained statistically significant ($P < 0.027$). Neither the fasting nor 2-h plasma glucose level was associated with glucagon suppression in Model 2 ($P \geq 0.159$). Further adjustment for changes in GLP-1 and GIP levels during the OGTT attenuated the association of insulin sensitivity with early glucagon suppression, but not with late glucagon suppression (Model 3). In this model, GLP-1 and GIP were not associated with early glucagon suppression ($P \geq 0.147$), but both were associated with late glucagon suppression ($P < 0.001$). A 50% increase in GLP-1 levels from 30 to 120 min was associated with 10% greater glucagon suppression, whereas a 50% increase in GIP levels from 30 to 120 min was associated with 1.5% less glucagon suppression.

DISCUSSION

The regulation of glucagon secretion is complex, and involves autocrine and paracrine mechanisms as well as intrinsic mechanisms in the α-cell related to glucose sensing (1). By exploring the relationship between insulin sensitivity and glucagon response in a large Danish cohort of individuals at low to high risk of diabetes, we found the following: *1*) individuals with screen-detected type 2 diabetes had higher circulating glucagon levels in the fasting state, and 30 and 120 min after oral glucose intake compared with individuals with NGT or prediabetes; *2*) lower insulin sensitivity (i.e., insulin resistance) was associated with higher fasting glucagon levels in a nonlinear manner; and *3*) insulin resistance, prediabetes, and screen-detected type 2 diabetes were associated with poorer glucagon suppression during the first 30 min after glucose intake, but slightly exaggerated glucagon suppression from 30 to 120 min, which suggests that the timing of glucose-stimulated glucagon suppression is particularly important for the maintenance of normoglycemia. Taken together, these results support our hypothesis that increased circulating levels of glucagon, together with increased insulin levels, are tightly coupled to a reduction of insulin sensitivity in the early stages of glucose dysregulation (9).

Impaired suppression of glucagon in response to glucose or meal challenges in patients with type 2 diabetes has been reported in several studies (2–5). We now show in a well-powered study that individuals with newly diagnosed type 2 diabetes only have impairment in the early

Table 3—Glucagon characteristics in individuals with different subtypes of screen-detected diabetes

	F-D (*N* = 80)	2h-D (*N* = 42)	F-2h-D (*N* = 37)	*P* value
Fasting glucagon (pmol/L)	8.8 (7.3; 10.6)	14.3 (11.8; 17.3)[a]	14.8 (12.9; 17)[a]	<0.001
30-min glucagon (pmol/L)	9.3 (8.0; 10.8)	13.9 (12.1; 16.1)[a]	15.9 (13.8; 18.3)[a]	<0.001
120-min glucagon (pmol/L)	5.6 (4.8; 6.5)	6.5 (5.4; 8.0)	6.9 (5.7; 8.3)	0.355
Glucagon suppression, 30 min (%)*	−6.2 (−21.6; 7.3)	2.3 (−10.7; 13.8)	−7.4 (−17.7; 2.1)	0.613
Glucagon suppression, 30–120 min (%)‡	41.0 (33.4; 47.7)	53.1 (47.1; 58.3)[a]	56.6 (51.5; 61.2)[a]	0.001
Glucagon suppression, 120 min (%)§	37.1 (28.7; 44.4)	54.2 (46.3; 60.8)[a]	53.4 (47.7; 58.5)[a]	<0.001

Data are geometric mean (95% CI), unless otherwise indicated. F-D, diabetes diagnosed by fasting glucose only; 2h-D, diabetes diagnosed by 2-h glucose only; F-2h-D, diabetes diagnosed by both fasting and 2-h glucose levels. [a]$P < 0.05$ vs. NGT. *Calculated as: $(1 - [\text{glucagon}_{30\ min}/\text{glucagon}_{0\ min}]) \times 100$. ‡Calculated as: $(1 - [\text{glucagon}_{120\ min}/\text{glucagon}_{30\ min}]) \times 100$. §Calculated as: $(1 - [\text{glucagon}_{120\ min}/\text{glucagon}_{0\ min}]) \times 100$.

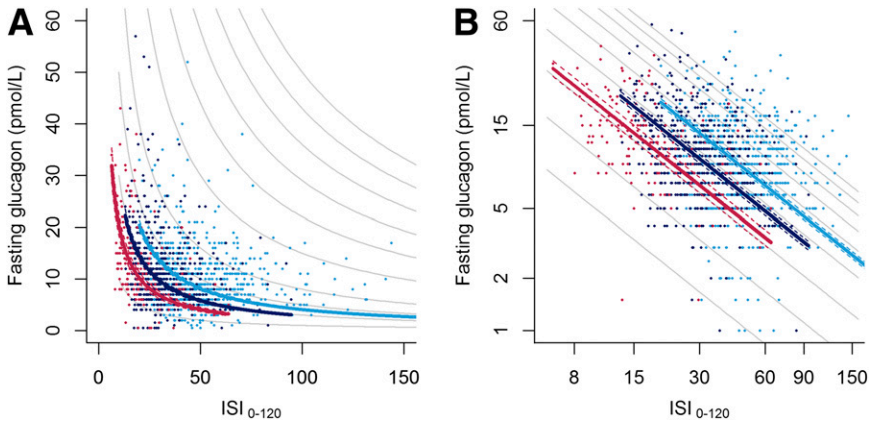

Figure 2—Fasting glucagon concentration as a function of insulin sensitivity on the original scale (*A*) and on the log-scale (*B*). Curves are plotted within the range of observed insulin sensitivity and shown for NGT (light blue), prediabetes (dark blue), and type 2 diabetes (red). The thin gray lines illustrate different levels of insulin sensitivity × fasting glucagon concentrations (hyperbolic function). $P < 0.001$ for all associations.

glucose-stimulated glucagon suppression. Their later relative glucagon suppression is normal or even exaggerated compared with individuals without diabetes, and this is particularly true for individuals in whom diabetes was diagnosed by 2-h glucose concentration after an oral glucose load. Yet, if the high post-OGTT glucose levels observed in this group are considered, it is clear that the late glucagon suppression is not sufficient to secure NGT. Therefore, hyperglycemia seems to be related mainly to impairment of the early glucagon response, which is analogous to the importance of defective early insulin secretion for hyperglycemia in type 2 diabetes (27). This combined early defect in both α- and β-cell secretion during the development of type 2 diabetes is of important pathophysiological interest and also supports the indication that the restoration of the early pancreatic responses is an important target for therapy.

The observed novel, nonlinear, relationship between fasting glucagon concentration and insulin sensitivity suggests that basal α-cell secretion depends on insulin sensitivity. It is well established that β-cell secretion depends on insulin sensitivity and is increased in a nonlinear manner during the development of insulin resistance (i.e., the underlying background for the generation of the disposition index) (16,28). However, our results also strongly indicate that the resulting glucose tolerance is jeopardized by simultaneous hypersecretion of the α-cells, eventually resulting in hyperglycemia in the presence of hyperinsulinemia—a feature well known in type 2 diabetes. A shift from the upper right corner toward the lower left corner of the

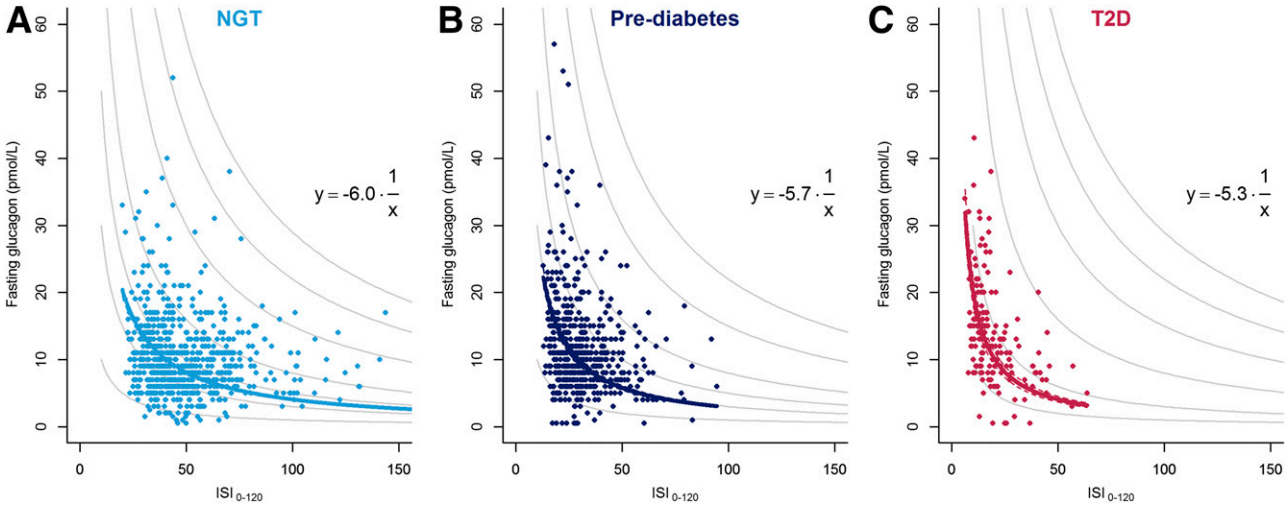

Figure 3—Fasting glucagon concentration as a function of insulin sensitivity stratified by NGT (light blue, *A*), prediabetes (dark blue, *B*), and type 2 diabetes (red, *C*). Curves are plotted within the range of observed insulin sensitivity. The thin gray lines illustrate different levels of insulin sensitivity × fasting glucagon concentrations (hyperbolic function).

Table 4—Estimated change in percentage (95% CI) in early and late glucagon suppression by a doubling in insulin sensitivity

	Early glucagon suppression		Late glucagon suppression	
	Estimate	*P* value	Estimate	*P* value
Model 1	28.0 (21.0; 35.0)	<0.001	−12.4 (−16.4; −8.4)	<0.001
Model 2	20.5 (6.3; 34.7)	0.005	−8.9 (−16.8; −1.0)	0.027
Model 3	11.5 (−0.9; 23.8)	0.069	−10.6 (−18.3; −2.9)	0.007

Model 1, adjustment for age, sex, and fasting glucagon (early suppression as outcome) or 30-min glucagon level (late suppression as outcome); model 2, further adjustment for fasting and 2-h plasma glucose level; and model 3, further adjustment for relative change in GIP and GLP-1 (from 0 to 30 min for early suppression as outcome and from 30 to 120 min for early suppression as outcome).

glucagon-insulin sensitivity relationship (Fig. 2*A*) is associated with a worsening of glucose tolerance status—exactly as seen for the insulin secretion disposition index (16,28). Unfortunately, from our cross-sectional study it is not possible to conclude whether the changes in basal glucagon secretion precede or parallel changes in insulin secretion in individuals who become insulin resistant.

Another novel finding of our study was that the timing of glucagon suppression after oral glucose intake is particularly important for the maintenance of normoglycemia. The postprandial glycemic response is dependent on complex, interdependent relationships between gastric emptying and glucose absorption, secretion and action of incretin hormones, insulin sensitivity, as well as insulin and glucagon release. The rates of gastric emptying and glucose absorption play particularly important roles for the magnitude of both the postprandial glycemic excursion and the incretin hormone responses (29,30). However, the negative-feedback mechanism, which limits the amount of nutrients released into the bloodstream during hyperglycemic states (31) makes it difficult to conclude whether delayed gastric emptying results in delayed glucagon suppression or whether a worsening of early glucagon suppression with resulting hyperglycemia leads to delayed gastric emptying (29). Thus, the precise mechanisms leading to increased basal glucagon levels and failing early glucose-stimulated glucagon suppression in response to insulin resistance are not clearly understood. Fasting hyperglucagonemia has previously been associated with insulin resistance as measured by gold standard methods in individuals without diabetes, suggesting that insulin resistance may be present at the level of the pancreatic α-cells (32). From studies in patients with maturity-onset diabetes of the young, it is clear that it is not the circulating glucose levels per se that drive glucagon secretion, but more likely the intracellular metabolism of glucose. In other words, it is likely that glucokinase serves as a metabolic glucose sensor in pancreatic α-cells, which then mediate a mechanism for direct regulation of glucagon release by extracellular glucose in the same way as in β-cells (33). However, α-cell secretion is also regulated by the gut incretin hormone GLP-1 (14), possibly indirectly by somatostatin (34). In the current study, GLP-1 was not associated with early glucagon suppression, whereas positive changes in GLP-1 levels from 30 to 120 min during the OGTT were associated with greater late glucagon suppression. This supports an inhibitory role of GLP-1 on glucagon secretion during hyperglycemia (14). Similarly, an increase in GIP levels from 30 to 120 min was associated with a poorer late suppression of glucagon. Although the effect size was small, the finding is in line with a stimulating role of GIP on glucagon secretion, as previously reported (15). The lack of association of GLP-1 and GIP with early glucagon suppression underscores the idea that the control of glucagon secretion is complex and multifactorial, involving a combination of paracrine, autocrine, hormonal, and autonomic neural mechanisms (13). In addition, recent evidence from patients who had undergone pancreatectomy suggests that glucagon may be secreted not only from the pancreas, but also from extrapancreatic tissue in humans, probably the gut (21). Whether gut-derived glucagon secretion is partly responsible for fasting hyperglucagonemia in insulin-resistant individuals warrants further studies.

One of the major strengths of this study is the large sample size and the 3-point OGTT, which enabled us to study subgroups of individuals with different patterns of glucagon suppression. Another strength is that we used a well-documented assay method with high specificity and sensitivity for glucagon, which was directed against the C terminus of the glucagon molecule and therefore mainly measures glucagon of pancreatic origin (35). Insulin sensitivity was not assessed by the gold standard clamp technique because of the large size of the cohort, which could appear as a limitation of our study. However, $ISI_{0–120}$ is a valid measure of insulin sensitivity across different categories of glucose tolerance status and obesity when compared with the gold standard clamp technique (26,30), supporting the validity of our results. Unfortunately, we were not able to assess the optimal time point for distinguishing between early and late glucagon suppression, since this would have required more measurements during the OGTT. The 30-min measurement in the ADDITION-PRO Study was chosen to be able to assess early insulin secretion, but we cannot exclude the possibility that samples for the measurement of glucagon taken 45 or 60 min after oral glucose intake would have given slightly different results.

In conclusion, we found a nonlinear inverse relationship between fasting glucagon levels and insulin sensitivity, which means that fasting glucagon concentrations increase exponentially with the deterioration of insulin

sensitivity. Interestingly, individuals with screen-detected diabetes exhibited a lack of glucagon suppression from 0 to 30 min, but their glucagon suppression from 30 to 120 min was normal or even exaggerated. Our findings suggest that the fasting hyperglucagonemia observed in individuals with type 2 diabetes is likely to develop in parallel with insulin resistance. A novel finding in our study is that the timing of glucagon suppression is crucial for the maintenance of normal glucose homeostasis. Although early glucagon suppression is associated with adequate insulin sensitivity and NGT, delayed glucagon suppression is associated with insulin resistance and a higher risk of type 2 diabetes. Future studies should examine whether an improvement in insulin sensitivity contributes to a restoration of the early pancreatic response. The specific mechanisms linking insulin resistance to increased fasting glucagonemia and delayed glucagon suppression also needs further study.

Acknowledgments. The authors thank the ADDITION-PRO study centers, the staff, and the participants for their important contribution to the study. The authors also thank the laboratory technicians Lene Albæk and Sofie P. Olesen at the University of Copenhagen.

Funding. The ADDITION-Denmark study was supported by the National Health Services in the counties of Copenhagen, Aarhus, Ringkøbing, Ribe, and Southern Jutland in Denmark; the Danish Council for Strategic Research; the Danish Research Foundation for General Practice; Novo Nordisk Foundation; the Danish Centre for Evaluation and Health Technology Assessment; the Diabetes Fund of the National Board of Health; the Danish Medical Research Council; and the Aarhus University Research Foundation. Additionally, the ADDITION-PRO study was funded by an unrestricted grant from the European Foundation for the Study of Diabetes/Pfizer for Research into Cardiovascular Disease Risk Reduction in Patients with Diabetes (grant 74550801), the Danish Council for Strategic Research, and internal research and equipment funds from Steno Diabetes Center. K.F. is supported by the Novo Nordisk Foundation. N.B.J. and D.R.W. are funded by the Danish Diabetes Academy, which is supported by the Novo Nordisk Foundation.

Duality of Interest. K.F., D.V., N.B.J., and M.E.J. are employed by Steno Diabetes Center A/S, a research hospital working in the Danish National Health Service and owned by Novo Nordisk A/S (Steno Diabetes Center receives part of its core funding from unrestricted grants from the Novo Nordisk Foundation and Novo Nordisk A/S), and hold shares in Novo Nordisk A/S. S.S.T., D.R.W., O.P., and T.H. hold shares in Novo Nordisk A/S. T.L. holds shares in Novo Nordisk A/S; has received, between 2000 and 2011, unrestricted grants for the ADDITION Study (screening and intensive treatment of type 2 diabetes in primary care) from public foundations and the medical industry, as follows: Novo Nordisk AS, Novo Nordisk Scandinavia AB, ASTRA Denmark, Pfizer Denmark, GlaxoSmithKline Pharma Denmark, Servier Denmark A/S, and HemoCue Denmark A/S. B.A. holds shares in Novo Nordisk A/S and is a board member of the Novo Nordisk Foundation. No other potential conflicts of interest relevant to this article were reported.

Author Contributions. K.F. developed the hypotheses and drafted the first version of the manuscript. D.V. developed the hypotheses, performed the statistical analyses, and drafted the first version of the manuscript. G.P. and B.A. conceived the idea and developed the hypotheses. S.S.T. developed the hypotheses and generated glucagon data. N.B.J., D.R.W., and T.L. designed the ADDITION-PRO Study. A.J., O.P., and T.H. designed the incretin and glucagon part of the ADDITION-PRO Study. M.E.J. contributed intellectually to the discussion of the results. J.J.H. conceived the idea, developed the hypotheses, and generated glucagon data. All authors reviewed and edited the manuscript and approved the final version of the manuscript. K.F. and D.V. 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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