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Clinical Research Article

Hyperglucagonemia in Pediatric Adiposity Associates With Cardiometabolic Risk Factors but Not Hyperglycemia

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Abstract

Context: In adults, hyperglucagonemia is associated with type 2 diabetes, impaired glucose tolerance, and obesity. The role of glucagon in pediatric overweight/obesity remains unclear.

Objective: We examined whether fasting concentrations of glucagon are elevated in youth with overweight/obesity and whether this associates with cardiometabolic risk profiles.

Methods: Analyses were based on the cross-sectional HOLBAEK study, including children and adolescents 6 to 19 years of age, with overweight/obesity from an obesity clinic group (n = 2154) and with normal weight from a population-based group (n = 1858). Fasting concentrations of plasma glucagon and cardiometabolic risk outcomes were assessed, and multiple linear and logistic regressions models were performed.

Results: The obesity clinic group had higher glucagon concentrations than the population-based group ($P < 0.001$). Glucagon positively associated with body mass index (BMI) standard deviation score (SDS), waist, body fat %, liver fat %, alanine transaminase (ALT), high-sensitivity C-reactive protein, homeostasis model assessment of insulin resistance, insulin, C-peptide, LDL-C, triglycerides, SDS of diastolic and systolic blood pressure, and was inversely associated with fasting glucose. The inverse relationship between glucagon and glucose was attenuated in individuals with high BMI SDS and high fasting insulin. Glucagon was associated with a higher prevalence of insulin resistance, increased ALT, dyslipidemia, and hypertension, but not with hyperglycemia. Glucagon was positively associated with fasting total glucagon-like peptide-1.

Conclusion: Compared with normal weight peers, children and adolescents with overweight/obesity had elevated concentrations of fasting glucagon, which corresponded to worsened cardiometabolic risk outcomes, except for hyperglycemia. This suggests hyperglucagonemia in youth may precede impairments in glucose regulation.

Key Words: adolescent, cardiometabolic risk, child, glucagon, hyperglycemia, obesity

Abbreviations: ALT, alanine aminotransferase; BMI, body mass index; BP, blood pressure; GLP-1, glucagon-like peptide-1; HbA_{1c}, glycated hemoglobin A_{1c}; HDL-C, high-density lipoprotein-cholesterol; HOMA-IR, homeostatic model assessment of insulin resistance; hs-CRP, high-sensitivity C-reactive protein; IQR, interquartile range; LDL-C, low-density lipoprotein-cholesterol; LLOQ, lower limit of quantitation; OR, odds ratio; SDS, standard deviation score.

Glucagon opposes the glucose-lowering actions of insulin and stimulates hepatic glucose production (1). Glucagon also mediates several nonglucose-related metabolic effects, including regulation of amino acid metabolism (ureagenesis) (2); stimulation of insulin secretion (3); break down of fatty acids and lipogenesis inhibition in the liver (4); potential reduction of food intake (5); increased energy expenditure (6); and possibly regulation of heart rate and contractibility (7), although the latter effects may not be physiological. The regulation of glucagon secretion is complex, involving a combination of paracrine, endocrine, nutritional, and autonomic factors (1). Inhibitors of glucagon secretion have been reported to include beta cell–derived factors such as amylin, insulin, and zinc; delta cell–derived somatostatin; alpha cell–derived glucagon feedback; gastrointestinal peptides, such as glucagon-like peptide-1 (GLP-1); and metabolic factors, including fatty acids and glucose. Conversely, stimulators of glucagon release may include amino acids such as alanine, glutamine, and tyrosine;

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gut-derived factors such as glucose-dependent insulinotropic polypeptide (GIP), oxyntomodulin, and ghrelin; as well as parasympathetic/sympathetic controlled mechanisms (8).

Hyperglucagonemia associates with numerous cardiometabolic risk factors and contributes to the hyperglycemic state in adults with type 2 diabetes (8). A recent study, which employed soft clustering of 32 clinical phenotypes in recently diagnosed adults with type 2 diabetes, revealed distinctive archetypes, wherein the archetype with obesity and insulin resistance associated with lower physical activity, higher subcutaneous and visceral fat, liver fat, fasting glucagon, and GLP-1, compared with the lean and insulin deficient archetype, which showed the opposite trends (9). Higher fasting concentrations of glucagon are also present in adults with obesity and normal glucose tolerance, suggesting that hyperglucagonemia manifests early in the development of glucose intolerance (10). Less is known regarding the role of glucagon in pediatric obesity (11-16); however, the appearance of higher fasting glucagon may likewise proceed deteriorations in glucose tolerance (15). Importantly, signs of hyperglucagonemia in early life could be a central indicator of obesity-related complications.

In the present study, we aimed to assess whether fasting plasma glucagon associates with cardiometabolic risk outcomes in a cross-sectional study of 4012 children and adolescents, aged 6 to 19 years, recruited to an obesity clinic or from the general population in Denmark. We examined whether these associations were modified by overweight/obesity and depend on fasting insulin concentrations. Lastly, we examined whether glucagon is associated with fasting GLP-1. We hypothesized that glucagon will be elevated in youth with overweight/obesity when compared to normal weight, and this will be indicative of worsened cardiometabolic risk profiles and will associate with fasting GLP-1.

Methods

Study Populations

The present study is based on data from The HOLBAEK Study, previously known as The Danish Childhood Obesity Biobank (17, 18). Two groups of children and adolescents were included: (1) an obesity clinic group (n = 2555), the members of which followed a multidisciplinary childhood obesity management program at Holbæk Hospital (17) and had a body mass index (BMI) > 90th percentile (BMI standard deviation score [SDS] > 1.28) according to Danish reference values (19); and (2) a population-based group (n = 2734), recruited from schools across 11 municipalities in Zealand, Denmark (18). Both groups were enrolled into The HOLBAEK Study between August 2007 and April 2019.

The exclusion criteria were: (1) age younger than 6 or older than 19 years (n = 177); (2) ethnicity other than Danish or North-European (self-reported country of origin and ancestry, n = 481); (3) diagnosed type 1 diabetes (n = 13); (4) diagnosed type 2 diabetes (n = 4); (5) treatment with medications including insulin, liraglutide, or metformin (n = 17); and (6) meeting type 2 diabetes criteria (20) based on the blood sample taken for this study (fasting plasma glucose > 7.0 mmol/L and/or glycated hemoglobin A_{1c} [HbA_{1c}] > 48 mmol/mol, n = 7). Additionally, individuals from the population-based group were excluded if they were underweight, defined as BMI < 10th percentile (BMI SDS < –1.28, n = 136) or had overweight/obesity defined as BMI > 90th percentile (BMI SDS > 1.28, n = 442) (19). After applying the exclusion criteria, 2154 remained in the obesity group and 1858 in the population-based group.

Ethics

According to the Declaration of Helsinki, informed consent was obtained from all participants. Written consent was obtained either from parents/legal guardians of participants younger than 18 years, or from the participants when 18 years or older. The study was approved by the Scientific Ethics Committee of Region Zealand, Denmark (protocol no. SJ-104) and by the Danish Data Protection Agency.

Anthropometrics and Blood Pressure

In the obesity clinic group, anthropometrics (height, weight, and waist circumference) and blood pressure (BP) were obtained as a part of a clinical examination, whereas the population-based group was assessed in a mobile laboratory by trained medical professionals, as previously described (21). BMI standard deviation scores were calculated according to a Danish reference (19). Mean values for the last 2 measurements of BP were calculated and converted to BP SDS based on age-, sex-, and height-specific reference values from the American Academy of Pediatrics (22).

Puberty Stage

In the obesity clinic, Tanner stage (23, 24) was evaluated by a pediatrician. In the population-based group, Tanner stage was self-evaluated using a questionnaire with picture pattern recognition. Self-assessment has been shown to accurately distinguish between the stages of prepuberty and puberty (25). Consequently, puberty stage in the obesity clinic (n = 1719) and population-based (n = 1341) was defined as either prepubertal (Tanner stage 1) or pubertal (Tanner stage 2-5) to make measures comparable.

Dual-Energy X-Ray Absorptiometry

Whole-body dual-energy x-ray absorptiometry (DXA) was performed and body fat % was quantified in a subset from both the obesity clinic (n = 1885) and population-based (n = 207) groups, using a GE Lunar Prodigy (DF+10031, GE Healthcare, Madison, Wisconsin, USA) until October 2009 and thereafter on a GE Lunar iDXA (ME+200179, GE Healthcare), as previously described (26).

Proton Magnetic Resonance Spectroscopy

Proton magnetic resonance spectroscopy (^{1}H-MRS) was performed and liver fat % was quantified in a subset of both the obesity clinic (n = 544) and population-based (n = 98) groups, using a 3T Achieva MR imaging system (Philips Medical Systems, Best, Netherlands), as previously specified (27).

Biochemical Analyses

Venous blood samples were collected from 7 to 9 am following an overnight fast of at least 8 hours. Fasting biochemical measurements described previously by our group include plasma alanine transaminase (ALT) (28), serum high-sensitivity C-reactive protein (hs-CRP) (29), serum insulin, serum C-peptide, plasma glucose, whole blood HbA_{1c} (30), plasma high-density lipoprotein cholesterol (HDL-C), plasma low-density lipoprotein cholesterol (LDL-C), plasma triglycerides (31), and plasma total GLP-1 (21).

To measure glucagon, blood samples were drawn in ice-cold EDTA tubes, separated by centrifugation within 20 minutes, and stored immediately at −80 °C until further analyses. Plasma glucagon was measured using the Mercodia Glucagon ELISA (RRID: AB_2737304, https://scicrunch.org/resources/Any/search?q=AB_2737304&l=AB_2737304, Cat. No. 10-1271-01, Uppsala, Sweden) (32). The assay was performed in duplicate and read on a SpectraMax iD3 (San Jose, CA, USA). The standard curve for glucagon had a range of 1.5 to 130 pmol/L, with a lower limit of quantification (LLOQ) of 0.75 pmol/L. Values below the LLOQ (n = 42) were assigned half the LLOQ (0.375 pmol/L). The interassay CV was 12.2% and the intra-assay CV was 12.1%.

Defining Cardiometabolic Risk Features

Insulin resistance was defined as homeostasis model assessment of insulin resistance (HOMA-IR) values above the 90th percentile for age and sex, based on the obesity clinic and population-based groups (30). HOMA-IR was calculated as insulin mU/L × glucose mM/22.5. Hyperglycemia was defined as fasting plasma glucose between 5.6 and 6.9 mmol/L or HbA_{1c} between 39 and 47 mmol/mol, according to the American Diabetes Association guideline for diabetes (20). Increased ALT was defined as fasting plasma ALT concentrations > 24.5 U/L in girls and > 31.5 U/L in boys, which was determined to be the optimal cutoff for identifying hepatic steatosis by our group (liver fat content of > 5% measured by proton magnetic resonance spectroscopy (^{1}H-MRS) in 458 children and adolescents) (28). Dyslipidemia was defined as values above the 95th percentile according to pediatric guidelines, corresponding to total cholesterol > 200 mg/dL (5.2 mM), LDL-C > 130 mg/dL (3.4 mM), triglycerides > 100 mg/dL (1.1 mM) for 0 to 9 years or > 130 mg/dL (1.5 mM) for 10 to 19 years of age, or HDL-C < 40 mg/dL (1.0 mM) (33). Hypertension was defined as a systolic or diastolic BP above the 95th percentile for age and sex, based on pediatric guidelines (34).

Statistical Analyses

Statistical analyses were performed in R version 4.1.2 (35). Normality of parameter distributions were evaluated. Data were reported as median (interquartile range [IQR]) for nonparametric variables and frequencies and percentages for categorical variables. The 2 groups were compared using Wilcoxon rank sum tests for continuous variables and χ^2 test for categorical variables. Statistical significance was set at $P < 0.05$.

Age- and sex-specific percentile curves for glucagon were calculated using the "Generalized Additive Models for Location, Scale and Shape" R package (https://cran.r-project.org/web/packages/gamlss/), using the Box-Cox transformation distribution family to account for skewness, with the best fit determined by the Akaike Information Criterion (36).

Linear regression models were used to evaluate associations between fasting glucagon as an indicator of cardiometabolic risk factors. Nonnormally distributed (right-skewed) cardiometabolic risk factors were naturally log-transformed. Estimated β-effect sizes and 95% CI were reported as the SD change in cardiometabolic risk factors per SD change in fasting glucagon to facilitate direct comparisons of the strength of associations. The obesity clinic and population-based groups were pooled. Potential covariates were assessed, and the following pooled models were performed Model 1: adjusted for age, sex, and BMI SDS; Model 2: Model 1 + puberty stage; and Model 3: Model 1 + fasting insulin. Model 2 was performed in a subset (76 % individuals) with available information on puberty stage. We were also interested in whether the relationship between fasting glucagon and cardiometabolic risk factors was modified by group, so a 2-way interaction model was applied (glucagon × group [obesity clinic vs population-based]), adjusted for age and sex. We further evaluated the relationship between fasting glucagon and fasting glucose, stratified by BMI quartiles, applying an interaction model (glucagon × insulin [high vs low]), adjusted for age and sex.

Logistic regression models were used to examine the relationship between glucagon as an indicator of cardiometabolic risk features (0/1), using a similar approach as the linear regression models for cardiometabolic risk factors.

To explore the relationship between glucagon as an indicator of total GLP-1, linear regression models were applied stratified by group and sex, adjusted for age and BMI SDS. Again, because the data did not meet the requirement for a normal distribution of model residuals, total GLP-1 was log-transformed prior to analysis.

Results

Characteristics of the Study Groups

The descriptive characteristics of 4012 individuals from the obesity clinic and population-based groups are provided in Table 1. There were no significant differences in age between groups. There were more boys in the obesity clinic group than in the population-based group and a higher percentage of individuals were in the prepubertal stage (both $P < 0.001$). Patients in the obesity clinic group had higher BMI SDS, waist, body fat %, liver fat %, fasting levels of ALT, hs-CRP, HOMA-IR, insulin, C-peptide, glucose, HbA_{1c}, LDL-C, triglycerides, SDS of diastolic and systolic BP, and lower HDL-C, than participants in the population-based group (all $P < 0.001$). Patients in the obesity clinic group exhibited worse cardiometabolic risk profiles, with a higher prevalence of insulin resistance, hyperglycemia, increased ALT, dyslipidemia, and hypertension than participants in the population-based group (all $P < 0.001$).

Patients in the obesity clinic group had higher fasting plasma glucagon concentrations (median 7.8; IQR, 5.5-10.8 pmol/L) than participants in the population-based group (median 5.5; IQR, 3.9-7.7 pmol/L; $P < 0.001$). Age- and sex-specific values for fasting glucagon are illustrated with 5th, 50th, and 95th percentile curves for the respective groups (see Supplementary Figure 1) (37).

Associations of Fasting Glucagon as an Indicator of Cardiometabolic Risk Factors

Fasting glucagon was positively associated with BMI SDS, waist, body fat %, liver fat %, ALT, hs-CRP, HOMA-IR, insulin, C-peptide, LDL-C, triglycerides, and diastolic and systolic BP SDS, and it was inversely associated with glucose, but fasting glucagon was not significantly associated with HbA_{1c} or HDL-C (Fig. 1 and Supplementary Table 1, Model 1) (37), adjusted for age, sex, and BMI SDS (except for BMI SDS, waist, and body fat % which were adjusted for age and sex only). The associations to cardiometabolic risk factors remained consistent when further adjusted for puberty stage, except for a

Table 1. Characteristics of the obesity clinic and population-based groups

Characteristic	n	Obesity clinic	n	Population-based	*P*
Age, years	2154	11.8 (9.6, 14.2)	1858	11.8 (9.0, 14.9)	0.83
Sex, boys, n (%)	2154	987 (45.8)	1858	749 (40.3)	< 0.001
Puberty stage, prepubertal, n (%)	1719	693 (40.3)	1341	441 (32.9)	< 0.001
Fasting plasma glucagon, pmol/L	2154	7.8 (5.5, 10.8)	1858	5.5 (3.9, 7.7)	< 0.001
Cardiometabolic risk factors					
BMI SDS	2154	2.86 (2.46, 3.31)	1858	0.11 (-0.41, 0.62)	< 0.001
Waist, cm	2070	91.0 (82.0, 102.0)	1844	64.0 (59.0, 71.0)	< 0.001
Body fat, %	1885	43.6 (40.2, 46.9)	207	24.4 (20.9, 29.5)	< 0.001
Liver fat, %	544	1.0 (0.5, 2.0)	98	0.5 (0.5, 0.5)	< 0.001
Plasma ALT, U/L	2106	23.0 (18.0, 31.0)	1819	20.0 (16.0, 23.0)	< 0.001
Serum hs-CRP, mg/L	1176	1.3 (0.5, 2.9)	548	0.4 (0.2, 0.8)	< 0.001
HOMA-IR, mIU/L	2049	3.8 (2.5, 5.6)	1812	2.0 (1.4, 2.8)	< 0.001
Serum insulin, pmol/L	2108	100.9 (68.2, 144.3)	1842	55.2 (39.3, 74.3)	< 0.001
Serum C-peptide, nmol/L	2056	0.8 (0.6, 1.1)	1836	0.5 (0.4, 0.7)	< 0.001
Plasma glucose, mmol/L	2055	5.0 (4.8, 5.3)	1826	5.0 (4.7, 5.2)	< 0.001
Whole blood HbA_{1c}, mmol/mol	2097	34.0 (32.0, 36.0)	1815	34.0 (32.0, 35.0)	< 0.001
Plasma HDL-C, mmol/L	2092	1.2 (1.0, 1.4)	1814	1.5 (1.3, 1.7)	< 0.001
Plasma LDL-C, mmol/L	2091	2.4 (2.0, 2.9)	1814	2.0 (1.7, 2.5)	< 0.001
Plasma triglycerides, mmol/L	2092	0.9 (0.7, 1.3)	1814	0.6 (0.5, 0.8)	< 0.001
DBP SDS	2059	0.19 (-0.26, 0.67)	1742	-0.22 (-0.61, 0.24)	< 0.001
SBP SDS	2059	0.67 (0.09, 1.29)	1742	0.35 (-0.14, 0.84)	< 0.001
Cardiometabolic risk features					
Insulin resistance, n (%)	2037	970 (47.6)	1768	118 (6.7)	< 0.001
Hyperglycemia, n (%)	2059	372 (18.1)	1791	111 (6.2)	< 0.001
Increased ALT, n (%)	2106	684 (32.5)	1819	194 (10.7)	< 0.001
Dyslipidemia, n (%)	2092	757 (36.2)	1814	153 (8.4)	< 0.001
Hypertension, n (%)	2059	347 (16.9)	1742	96 (5.5)	< 0.001

Continuous values are shown as medians (IQR) and categorical variables are presented as frequencies, n (%). Puberty stage defined as prepubertal (Tanner stage 1) vs pubertal (Tanner stage 2-5). Statistical analysis was performed using Wilcoxon rank sum tests or χ^2 tests.
Abbreviations: ALT, alanine aminotransferase; DBP, diastolic blood pressure; HbA_{1c}, glycated hemoglobin A_{1c}; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance; hs-CRP, high-sensitivity C-reactive protein; LDL-C, low-density lipoprotein cholesterol; SBP, systolic blood pressure; SDS, standard deviation score.

significant inverse association to HDL-C (β −0.03, $P = 0.04$, formerly nonsignificant) and SBP SDS which was attenuated (β 0.02, $P = 0.21$, formerly significant) (Supplementary Table 1, Model 2) (37). When further adjusted for fasting insulin, the previous results remained consistent, apart from liver fat % which was attenuated (β 0.02, $P = 0.50$, formerly significant) and an inverse association with HbA_{1c} (β −0.05, $P = 0.006$, formerly nonsignificant) (Supplementary Table 1, Model 3) (37).

Group (obesity clinic vs population-based) modified the relationship between glucagon and cardiometabolic risk factors (Supplementary Table 2, Model 1) (37). Significant interactions were detected for hs-CRP, HOMA-IR, insulin, C-peptide, LDL-C, triglycerides, and diastolic and systolic BP SDS (all $P_{interaction} < 0.05$), with larger β-effect sizes in the obesity clinic group compared with the population-based group, adjusted for age and sex. Liver fat % ($P_{interaction} = 0.06$) and ALT ($P_{interaction} = 0.05$) showed similar trends of larger β-effect sizes in the obesity clinic group but did not differ significantly in slopes. Significant interactions were also observed for glucose and HbA_{1c} (both $P_{interaction} < 0.05$), with larger negative β-effect sizes in the population-based group compared to the obesity clinic group. No significant interactions were observed for HDL-C ($P_{interaction} = 0.70$).

Association of Fasting Glucagon as an Indicator of Fasting Glucose Stratified by BMI SDS Quartiles, Modified by Fasting Insulin

To further examine the inverse relationship between glucagon and glucose, individuals were stratified by BMI SDS quartiles and an interaction model (glucagon × insulin [high vs low]) was applied, adjusted for age and sex (Fig. 2). There was a significant interaction ($P_{interaction} = 7.8E-04$) in the highest BMI quartile, by which glucagon was not significantly associated to glucose in individuals with high insulin (β −0.04, $P = 0.31$), but was inversely associated in those with low insulin concentrations (β −0.23, $P = 4.2E-07$).

Associations of Fasting Glucagon as an Indicator of Cardiometabolic Risk Features

A 1-SD increase in glucagon was associated with a higher prevalence of insulin resistance (odds ratio [OR] 1.31, $P = 9.1E-10$), increased ALT (OR 1.37, $P = 4.0E-14$), dyslipidemia (OR 1.20, $P = 8.2E-06$), and hypertension (OR 1.17, $P = 0.001$), but was not significantly associated with hyperglycemia (OR 0.92, $P = 0.09$) adjusted for age, sex, and BMI SDS (see Fig. 3, Supplementary Table 3, Model 1) (37). Results remained consistent when further adjusted for puberty stage, except for

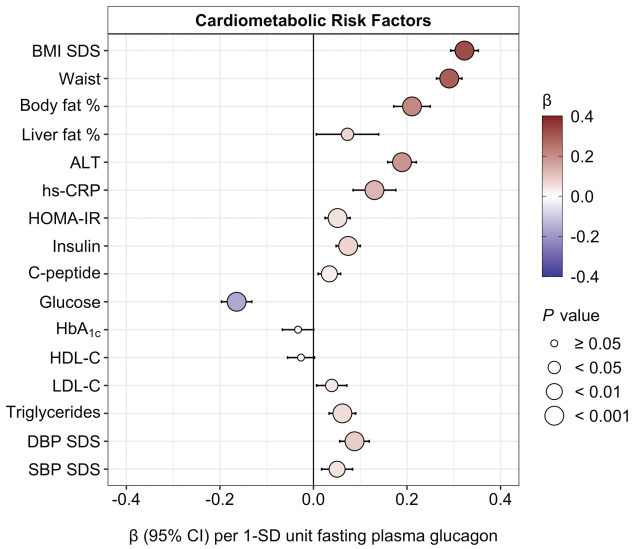

Figure 1. Estimated regression β-effects (95% CI) for associations of fasting plasma glucagon as an indicator of cardiometabolic risk factors in a pooled model, adjusted for age, sex, and BMI SDS. Cardiometabolic risk factors: BMI SDS, waist, and body fat % were not adjusted for BMI SDS. Cardiometabolic risk factors were nonnormally distributed (right-skewed) and log-transformed, except for BMI SDS, DBP SDS, and SBP SDS. Abbreviations: ALT, alanine aminotransferase; DBP, diastolic blood pressure; HbA_{1c}, glycated hemoglobin A_{1c}; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance; hs-CRP, high-sensitivity C-reactive protein; LDL-C, low-density lipoprotein cholesterol; SBP, systolic blood pressure; SDS, standard deviation score.

hypertension, which became attenuated (OR 1.11, $P = 0.07$, formerly significant) (Supplementary Table 3, Model 2) (37). When controlling for fasting insulin, higher glucagon concentrations remained associated with a higher prevalence of increased ALT, dyslipidemia, and hypertension, but became significantly associated with a lower prevalence of hyperglycemia (OR 0.81, $P = 1.7E-04$, formerly nonsignificant) (Supplementary Table 3, Model 3) (37).

The modifying effect of group on the relationship between glucagon and cardiometabolic risk features was evaluated (Supplementary Table 4, Model 1) (37). A significant interaction for hyperglycemia ($P_{interaction} = 0.02$) was observed, with a lower OR in the population-based group (OR 0.68, $P = 0.009$) compared to the obesity clinic group (OR 0.99, $P = 0.88$), adjusted for age, and sex. For insulin resistance, a higher OR was observed in the obesity clinic group (OR 1.46, $P = 4.9E-16$), compared to the population-based group (OR 1.14, $P = 0.27$), yet the slopes did not significantly differ ($P_{interaction} = 0.05$). No significant interactions were detected for increased ALT, dyslipidemia, and hypertension (all $P_{interaction} > 0.05$).

Associations of Fasting Glucagon As an Indicator of Fasting Total GLP-1

Glucagon was positively associated with fasting total GLP-1 in both girls and boys from the obesity clinic and the population-based groups ($P < 0.001$), when adjusted for age and BMI SDS (see Supplementary Figure 2) (37).

Discussion

In this study we demonstrate that children and adolescents with overweight/obesity have elevated concentrations of fasting glucagon, compared with normal weight peers. Glucagon was positively associated with BMI SDS, waist, body fat %, liver fat %, ALT, hs-CRP, HOMA-IR, insulin, C-peptide, LDL-C, triglycerides, and SDS of diastolic and systolic BP, and inversely associated with glucose, but did not significantly associate with HbA_{1c} or HDL-C. The inverse relationship between fasting glucagon and fasting glucose was attenuated in those with high BMI SDS and high fasting insulin. Higher glucagon concentrations were associated with a higher prevalence of insulin resistance, increased ALT, dyslipidemia, and hypertension, but not with hyperglycemia. Lastly, glucagon was positively associated with total GLP-1 concentrations in both boys and girls from both the obesity clinic and population-based groups. Together, these findings demonstrate that fasting glucagon is an indicator of adverse cardiometabolic risk traits in children and adolescents with overweight/obesity but does not associate with hyperglycemia in this age group.

Despite the importance of glucagon on cardiometabolic health, only a limited number of studies have focused on the role of glucagon during childhood and adolescence (11-16). A study including 65 adolescents observed elevated fasting glucagon concentrations in those with type 2 diabetes, followed by individuals with obesity, when compared to normal weight participants, which associated with a decreased suppression of glucagon levels in response to an oral glucose tolerance test (11). In cross-sectional study of 190 children and adolescents with obesity and normal weight, fasting glucagon was positively associated with BMI SDS, waist, subcutaneous and visceral adipose tissue, liver fat %, fasting insulin, triglycerides, and free fatty acids, but was not significantly associated with fasting glucose concentrations (14). A recent study comparing 350 adults to 66 youth aged 10-19 years, with impaired glucose tolerance or recently diagnosed type 2 diabetes, found that fasting glucagon was positively associated with fasting glucose in adults, whereas this relationship was inversed in youth, although not significantly (16). Furthermore, at matched glucagon concentrations, youth exhibited higher C-peptide concentrations compared to adults, suggesting higher sensitivity of beta cells to glucagon in youth (16). This highlights the intriguing finding in the present study in which fasting glucagon inversely associates with fasting glucose, but this relationship depends on the degree of adiposity and level of fasting insulin. This concept should be further explored to examine at which stage hyperglucagonemia begins to associate with hyperglycemia in young adulthood.

The concept of a liver–alpha cell axis may provide an explanation for the link between metabolic syndrome and hyperglucagonemia, with glucagon regulating amino acid turnover and amino acids regulating alpha cell growth and secretion (8). Support for this theory comes from the ADDITION-PRO study, as circulating levels of amino acids (alanine, glutamine, and tyrosine) associated with fasting glucagon in 1408 adults with normal and impaired glucose tolerance (38). These findings were replicated in an independent cohort of women with low to high risk for type 2 diabetes, where even lower levels of liver fat % were associated with hyperglucagonemia, reflecting impairments of the liver–alpha cell axis (39). It is hypothesized that a similar relationship may exist in youth, since the present study found a positive association between fasting glucagon, liver fat %, and ALT. However, measurements of amino acids are lacking. In line with this, a retrospective study that included 26 children with obesity and hyperlipidemia found that amino acids, including

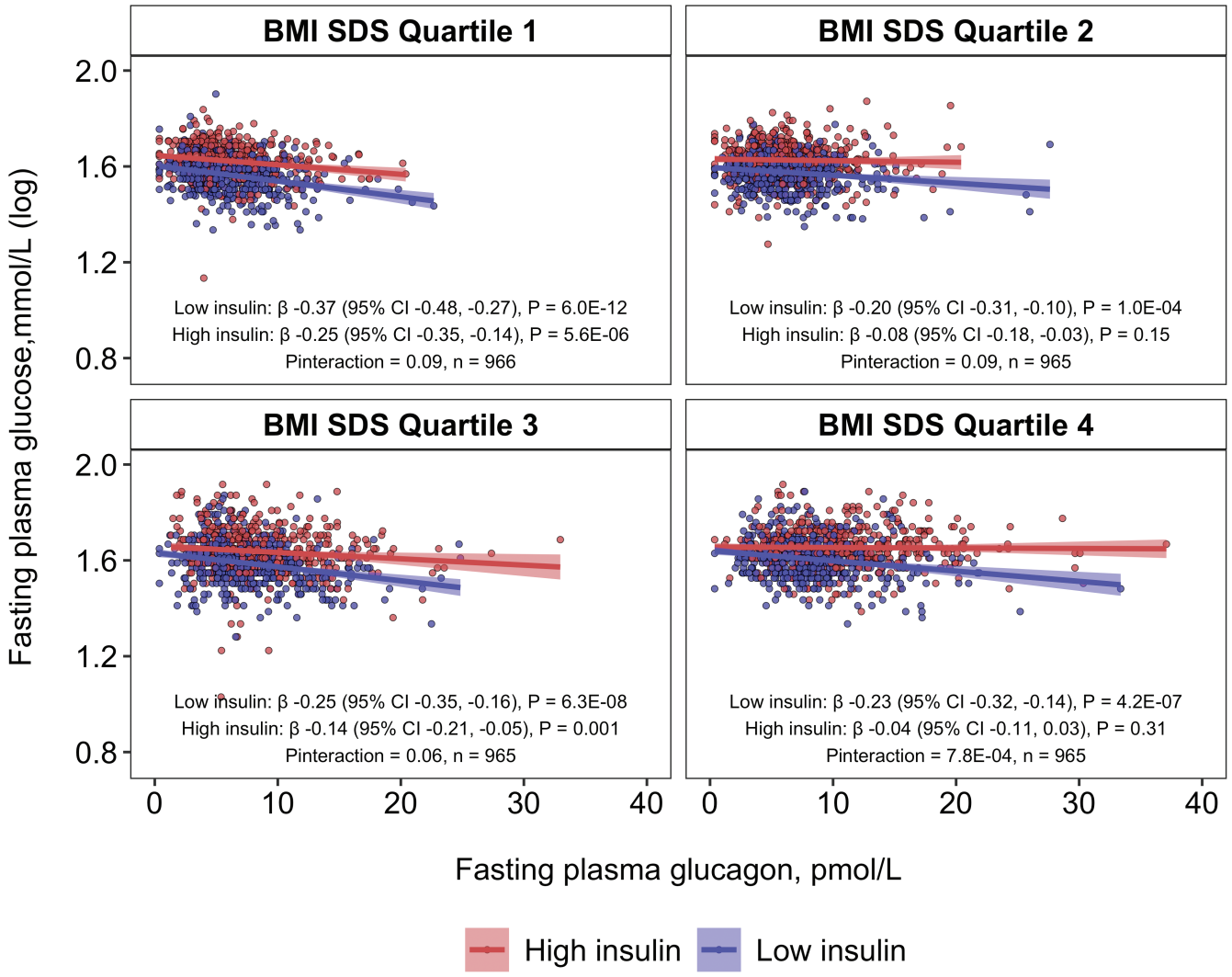

Figure 2. Estimated regression β-effects (95% CI) for associations of fasting plasma glucagon as an indicator of fasting plasma glucose, stratified by BMI SDS quartiles, in an interaction model (fasting glucagon × fasting insulin [High vs Low]), adjusted for age and sex. Fasting plasma glucose was nonnormally distributed and log-transformed. Median BMI SDS for Quartile 1 = -0.37, Quartile 2 = 0.72, Quartile 3 = 2.53, Quartile 4 = 3.33. High insulin (red) and Low insulin (blue) groups were defined by median fasting insulin concentration in each quartile. Abbreviation: SDS, standard deviation score.

alanine, associated with BMI and HOMA-IR, yet fasting glucagon was not assessed (40).

The regulation of glucagon secretion is multi-faceted and partly modulated by intestinal peptides, such as GLP-1 (which inhibits), oxyntomodulin (enhances), and glucose-dependent insulinotropic polypeptide (GIP) (enhances) (8). In the present study, a positive association between fasting glucagon and fasting total GLP-1 was observed. A similar finding has been reported in adults with and without type 2 diabetes (n = 1049) from the IMI DIRECT study (9, 41). Likewise, in children and adolescents with newly diagnosed type 1 diabetes (n = 257), GLP-1 concentrations were positively associated with glucagon release in response to a carbohydrate rich meal (42).

There are several strengths and limitations to the current study. A major strength of this study is the large number of participants with comprehensive cardiometabolic risk profiling. Participants were recruited from the same geographical area and individuals with diseases or intake of medications related to obesity or diabetes were excluded. A well-documented and validated assay with high specificity and sensitivity for glucagon was used (32, 43) and samples were assayed in duplicate. One limitation of the study is the inability to assess temporality with progression of overweight/obesity during childhood and adolescence, as only a single time point was collected, limiting the ability to draw some conclusions, including meal-related or day-to-day variation. Additionally, due to the inherent cross-sectional study design, causality cannot be established.

Conclusions and Future Directions

In the present study, we demonstrated that elevated fasting glucagon concentrations were present in children and adolescent with overweight/obesity, which associated with worsened cardiometabolic risk outcomes, including insulin resistance, increased ALT (representative of hepatic steatosis), dyslipidemia, and hypertension, but was not associated with hyperglycemia. The causality behind hyperglucagonemia and cardiometabolic risk cannot be established in the present study, but it is most likely multi-dimensional, where amino acids and gastrointestinal peptides could be of key importance (38). The genetic influence on glucagon secretion has

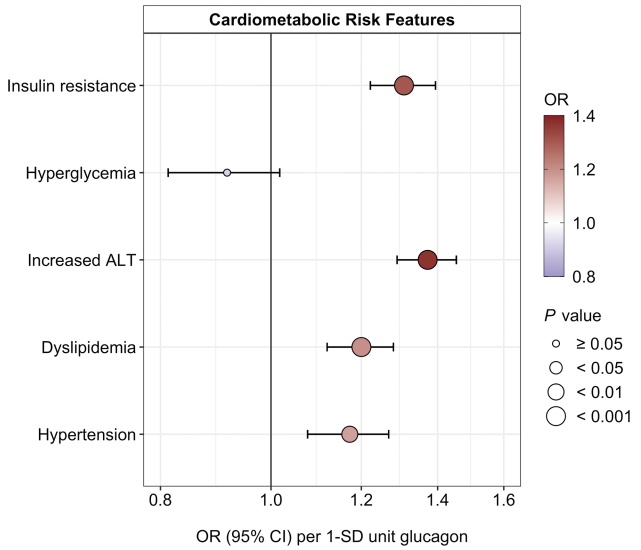

Figure 3. Estimated OR (95% CI) for associations of fasting plasma glucagon as an indicator of cardiometabolic risk features in a pooled model, adjusted for age, sex, and BMI SDS. Abbreviations: ALT, alanine aminotransferase; OR, odds ratio; SDS, standard deviation score.

been studied in a few adult populations (44, 45), yet genetic data in pediatric populations has yet to be utilized. Longitudinal studies are needed to study the progression of hyperglucagonemia during the development of pediatric overweight/obesity and how this may advance to hyperglycemia at later stages in life.

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Disclosures

The authors have nothing to disclose.

Clinical Trial Information

The HOLBAEK Study, formerly known as The Danish Childhood Obesity Biobank, is registered with ClinicalTrials.gov identifier number NCT00928473, https://clinicaltrials.gov/ct2/show/NCT00928473 (registered June 2009).

Data Availability

Restrictions apply to the availability of some or all data generated or analyzed during this study to preserve patient confidentiality. The corresponding author will on request detail the restrictions and any conditions under which access to some data may be provided.

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