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Fasting glucagon concentrations are associated with longitudinal decline of β-cell function in non-diabetic humans

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Abstract

Purpose.—Abnormal glucagon concentrations are a feature of prediabetes but it is uncertain if α-cell dysfunction contributes to a longitudinal decline in β-cell function. We therefore sought to determine if a decline in β-cell function is associated with a higher nadir glucagon in the postprandial period or with higher fasting glucagon.

Methods.—This was a longitudinal study in which 73 non-diabetic subjects were studied on 2 occasions 6.6 ± 0.3 years apart using a 2-hour, 7-sample oral glucose tolerance test. Disposition Index (DI) was calculated using the oral minimal model applied to the measurements of glucose, insulin, C-peptide concentrations during the studies. We subsequently examined the relationship of glucagon concentrations at baseline with change in DI (used as a measure of β-cell function) after adjusting for changes in weight and the baseline value of DI.

Results.—After adjusting for covariates, nadir postprandial glucagon concentrations were not associated with changes in β-cell function as quantified by DI. On the other hand, fasting glucagon concentrations during the baseline study were inversely correlated with longitudinal changes in DI.

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Data Availability:
Deidentified datasets will be made available on request from the authors after all necessary approvals and agreements are obtained.

Conclusions.—Defects in α-cell function, manifest as elevated fasting glucagon, are associated with a subsequent decline in β-cell function. It remains to be ascertained if abnormal α-cell function contributes directly to loss of β-cell secretory capacity in the pathogenesis of type 2 diabetes.

Keywords

α-cell function; β-cell function; glucagon; insulin action; prediabetes

1.0 Introduction

Type 2 diabetes is characterized by defects in both β-cell and α-cell function. When insulin secretion is decreased and / or delayed, post-prandial glucagon concentrations mimicking those observed in type 2 diabetes raise glucose concentrations by increasing endogenous glucose production [1, 2]. In addition, glucagon expression in the islet is increased in prediabetes and diabetes [3]. Previously, α-cell dysfunction was thought to occur as a secondary consequence of β-cell dysfunction during the development of type 2 diabetes [4]. However, fasting glucagon is elevated in insulin-resistant subjects [5]. More recently, Faerch et al. suggested that elevated glucagon was associated with measures of impaired insulin action but not with impaired insulin secretion [6]. Indeed, in insulin-resistant mice, ceramide accumulation in α-cells impairs insulin-mediated suppression of preproglucagon mRNA [7]. Examination of a large cohort of non-diabetic subjects also demonstrated an inverse relationship of glucagon concentrations with insulin action [8]. However, due to the cross-sectional nature of these observations, firm conclusions as to the role of α-cell dysfunction in prediabetes cannot be made.

In non-diabetic subjects genetically predisposed to type 2 diabetes, glucagon suppression in response to an oral [9] or intravenous [10] challenge is impaired. If defective postprandial suppression of glucagon could drive the development of hyperglycemia, this might suggest a role for glucagon antagonism [11] in preventing the progression of prediabetes. We therefore sought to determine if higher fasting and postprandial glucagon concentrations are associated with longitudinal decline in β-cell function as quantified by the Disposition Index (DI) [12]. To test our hypotheses, we studied non-diabetic subjects using a 75g oral glucose tolerance test (OGTT) administered twice over a ~ 7 year period. We report that fasting, but not nadir, glucagon concentrations during the ‘Baseline’ study are inversely correlated with the change in overall β-cell function (DI) measured during the ‘Follow-Up’ study. These data suggest that fasting glucagon concentrations are associated with a subsequent decline in β-cell function.

2.0 Research Design and Methods

2.1 Subjects, Experimental Design, Calculations and Statistical Analysis.

Participants in previously published studies utilizing a standardized 7-sample, 2 hour, 75g OGTT were invited to participate in the current study. The study was performed after approval by the Mayo Institutional Review Board and informed, written consent was obtained. The OGTT was repeated (and analyzed) as outlined in the Supplementary Data.

Net insulin action (S_I) and β-cell responsivity indices calculated using the oral minimal model were used to derive Disposition index (DI) as reviewed previously [12]. Please refer to Supplementary Data for details.

All data are presented as Mean ± SEM unless otherwise noted. Multivariable regression analysis adjusting for the effects of variation in the weight change and the time interval between studies was performed. The target parameter was the fractional change (e.g. in the case of DI: $\Delta DI = [(DI_{Present} - DI_{Baseline})/ DI_{Baseline}]$). Please refer to Supplementary Data for details.

3.0 Results

3.1 Subject Characteristics

We studied a total of 73 nondiabetic subjects whose characteristics at the time of the 'Baseline' and 'Follow-Up' studies are described in Supplementary Table 1.

3.2 Relationship of Fractional Change in Disposition Index (ΔDI) with 'Baseline' nadir and fasting glucagon

Nadir glucagon in the Baseline experiments was not associated with ΔDI over the period of observation after adjustment for covariates (Supplementary Figure 1). 'Baseline' fasting glucagon was inversely correlated with ΔDI after adjustment for covariates. Spearman Correlation also produced similar results ($R = -0.28$, $p = 0.02$ – Figure 1).

The fractional change in insulin action (ΔS_I) was inversely correlated with 'Baseline' fasting glucagon (Supplementary Figure 2 – Panel A) but this was not true for β-cell responsivity (ΔΦ – Panel B).

3.3 Relationship of Δ 120 minute glucose with 'Baseline' fasting glucagon

'Baseline' fasting glucagon correlated with fractional change in 120 minute glucose after OGTT when adjusted for covariates (Figure 2). Spearman Correlation also produced similar results ($R = 0.28$, $p = 0.02$).

Post hoc we divided the cohort into subjects who experienced a >10 (symmetrical) % increase in 120 minute glucose over time versus the remainder. Baseline' fasting glucagon concentrations were higher in the subjects who had a > 10% increase in 2 hour glucose over time than those who had a ≤ 10% increase in 2 hour glucose ($p = 0.02$) during the 'Baseline' study (Supplementary Figure 3 – Panel A). This pattern was maintained during the 'Follow-Up' series of experiments ($p = 7 \times 10^{-4}$ – Panel B). The characteristics of the participants in each group as characterized by changes in their glucose tolerance are described in Supplementary Table 2.

4.0 Discussion

In this small longitudinal study, we demonstrate that fasting glucagon concentrations were inversely correlated with a subsequent decline in β-cell function and glucose tolerance. It is notable that the relationship of 'Baseline' fasting glucagon with longitudinal changes in DI

is not explained by a relationship with DI during the 'Baseline' study. Indeed, initial DI (as well as insulin action and β-cell responsivity) did not differ between subjects categorized by the presence or absence of worsening glucose tolerance (Supplementary Table 2). Taken together, these data suggest that α-cell function is altered early in the evolution of glucose dysregulation, prior to (and independently of [8]), defects in insulin secretion. This α-cell dysfunction is associated with longitudinal decline in β-cell function.

Glucagon secretion and α-cell function have been relatively ignored aspects of islet dysfunction in prediabetes. Indeed our study is relatively unique in the ability to characterize changes in post-(oral) challenge glucagon concentrations over time. However, there are limitations that need to be acknowledged.

The cohort is relatively small and the effect of glucagon on endogenous glucose production was not measured. Glucagon raises endogenous glucose production [13] especially in situations where insulin secretion is impaired [1, 2]. In the absence of tracer-based measures of glucose metabolism, we can only speculate that this is the underlying reason for the longitudinal decline in glucose homeostasis, since glucagon does not impair peripheral glucose uptake [1].

Although the group experiencing decline in glucose tolerance tended to be heavier and older at baseline, these differences were not statistically significant (Supplementary Table 2). In addition, the 120 minute glucose values at baseline were lower in the group who experienced worsening of glucose tolerance. Other factors including age, absolute weight and weight gain did not differ between subjects who did or did not have deterioration in their glucose tolerance as measured by 120 minute glucose.

An important limitation is that the glucagon assay used in this series of experiments cross-reacts with proglucagon-derived peptide fragments [14]. Therefore, it is possible that these, rather than fasting glucagon, are markers of future decline in islet function. However, despite differences in absolute concentrations of fasting and nadir glucagon there is good correlation between these assays and the assay we used in this experiment [14–17]. Nevertheless, future studies using immunoassays with greater specificity for glucagon will be needed to test this possibility.

A final consideration is that in the *post hoc* analysis grouped by change in glucose tolerance, there was a preponderance of male subjects (in the group experiencing a > 10% rise in 120 minute glucose). Men have consistently been shown to have higher fasting glucagon (and glucose) concentrations compared to women [8, 18, 19], however, baseline DI did not differ between sexes. Further study in larger, better characterized cohorts balanced for sex will be required to confirm this observation and provide mechanistic insights into how elevated fasting glucagon might contribute to a decline in β-cell function.

In conclusion, these data suggest that defects in α-cell function, manifest as elevated fasting glucagon, could drive subsequent decline in insulin action and overall β-cell function. These findings require replication in a larger, independent cohort that addresses the limitations of the current study prior to targeted manipulation of glucagon secretion or signaling in prediabetes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

DI	Disposition Index
mRNA	messenger Ribonucleic Acid
OGTT	Oral Glucose Tolerance Test
SD	Standard Deviation
SEM	Standard Error of the Mean
S_i	Insulin Action
Φ	Total β-cell responsivity to glucose
ϕ_s	Static β-cell responsivity to glucose
ϕ_d	Dynamic β-cell responsivity to glucose

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Highlights

- Genetic evidence implicates impaired glucagon suppression in diabetes risk
- Could higher glucagon concentrations predict beta-cell dysfunction?
- Over a 7-year period higher fasting glucagon predicted a decline in β-cell function
- This is independent of weight changes over the period of observation

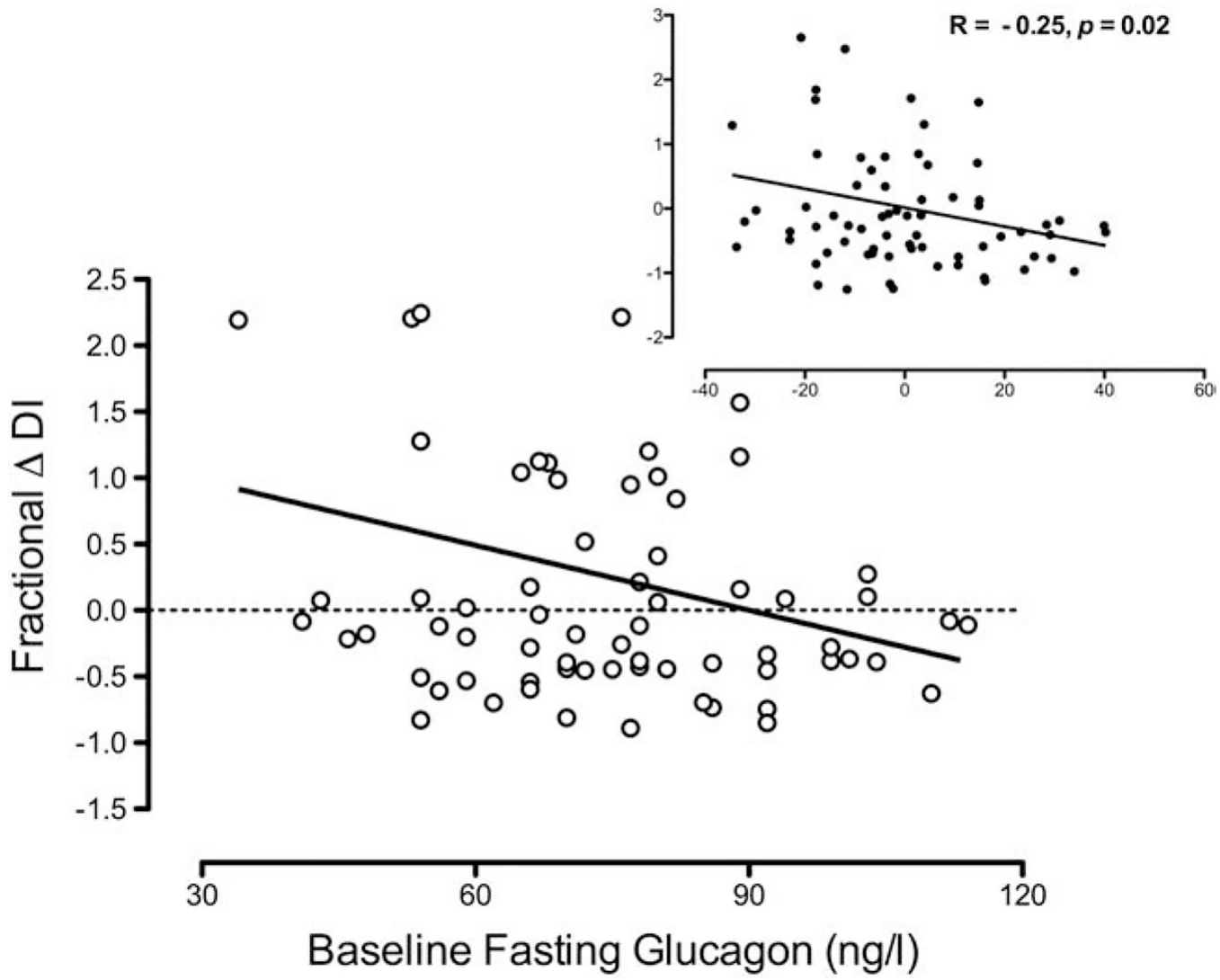

Figure 1:

Correlation of fractional change in Disposition Index (DI) in the 'Follow-Up' series of experiments with fasting glucagon concentrations in the 'Baseline' series of experiments. Inset panels represent residuals after adjusting for the covariates of weight change and time interval between studies.

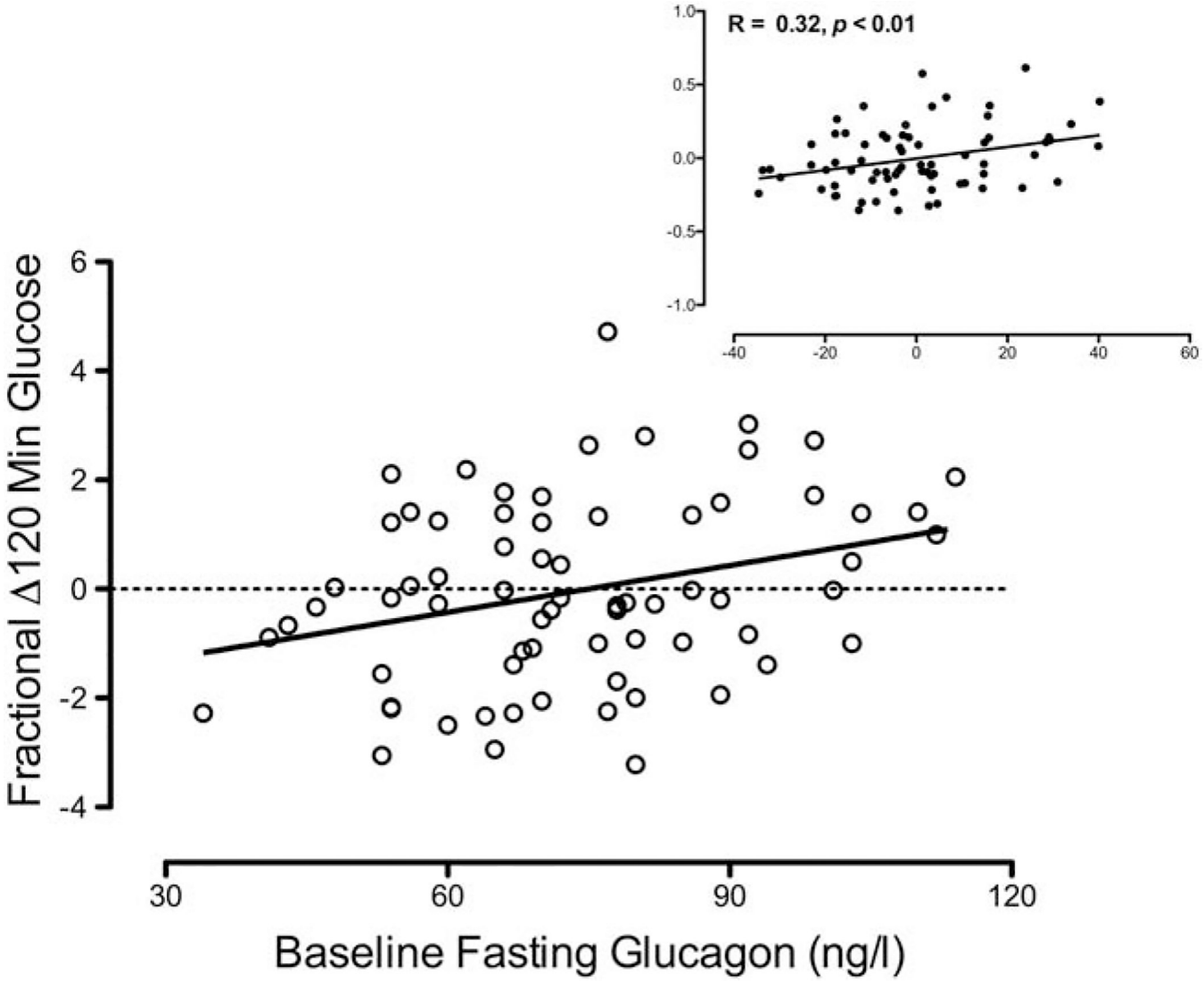

Figure 2:

Correlation of fractional change in 120 minute glucose in the 'Follow-Up' series of experiments with fasting glucagon concentrations in the 'Baseline' series of experiments. Inset panels represent residuals after adjusting for the covariates of weight change and time interval between studies.