Altered Glucagon Response to Oral Glucose in Individuals at Different Stages of Type 1 Diabetes Development

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Abstract

Context

Autoimmune destruction of beta cells and their functional decline precedes the clinical onset of type 1 diabetes. However, altered alpha-cell function and hyperglucagonemia may contribute to the development of hyperglycemia and ketoacidosis at onset.

Objective

In this cross-sectional study, we analyzed glucagon concentrations during an oral glucose tolerance test (OGTT) in individuals at early stages of type 1 diabetes to understand the role of alpha-cell function in the disease process.

Methods

We recruited 47 participants, aged 4–25 years, from the Finnish Diabetes Prediction and Prevention (DIPP) study, categorized them into the following groups: islet autoantibody (IAb) negative, single IAb positive, and stages 1-3 of type 1 diabetes. Glucagon levels were measured during a six-point OGTT using a conventional radioimmunoassay, alongside insulin, C-peptide, glucose and glucagon-like peptide-1 (GLP-1).

Results

Fasting plasma glucagon levels increased with disease progression. The longitudinal patterns of glucagon concentrations during the OGTT differed significantly between groups, with a paradoxical 15-minute glucagon increase observed only in individuals at early stage 3 of type 1 diabetes.

Conclusion

These findings highlight the need for prospective studies to further elucidate the role of alpha-cells in disease progression and support testing pharmacotherapies aimed at improving both alpha- and beta-cell functions during disease development.

Introduction

The progressive decline of beta-cell function in the pancreatic islets leads to type 1 diabetes resulting in lifelong dependency on exogenous insulin and glucose monitoring. This immune-mediated beta-cell impairment originates from islet inflammation and leads to the early development of autoantibodies (1,2). Based on islet autoantibodies (IAb) and glucose metabolism, type 1 diabetes progresses through distinct presymptomatic stages (3): stage 1, with two or more IAb and normoglycemia; stage 2, with multiple IAb and dysglycemia; and stage 3, meeting the diagnostic criteria for diabetes, which can be further subdivided into stage 3a and 3b, based on residual beta-cell function or disease duration (4).

Beyond beta-cell loss, alpha-cell dysfunction in diabetes was recognized over 50 years ago (5), with subsequent studies reporting glucagon excess in type 1 diabetes (6). In rodent models, deleting the glucagon receptor or inhibiting hyperglucagonemia prevents diabetes (7). Mechanistically, hyperglucagonemia in type 1 diabetes aligns with the intra-islet hypothesis, which suggests that intra-islet insulin inhibits glucagon release (8). Beta-cell loss and reduced insulin production may elevate glucagon, stimulating hepatic gluconeogenesis and glycogenolysis, increasing plasma glucose levels. Glucagon secretion is regulated by glucose, insulin, somatostatin from the delta-cells and incretins, including glucagon-like peptide-1 (GLP-1), glucagon-like peptide-2 (GLP-2), and glucose-dependent insulinotropic polypeptide (GIP), responding to plasma glucose, food intake, and exercise (6,9,10).

Compared to beta-cell function, alpha-cell dysfunction in early stages of type 1 diabetes is poorly understood. In healthy individuals, rising plasma glucose suppresses glucagon secretion via insulin and other intra-islet signals. In type 1 diabetes, glucagon regulation fails, potentially causing abnormal glucagon responses to hypoglycemia, glucose boluses or meals (11). Progressive hyperglucagonemia (12,13) has been observed after diagnosis, and abnormal alpha-cell function has been reported in individuals with dysglycemia as well as in IAb-positive adults (14,15). Previous studies have suggested that glucagon could be more involved in the disease process than previously acknowledged (16). Moreover, the genetic expression of α-cells is altered in type 1 diabetes, while the

gene expression profile remains normal in the remaining β-cells (17). However, whether glucagon secretion is affected in the early stages of diabetes remains unclear.

We hypothesized that the glucagon response to oral glucose is altered in the early stages of type 1 diabetes. Therefore, we aimed to elucidate glucagon concentration patterns in children and adolescents during the early stages of the disease.

Research Design and Methods

Study participants

Participants were recruited between June 2020 and November 2021 from the Finnish Type 1 Diabetes Prediction and Prevention (DIPP) study, a prospective birth cohort study (ClinicaTrials.gov no NCT3269084). Children with HLA-conferred risk for type 1 diabetes were followed regularly(18,19), with islet autoantibody (IAb) screening against insulin (IAA), glutamate decarboxylase (GADA), insulinoma-associated antigen 2 (IA-2A) and zinc transporter 8 (ZnT8) at 3, 6, 12, 18, and 24 months, and annually until age 15-(19–22). After seroconversion, visits occurred every 3 months (23). Individuals with ≥2IAb underwent oral glucose tolerance tests (OGTT) and HbA1c measurements every 3-6 months.

Participants were categorized into five groups based on OGTT and IAb results (Supplementary Figure 1 (31)), as previously described (24,25): 1) no IAb and normal glucose metabolism (0 IAb), 2) one IAb and normal glucose metabolism (1 IAb), 3) multiple IAb and normal glucose metabolism (stage 1), 4) multiple IAb and dysglycemia (stage 2), and 5) early stage 3 type 1 diabetes based on two consecutive OGTTs.

This substudy used the same cohort as Kontola et al. (24) on continuous glucose monitoring (CGM). Power calculations were based on mean evening glucose as the primary outcome. We excluded pregnant individuals and individuals with BMI, or ISO-BMI, greater than 35. In addition, prior to the OGTT, all individuals had to be asymptomatic, healthy, and not receiving any treatment that would affect glucose metabolism.

The study was approved by the Ethics Committee of the Hospital District of Northern Ostrobothnia, Oulu, Finland (T08/034/19). Written informed consent was obtained from participants and/or their guardians.

Laboratory analysis

Oral Glucose Tolerance Test (OGTT): Six-point OGTTs (23) were conducted between 8-10 AM with blood samples collected at 0, 15, 30, 60, 90, and 120 minutes. Participants fasted overnight (> 10 hours) before ingesting 1.75g/kg glucose (max 75 g for those > 43kg, Glucosepro, Mediq, Finland) within 5 minutes.

Biochemical Measurements: Plasma insulin, C-peptide and HbA1c were measured using electrochemiluminescence immunoassays (ECLIA, Roche Diagnostics, Basel, Switzerland) at Turku University Hospital: Glucose was analyzed enzymatically. HbA1c values were converted to % using the ADA converter (https://ngsp.org/convert1.asp).

Blood was collected into precooled EDTA tubes, kept on ice, centrifuged (10 min, 2000 x g, 4 °C) within 15 min, plasma aliquoted and stored at -20 °C or -80 °C for long-term storage.

Glucagon Measurement: Glucagon was quantified using a validated radioimmunoassay (EURIA-Glucagon, Euro Diagnostica AB, Sweden) at an accredited laboratory (Vita Laboratories, Helsinki, Finland). The assay correlates with WHO standard 69/194 and uses an antiserum (26,27) that specifically recognizes pancreatic glucagon (1-29) with low cross-reactivity (<0.1% with GLI/GLP-1; <0.7% with oxyntomodulin). Intra-assay CV ranged from 4.8-8.1%, total variation 3.9-8.3%, and recovery 97.6%. The assay has been validated against other methods and shows expected fasting glucagon levels (<60 pmol/L/ 206 ng/L) in healthy individuals and decreased responses during OGTT (28,29).

Islet autoantibodies (IAA, IA-2A, GADA) were analyzed at the DIPP and PEDIA laboratories as previously described (30). Glucagon, insulin, C-peptide, and active GLP-1 were also measured using a MILLIPLEX kit (HDIAB-34K-PMX5, RRID:AB 3717320, Sigma Aldrich, Germany) for comparison.

Statistical analysis

Changes in mean glucagon, C-peptide and insulin values over OGTT time points were compared using a linear mixed-effects model for repeated measurements, with time (within, categorical), stage (between), and their interactions as fixed effects. Participant was included as a random effect with a compound symmetry covariance structure. Analyses were performed using IBM SPSS Statistics version 29 (IBM Corp, Armonk, NY) and SAS v9.4 (SAS Institute Inc., Cary, NC). Figures were generated using GraphPad Prism v10.1.0 (GraphPad Software, Boston, Massachusetts).

Normality was assessed using D'Agostino Pearson and Shapiro-Wilk tests with QQ-plots, variance equality with Brown Forsythe test, and one-way ANOVA with Tukey's post hoc test was used for group comparisons; Fisher's exact test for analyzing frequencies. Skewed C-peptide and insulin values were log-transformed. Baseline glucose medians (Table 1) remained non-normal after transformation and were analyzed using Kruskall-Wallis test.

Results

Study participants

An overview of the study and participant classification is shown in Supplementary Figure 1 (31). The 47 participants (aged 4-25 years) were healthy and asymptomatic, with BMI or ISO-BMI within the normal range and no differences in pubertal status between the groups. Five individuals were over 18 years old, and their BMIs were between 21.7 and 26.7. They were not included in the ISO-BMI data in Table 1. Mean HbA1c ranged from 32 to 45 mmol/mol (5.1% 6.3%), increasing progressively from stage 1 to 3. It was lower in the 0 IAb and 1 IAb groups compared to stage 1. In stage 3 (pre-insulin treatment), the mean HbA1c was 45.1 mmol/mol (range: 39-53 mmol/mol; 5.7%-7.0%), consistent with very early stage 3 type 1 diabetes (Table 1). Information about comorbidities in addition to type 1 diabetes was collected. One individual in the stage 2 group had autoimmune hypothyroidism. Two individuals had celiac disease, one in stage 2 and one in stage 3. Four individuals had

asthma, one in each group except the 1 IAb group. All of these individuals were asymptomatic and had well-controlled treatment, and there were no statistically significant differences in disease frequencies between study groups.

Glucagon Concentration During OGTT

Modeled glucagon responses during OGTT across type 1 diabetes stages are shown in Figure 1. The glucagon trajectory over 2 hours differed significantly between groups ($p=0.0159$ for group-by-time interaction). Fasting glucagon was highest in stage 3, with a significant difference between stage 3 and 1 (mean difference 38 ng/L [95% CI 12.2-63.8 ng/L], $p<0.01$) (Table 2, Supplementary Table 1 (31)). In the linear mixed-effects model, glucagon concentrations were significantly higher at all time points in stage 3 compared to stage 1. The greatest glucagon increase occurred in the stage 3 group, with 7 out of 8 individuals showing an inappropriate rise at 15 minutes post-glucose bolus. At this time point, stage 3 differed significantly from all the other groups (Figure 1A, 1B, Supplementary Table 1 (31)). We also conducted a correlation analysis of glucagon and glucose level changes between consecutive timepoints, but no statistically significant correlations were found.

Glucose, Insulin and C-Peptide Responses During OGTT

Figure 2 presents glucose, insulin and C-peptide responses during the OGTT. The most notable differences were between stage 3 and all other groups. Pairwise comparison at all time points are presented in Supplementary Table 1 (31).

Glucose peaked at 60 minutes in Stage 2 and 90 minutes in stage 3, whereas in other groups it peaked at 30 minutes (Supplementary Table 2 (31)). Insulin and C-peptide were lower in stage 2 and 3 (Figure 2 and Supplementary Tables 1-4 (31)). Glucagon-to-insulin ratio was highest in stage 3 at all timepoints, with significant differences compared to the 0 IAb and 1 IAb groups at 30 minutes, and between stage 2 and 3 at 60 and 90 minutes (Supplementary Table 5 (31), Figure 2).

GLP-1 Concentrations During OGTT at Different Stages of Type 1 Diabetes

To explore whether GLP-1 contributed to glucagon dysregulation, we assessed active GLP-1 concentrations during OGTT. Levels were highest in the 0 IAb and 1 IAb groups, but no significant differences were observed between groups ($p = 0.36$, Supplementary Figure 2, Supplementary Table 6 (31)). A positive correlation between GLP-1 and glucagon was found at 30 ($r = 0.70$, $p = 0.01$) and 60 minutes ($r = 0.72$, $p = 0.020$) in stage 1, and at 60 ($r = 0.80$, $p = 0.01$) and 120 minutes ($r = 0.65$, $p = 0.04$) in stage 2.

Conclusions

While beta-cell decline is central to type 1 diabetes, this study highlights that increased plasma glucagon levels may contribute to early disease stages, offering insights into pathogenesis and potential therapeutic targets. Using OGTT to simulate real-world postprandial metabolism, we identified early abnormalities in glucagon regulation. To our knowledge, plasma glucagon responses during OGTT have not been examined at such early prediabetes stages.

A paradoxical rise in glucagon occurred in asymptomatic individuals at early stage 3 type 1 diabetes, before insulin therapy. Mild fasting hyperglucagonemia was also observed prior stage 3, suggesting inappropriate glucagon secretion begins before clinical disease onset. Simultaneous decreases in insulin secretion in stage 2 and loss of the early insulin peak in stage 3 were evident. The reduced insulin response in stages 2 and 3 can lead to blunted or delayed glucagon suppression resulting in hyperglucagonemia. According to the intra-islet insulin hypothesis, reduced insulin indirectly promotes glucagon release via alpha-cell dysregulation. Hyperglucagonemia was defined in relation to the other stages and/or values exceeding normal range (>209 ng/L). In individuals with newly diagnosed type 1 diabetes, hyperglucagonemia has also been reported, with exaggerated mixed-meal responses worsening over time (13), supporting the bihormonal hypothesis that both insulin deficiency and hyperglucagonemia contribute to hyperglycemia progression (32).

Our results also support the notion that hyperglucagonemia is not simply driven by exogenous insulin. Kramer et al. reported a paradoxical increase in glucagon following a glucose bolus in individuals with longstanding type

1 diabetes, which was not corrected by restoring euglycemia prior to testing (33). Furthermore, glucagon response remains abnormal even after the restoration of local insulin secretion (34).

Hyperglucagonemia and impaired postprandial glucagon suppression, well-established in type 2 diabetes, may involve liver dysfunction affecting the liver-alpha cell axis (35). In type 1 diabetes, reduced insulin and increased somatostatin (36) as well as intrinsic alpha-cell changes or disrupted paracrine regulation (37), may contribute to glucagon dysregulation, but these mechanisms were not evaluated in our study.

The cause of alpha-cell dysfunction remains unclear. Is it a consequence of beta-cell autoimmunity, disrupted alpha-beta-delta-cell interactions, hyperglycemia, reduced intra-islet insulin, or impaired incretin signaling? In our study, paradoxical glucagon increases were seen only in early stage 3 individuals with the lowest insulin levels, emphasizing the role of intra-islet insulin in glucagon regulation. Moreover, our study revealed that changes in glucagon levels during OGTT do not appear in IAb positive individuals unless they have already developed dyslgycemia or type 1 diabetes. This would indicate that alpha-cell dysfunction is not caused by islet autoimmunity alone. Previous animal studies have demonstrated, that glucagon stimulates insulin secretion through glucagon- and GLP-1 receptors located in the beta cell surfaces (38). While hyperglucagonemia in newly diagnosed patients has been attributed to inadequate insulin therapy (9), our findings show inappropriate glucagon responses and mild fasting hyperglucagonemia before insulin initiation. On the other hand, in individuals with established type 1 diabetes, postprandial elevations in glucagon appear independent of residual beta-cell function, as shown by similar glucagon responses regardless of disease duration or fasting C-peptide levels(39). In healthy individuals, glucagon secretion is suppressed in response to an increase in insulin secretion leading to normal glucose homeostasis. In our study, glucagon levels increased from the 0-15 minute timepoint in individuals with asymptomatic type 1 diabetes. Could our finding be explained by the attempt to stimulate insulin via glucagon secretion (38,40)? Could this also explain the positive correlation between GLP-1 and glucagon secretion in stages 1 and 2?Beyond insulin and hyperglycemia, incretins such as GLP-1, regulate alpha- and beta-cells simultaneously(41). In our study, similar to prior studies, GLP-1 levels did not differ statistically significantly

between groups (42,43). A trend toward lower GLP-1 levels during OGTT was observed in individuals at stages 1, 2 and 3, suggesting a possible decline in GLP-1 as type 1 diabetes progresses and individuals develop multiple IAbs. A preserved incretin response in the 0 and 1 IAb groups was detected. To clarify whether GLP-1 is altered early in the disease process during OGTT, larger studies in early-stage individuals with normal glucose metabolism are warranted. However, diminished incretin effect has been demonstrated in individuals with type 2 diabetes even in physiological levels of GIP and GLP-1 (44).

This study has several limitations. The relatively small cohort may limit statistical power and warrants replication in larger studies. We would also like to acknowledge the exploratory nature of these glucagon findings, because glucagon was a secondary outcome in our power calculations. Multiple groups and time points increase the risk of Type 1 error, though predefined contrasts were used. All participants had a high genetic risk of type 1 diabetes, potentially limiting generalizability. The cross-sectional design precludes conclusions on disease progression. Finally, glucagon was measured via conventional radioimmunoassay (RIA), which is less sensitive than newer enzyme-linked immunosorbent assays (ELISA) and may have reduced our ability to detect subtle group differences (28,29).

In summary, we show that paradoxical glucagon responses occur before the clinical onset of diabetes and initiation of insulin treatment. Consistent with previous studies, fasting glucagon levels are modestly elevated in stage 2 compared to earlier stages (15). These findings underscore the need for prospective studies to clarify the role of alpha-cells and GLP-1 secretion in early disease and support the exploration of therapies targeting both alpha- and beta-cell function in the development of type 1 diabetes.

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Authors' Contributions

H.K., J.K., J.T., and R.V. designed the study, H.K., L.C., E.L., and J.K. collected and analyzed the data. H.K. and J.K. wrote the article. H.K., L.C., E.L., J.T., R.V., J.J.K., and J.K. reviewed/edited the article. Guarantor of this article is J.K.

Author Disclosure Statement

The authors state no conflict of interest.

Data and resource availability

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

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Demographics of study participants

Group	Total	0 IAb	1 IAb	Stage 1	Stage 2	Stage 3	P-value
Gender N (Male)	46(16)	8(1)	6(2)	12(6)	12(3)	8(4)	0.38
Age (years) (range)	11.7 (3.9-25.4)	9.3 (3.9-15.2)	10.8 (7.4-13.1)	11.3 (5.1-25.4)	14.1 (4.0-20.2)	11.6 (4.5-19.7)	0.30
HbA1c (mmol/mol)	36.76(5.45)	35.83(3.19)*	35.00(2.83)*	32.45(2.02)*	36.40(3.24)*	45.13(5.00)	<0.0001
HbA1c (%)	5.5(0.5)	5.4(0.3)*	5.4(0.2)*	5.1(0.2)*	5.5(0.3)*	6.3(0.5)	<0.0001
Time from seroconversion (years) (range)	7.5 (0.0-20.9)	No Islet autoantibodies	5.6 (0.4-11.4)	7.9 (2.0-20.9)	7.8 (0.8-15.0)	8.0 (0.0-16.3)	0.79
ISO-BMI**	22.40(2.66)	22.05(2.66)	22.30(2.95)	22.80(3.28)	22.46(2.31)	21.83(3.12)	0.99

Table 1. Data are shown as mean and SD unless stated otherwise. The 0 IAb and 1IAb mean participants with 0 and 1 islet autoantibodies respectively. Stage 1 individuals have two or more islet autoantibodies and normal glucose tolerance, stage 2 have two or more islet autoantibodies and dysglycemia, and stage 3 participants have early asymptomatic type 1 diabetes. P-values indicate the ANOVA results from testing group means. *Indicates a statistically significant difference ($p<0.005$) between the denoted group and stage 3. ISO-BMI is used to evaluate children's and adolescents' BMI instead of regular BMI. ISO-BMI takes gender and age into account. Regular BMI was used for participants over 18 years of age, their BMIs were between 21.7 and 26.7. and they were not included in this table.

Table 2.

Glucagon means with 95% CI during OGTT (ng/L)

Groups Time	0 IAb	1 IAb	Stage 1	Stage 2	Stage 3
0 min.	166.0 (145.7-186.3)	165.7 (142.6-188.7)	154.8 (138.5-171.1)*	177.5 (161.2-193.8)	192.8 (172.8-212.7)
15 min.	152.6 (132.6-172.6)*	162.5 (139.5-185.6)*	146.1 (129.6-162.6)*	167.5 (151.2-183.8)*	212.5 (192.5-232.5)
30 min.	142.6 (122.7-162.6)*	146.5 (123.5-169.6)*	139.7 (123.4-156.0)*	161.9 (145.6-178.2)	183.3 (163.3-203.2)
60 min.	145.1 (125.2-165.1)	142.3 (119.3-165.4)	137.0 (120.7-153.3)*	149.0 (132.7-165.3)	169.0 (149.0-189.0)
90 min.	143.3 (123.3-163.2)*	146.8 (123.8-169.9)	136.7 (120.3-153.2)*	150.2 (134.0-166.5)	174.9 (154.9-194.8)
120 min.	147.0 (127.0-167.0)	146.0 ((123.0-169.1)	140.3 (124.0-156.6)*	148.5 (132.2-164.8)	170.9 (150.9-190.8)

Time*Group P=0.0159

Table 2.

Glucagon means with 95% CI during OGTT from linear mixed effects model analysis. The 0 IAb and 1IAb refer to subjects with 0 and 1 islet autoantibodies respectively. Stages 1, 2 and 3 represent different stages of type 1 diabetes. Stage 1 has two or more islet autoantibodies and normal glucose tolerance, stage 2 has two or more islet autoantibodies and dysglycemia, and stage 3 subjects have early asymptomatic type 1 diabetes. *Indicates a statistically significant difference between Stage 3 and denoted group. All pairwise comparison p-values are presented in Supplement Table 1 (31).

Figure 1. Glucagon response during the oral glucose tolerance test (OGTT) in participants at various early stages of type 1 diabetes

Panel A displays the means with a 95% CI for glucagon (ng/mL) during the OGTT at distinct time points. Participants with one islet autoantibody (IAb) are represented by light green circles, while those without any (0IAb) are shown with dark green circles. Stages 1-3 of type 1 diabetes are depicted as follows: yellow circle for stage 1, light red for stage 2, and red for stage 3 individuals. "N" denotes the number of participants. Panel B shows covariance analysis results of the glucagon difference between 0 and 15-minute timepoints. The mean difference is shown by black lines and raw values are indicated by colored dots. Panel C and D show individual raw glucagon values during OGTT at baseline before oral glucose bolus and at 15 minutes after the glucose bolus, respectively. The stage 3 group consists of asymptomatic participants at the time of diabetes diagnosis, before the start of insulin treatment. The p-value indicates the statistically significant difference between groups over time as analyzed by the linear mixed model. The asterisk (*) in panel A indicates a statistically significant difference between stage 3 and the 0 IAb group during the OGTT in linear mixed effects model analysis. The asterisk (*) in panel B indicates a statistically significant difference between stage 3 and the denoted group.

Figure 2. Glucose, Insulin, and C-peptide levels and glucagon insulin ratio during OGTT in participants at various early stages of type 1 diabetes

Means of (A) glucose (mmol/L), (B) C-peptide and (C) insulin levels, and (D) glucagon-to-insulin raw value ratio during the OGTT. Means were analyzed using linear mixed effects model. P-values indicate statistically significant differences between groups over time. Error bars show 95% CI, with colored circles representing means. Light green circles represent participants with one islet autoantibody (1 IAb), while those without any (0 IAb) are shown with dark green circles. The stages 1-3 of type 1 diabetes are depicted as follows: yellow circle for stage 1, light red for stage 2, and red for stage 3 individuals. The asterisk (*) in panels A, B and C indicates a statistically significant difference between the stage 3 and 0 IAb groups during the OGTT. In panel D, the asterisk (*) indicates a statistically significant difference between group means. "N" denotes the number of

participants. More in-depth analysis results are shown in Table 2, with additional parameters presented in Supplementary Tables 1, 2, 3, 4 and 5 (31).

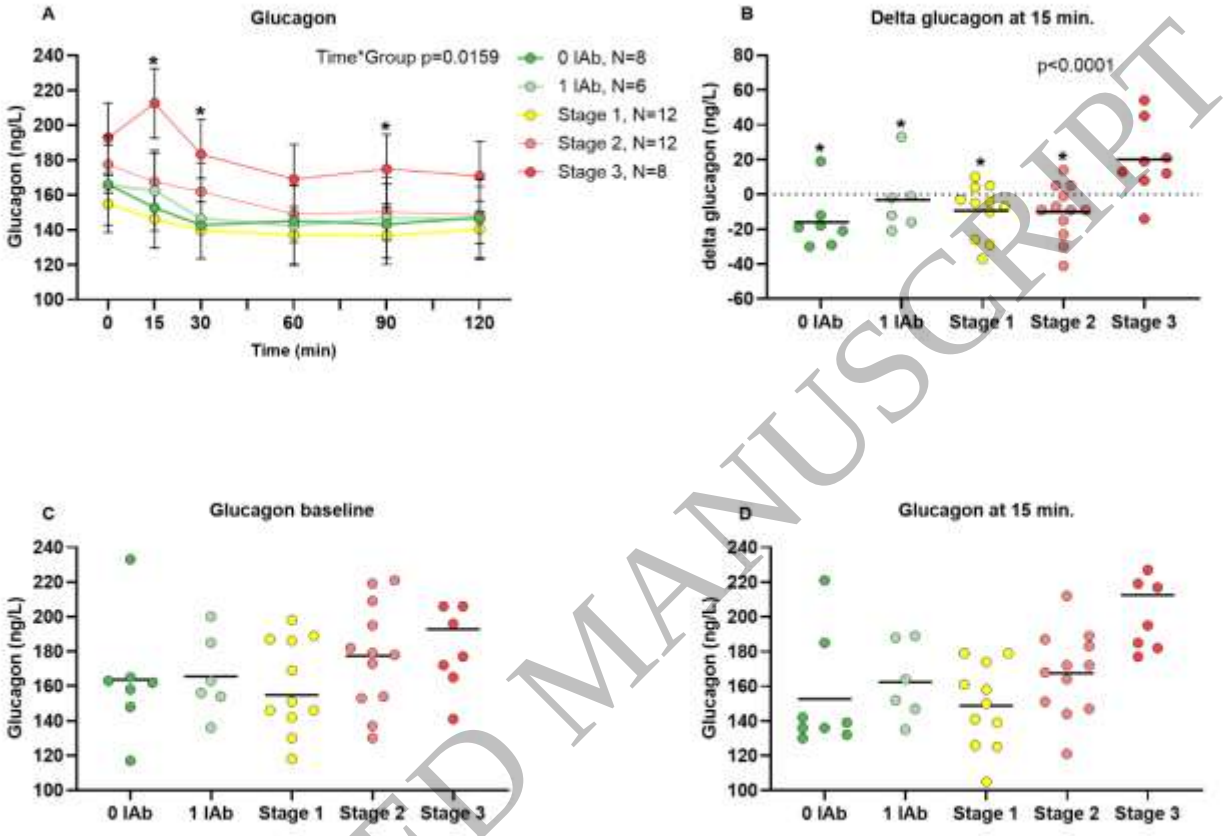

Figure 1
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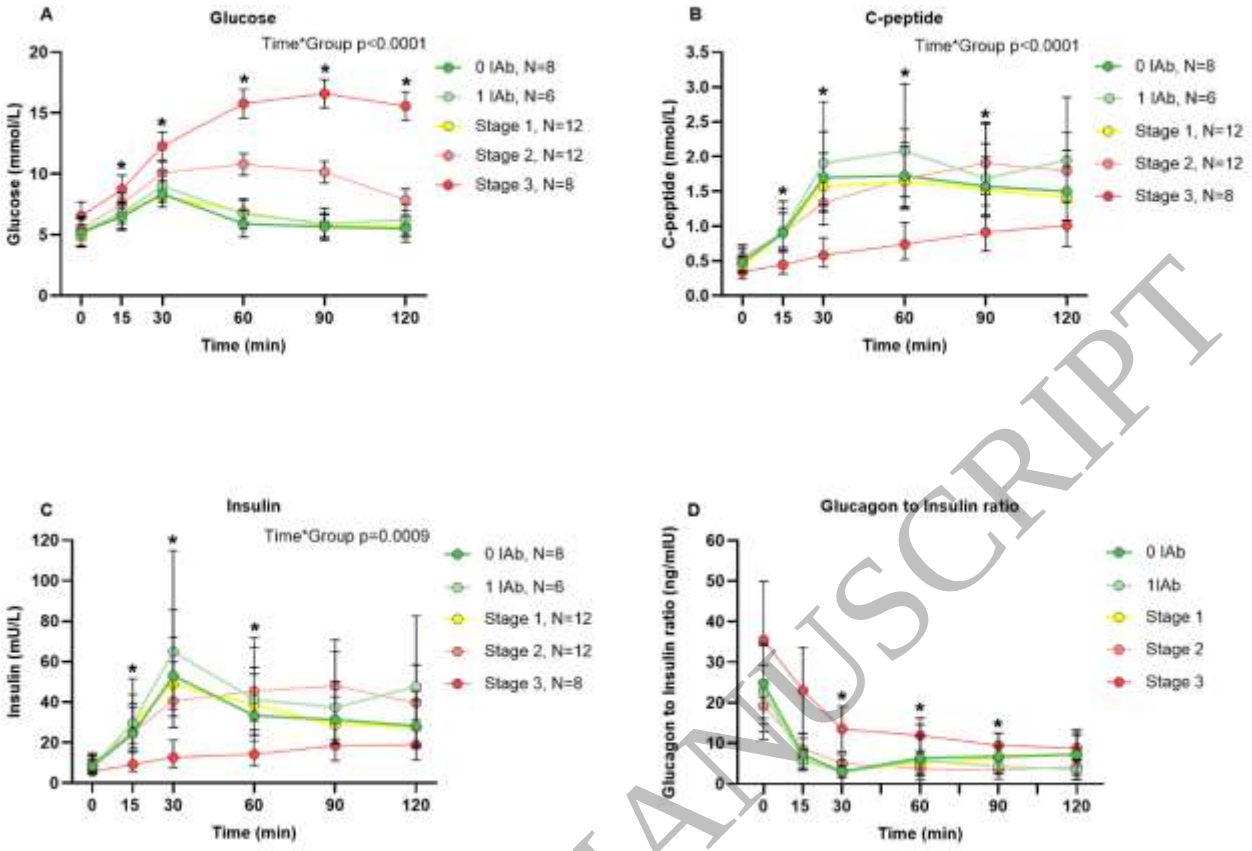

Figure 2
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