

Fasting plasma GLP-1 associates with overweight/obesity and cardiometabolic risk factors in children and adolescents

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Abbreviations: ALT: alanine aminotransferase, BF%: body fat percent, BMI: body mass index, CI: confidence interval, CMR: cardiometabolic risk, DBP: diastolic blood pressure, GLP-1: glucagon-like peptide-1, HbA1c: hemoglobin A1c, HDL-C: high-density lipoprotein cholesterol, HOMA-IR: homeostasis model assessment of insulin resistance, hs-CRP: high-sensitivity C-reactive protein, IQR: interquartile range, LDL-C: low-density lipoprotein cholesterol, OR: odds ratio, SBP: systolic blood pressure, SDS: standard deviation score, WC: waist circumference.

Abstract

Context: The importance of fasting GLP-1 in altered metabolic outcomes has been questioned.

Objective: To assess if fasting GLP-1 differs in children and adolescents with overweight/obesity compared to a population-based reference, and whether concentrations predict cardiometabolic risk (CMR) factors.

Methods: Analyses were based on The Danish Childhood Obesity Data- and Biobank, a cross-sectional study including children and adolescents, 6-19 years old, from an obesity clinic group (n = 1978) and from a population-based group (n = 2334). Fasting concentrations of plasma total GLP-1 and quantitative CMR factors were assessed. The effects of GLP-1 as a predictor of CMR risk outcomes were examined by multiple linear and logistic regression modelling.

Results: The obesity clinic group had higher fasting GLP-1 concentrations (median 3.3 pmol/L; interquartile range 2.3–4.3) than the population-based group (2.8 pmol/L; 2.1–3.8; $P < 2.2E-16$). BMI SDS, waist circumference and total body fat percentage were significant predictors of fasting GLP-1 concentrations in boys and girls. Fasting GLP-1 concentrations positively associated with HOMA-IR, fasting values of insulin, hs-CRP, C-peptide, triglycerides, ALT, HbA1c and SDS of diastolic and systolic blood pressure. A 1-SD increase in fasting GLP-1 associated with an increased risk of insulin resistance (Odds ratio [OR] 1.59), dyslipidemia (OR 1.16), increased ALT (OR 1.14), hyperglycemia (OR 1.12) and hypertension (OR 1.12).

Conclusions: Overweight/obesity in children and adolescents is associated with increased fasting plasma total GLP-1 concentrations, which was predictive of higher cardiometabolic risk factors.

Key words: GLP-1, obesity, children, adolescents, cardiometabolic risk factors

Introduction

Glucagon-like peptide-1 (GLP-1) is a peptide hormone with numerous physiological functions, including enhanced glucose-sensitivity of pancreatic β -cells, satiety, suppression of gastric emptying, and possibly cardio-, and neuroprotection (1). GLP-1 is a product of proglucagon gene (*Gcg*), expressed in the enteroendocrine L-cells of the intestine, in the α -cells of the pancreas, and in a set of neurons in the brain stem (2). GLP-1 exerts insulinotropic actions via two mechanisms, 1) GLP-1 secreted from the gut activates receptors expressed in the nodose ganglions of the brain, which potentiates glucose-dependent insulin secretion through the vagus nerves and 2) GLP-1 secreted from the pancreatic α -cells activates receptors expressed in the β -cells in a paracrine-dependent manner (3). The majority of circulating GLP-1 is derived from the intestinal L-cells, which increase in density from the proximal to the distal colon, with the highest concentration located in the rectum (4). The early phase GLP-1 response has largely been attributed to secretion from the proximal intestine shortly after a meal. It would seem unlikely that GLP-1 secreted from the colon, long after a meal could contribute to altered metabolic outcomes (5). However, in addition to nutrient-stimulated GLP-1 response, a basal (tonic) secretion seems to occur, as infusions of somatostatin, a potent inhibitor of L-cell secretion (6), lowers basal concentrations of fasting GLP-1 (7). Selective reduction of *Gcg* expression in the distal gut of mice, reduces fasting active GLP-1, together with impaired oral glucose tolerance, and accelerated gastric emptying, but normal food intake/body weight (8). Selective stimulation of colonic L-cells in mice, increases fasting insulin concentrations and improve glucose tolerance (5). In a murine model of chronic hyperinsulinemia, higher concentrations of insulin corresponded to higher fasting GLP-1 and impaired GLP-1 secretion following an oral glucose challenge (9). Moreover, left colectomy patients exhibit a higher hazard ratio of clinically recorded T2D, compared with non-colectomy patients (10). Colectomy patients have also been found to exhibit higher insulin and C-peptide levels compared to patients with resections of the small intestine, inactive Crohn's patients (no surgery), and healthy controls, but no differences in active GLP-1 concentrations (11). Together, these findings suggest that colonic GLP-1 secretion may contribute to altered metabolic phenotypes, potentially in an insulin dependent manner.

A discrepancy exists in adults with T2D and obesity, where both no differences (12,13) as well as higher (14,15) concentrations of fasting plasma total GLP-1 have been observed. Obesity during childhood increases the risk of long-term obesity (16-18), as well as the risk for cardiometabolic complications and mortality into adulthood (19-23). It remains unclear whether fasting GLP-1 concentrations are altered in pediatric obesity and if this may play a role in modifying cardiometabolic risk outcomes. A limited number of small studies have examined changes in fasting GLP-1 concentrations in children and adolescents with obesity (24-26). Similar concentrations of fasting active GLP-1 were observed in children with obesity (median 6.2 pmol/L; interquartile range (IQR) 3.3 – 9.1; N = 42) versus normal weight (NW) (5.7; 3.3 – 8.1; N = 16; $P = 0.56$), however GLP-1 concentrations positively associated with insulin levels and homeostasis model assessment of insulin resistance (HOMA-IR) (24). Following 1 year of significant weight loss, fasting active GLP-1 concentrations were lower compared to baseline and positively associated with insulin and HOMA-IR (24). A study investigating GLP-1 response to a standardized breakfast and lunch found higher fasting total GLP-1 concentrations in children with overweight (mean [standard error]: 23.5 [4.9] pmol/L; N = 12) compared to NW children (11.5 [2.4]; N = 20; $P = 0.02$) (25). Adolescents with obesity and normal glucose tolerance had higher fasting active GLP-1 concentrations (median 0.53 pmol/L; IQR 0.32 – 0.75; N = 23), than the adolescents with obesity and impaired glucose tolerance (0.25; 0.23 – 0.44; N = 19), obesity and type 2 diabetes mellitus (T2DM) (0.20; 0.13 – 0.43; N = 4), and NW adolescents (0.32; 0.18 – 0.53; N = 19, $P < 0.05$) in the Uppsala Longitudinal Study of Childhood Obesity (26).

The aim of the present study is to investigate whether fasting plasma concentrations of total GLP-1 in children and adolescents with overweight/obesity differs in comparison to a population-based control group. The second aim was to evaluate whether fasting GLP-1 concentrations predict cardiometabolic risk (CMR) factors. It was hypothesized that children and adolescents with overweight/obesity would

have lower fasting concentrations of GLP-1 compared to the general population, which will associate with worsened CMR factors.

Materials and Methods

Study populations

Two groups of children and adolescents were included in the present study: (1) an obesity clinic group (N = 1978), the members of which followed a multidisciplinary obesity treatment program at Copenhagen University Hospital Holbæk (BMI >90th percentile) and (2) a population-based group (N = 2334), recruited from schools and high schools in 11 municipalities in Zealand, Denmark. Both groups were enrolled in The Danish Childhood Obesity Data- and Biobank between August 2007 and April 2019, and have been previously described (27-29). Exclusion criteria were: (a) age younger than 6 or older than 19 years; (b) ethnicity other than Danish or North-European (self-reported country of origin and ancestry); (c) diagnosed type 1 diabetes mellitus (T1DM); (d) diagnosed T2DM; (e) treatment with insulin, Liraglutide, or Metformin, and (f) meeting T2DM criteria based on the blood sample taken for this study (fasting plasma glucose > 7.0 mmol/L and/or HbA1c > 49 mmol/mol).

Ethics

According to the Declaration of Helsinki, informed consent was obtained from all participants, while written consent was obtained either from parents 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

Anthropometrics included height, weight, body mass index standard deviation score (BMI SDS), waist circumference (WC), as well as measurements of blood pressure (BP). In the obesity clinic group, anthropometrics were obtained as a part of a clinical examination, whereas the population-based group was examined in a mobile laboratory by trained medical professionals. Height was measured by a stadiometer to the nearest 0.1 cm. With the participants wearing light clothing without shoes, weight was measured to the nearest 0.1 kg using a Tanita Digital Medical Scale, WB-110 MA (Tanita Corp., Tokyo, Japan) for the obesity clinic group, and a Tanita BC-418 Scale (Tanita Corp., Tokyo, Japan) for the population-based group. To calculate BMI SDS, the Lambda-Mu-Sigma (LMS) method was used, based on a Danish reference population (30). Waist circumference was measured post-exhalation in a standing position at umbilical level, measured to the nearest 0.5 cm. BP was measured three times on the right upper arm after five minutes of rest with the subject in supine position using the oscillometric device Omron 705IT (Omron Health Care Co., Ltd., Kyoto, Japan). An average values of the last two measurements was calculated and converted to BP SDS based on age-, sex- and height-specific reference values from the American Academy of Pediatrics (31).

Biochemical analyses

A venous blood sample was collected from all participants between 7 to 9 AM, following an overnight fast (32,33). The participants were offered a local anesthetic prior (lidocaine/prilocaine mixture, EMLA, AstraZeneca, Sweden). Immediately after collection, samples were processed and stored at -80°C until further analysis. The Department of Clinical Biochemistry, Copenhagen University Hospital Holbæk performed analyses of all biomarkers, except for fasting plasma total GLP-1 measured at the Novo Nordisk Foundation Center for Basic Metabolic Research, University of Copenhagen and serum high-sensitivity C-reactive protein (hs-CRP) measured at the Statens Serum Institute, Denmark (34). Fasting biochemical measurements described previously by our group include serum insulin, serum C-peptide, plasma glucose, and whole blood HbA1c (33), plasma high-

density lipoprotein cholesterol (HDL-C), plasma low-density lipoprotein cholesterol (LDL-C), plasma triglycerides (TG) (32), plasma alanine transaminase (ALT) (35) and serum hs-CRP (34). Plasma concentrations of GLP-1 were measured using a total GLP-1 enzyme-linked immunosorbent assay (ELISA) (Merckodia, Uppsala, Sweden) (36). The analyses were performed in duplicate and run on the SpectraMax iD3 (San Jose, CA, USA). Outliers were automatically re-measured. The inter- and intra-assay coefficient of variation (CV) was 3.0% and 3.4%, in the concentration range (0.904-940 pmol/L). The lower limit of quantification (LLOQ) of the assay is 1.0 pmol/L, values below this (N = 5) were assigned half the LLOQ, 0.5 pmol/L.

Dual-energy x-ray absorptiometry

Whole-body Dual-energy X-ray absorptiometry (DXA) was performed on 1754 patients from the obesity clinic group and 291 participants from the population-based group, on 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 (37), yielding data on body fat percent (BF%) (38,39).

Defining cardiometabolic risk profiles

We defined insulin resistance as HOMA-IR values above the 90th percentile for age and sex (33). HOMA-IR was calculated as $\text{insulin (mU/L)} \times \text{glucose (mM)} / 22.5$. We defined dyslipidemia as values beyond the 95th percentile according to the American Heart Association (40), corresponding to: total cholesterol above 5.2 mM (200 mg/dL), LDL-C above 3.4 mM (130 mg/dL), HDL-C below 0.9 mM (35 mg/dL), and/or triglycerides above 1.7 mM (150 mg/dL). Increased ALT was defined as ALT above 24.5 U/L in girls and ALT above 31.5 U/L in boys, which was determined to be the optimal cut-off for identifying hepatic steatosis by our group (liver fat content of > 5% measured by proton magnetic resonance spectroscopy in 458 children and adolescents) (35). We defined

hyperglycemia as fasting blood glucose between 5.6 - 6.9 mmol/L and/or HbA1c between 39 - 47 mmol/mol, according to the American Diabetes Association guideline for prediabetes (41). We defined hypertension as a systolic blood pressure (SBP) and/or diastolic blood pressure (DBP) above the 95th percentile for age and sex (42).

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Statistical analyses

Statistical analyses were performed in R statistical software, version 4.0.2 (43). Normality of data was evaluated by Q-Q plots and histograms. Continuous variables were described as median with IQR for non-normally distributed variables and categorical variables were presented as frequencies and percentages (%). *P*-values for continuous variables between the two groups were determined by Wilcoxon rank-sum tests and categorical variables were assessed by chi-squared tests.

Age- and sex specific percentile curves for fasting total GLP-1 concentrations were calculated using the R ‘Generalized Additive Models for Location, Scale and Shape’ package (<https://cran.r-project.org/web/packages/gamlss/>) (44), using the Box-Cox transformation distribution family to account for skewness, with the best fit determined by the Akaike Information Criterion (45) (Suppl. Fig. 1) (46). Linear regression models were performed to calculate effect sizes and 95% confidence intervals (CIs) for adiposity measures as a predictor of fasting plasma total GLP-1 concentrations according to sex (Suppl. Fig. 2) (46).

Linear regression models were performed to calculate effect sizes and 95% CIs for fasting GLP-1 concentrations as a predictor of CMR factors (Fig. 1, Suppl. Table 1) (46). CMR factors included anthropometric measurements (BMI SDS, WC, and BF% by DXA), blood pressure (SBP SDS and DBP SDS), measures of glucose metabolism (HOMA-IR, insulin, C-peptide, glucose, and HbA1c), lipid profile (HDL-C, LDL-C, TG), liver and inflammation markers (ALT and hs-CRP, respectively). Non-normally distributed CMR factors were log-transformed. The obesity clinic and population-based groups were pooled to estimate the effect sizes for CMR factors. Pooled linear regression formulas in R were: $\text{glm}(\text{CMR factor} \sim \text{GLP-1 (SD-units)} + \text{age} + \text{sex} + \text{BMI SDS})$. Interaction analyses were performed to estimate whether the group indicator (Obesity clinic group/Population-based group) modifies the

association between GLP-1 and CMR factors. Interaction linear regression formulas in R were: $\text{glm}(\text{CMR factor} \sim \text{GLP-1 (SD-units)} + \text{group indicator variable (0/1)} + \text{group indicator variable} \times \text{GLP-1 (SD-units)} + \text{age} + \text{sex} + \text{BMI SDS})$.

Logistic regression was used to calculate odds ratios (ORs) and 95% CIs of fasting total GLP-1 as a predictor of CMR features (Fig. 2, Suppl. Table 2) (46). CMR features were defined as the absence or presence of insulin resistance, dyslipidemia, increased ALT, hyperglycemia, and hypertension. Pooled logistic regression formulas in R: $\text{glm}(\text{CMR feature (0/1)} \sim \text{GLP-1 (SD-units)} + \text{age} + \text{sex} + \text{BMI SDS})$. Interaction logistic regression formulas in R were: $\text{glm}(\text{CMR feature (0/1)} \sim \text{GLP-1 (SD-units)} + \text{group indicator variable (0/1)} + \text{group indicator variable} \times \text{GLP-1 (SD-units)} + \text{age} + \text{sex} + \text{BMI SDS})$.

Additional linear and logistic regression models were performed to examine the role of fasting insulin concentrations, in relation to GLP-1 as a predictor of CMR (Suppl. Table 3 & 4) (46).

Statistical significance was set at $P < 0.05$. Correction for multiple testing was controlled for using the Bonferroni-Holm method for all regression modelling.

Results

Characteristics of the study groups

There were no significant differences in age between the two groups (Table 1). Sex differed across groups ($P = 4.1\text{E-}04$). Patients in the obesity clinic group had higher height ($P = 2.6\text{E-}05$), weight, BMI SDS, WC, and BF% than participants in the population-based group (all $P < 2.2\text{E-}16$). Patients in the obesity

clinic group exhibited worse cardiometabolic risk profiles, with a higher incidence of insulin resistance, dyslipidemia, increased ALT, hyperglycemia, and hypertension, than the participants in the population-based group (all $P < 2.2E-16$).

Patients in the obesity clinic group had higher fasting plasma total GLP-1 concentrations (median 3.3 pmol/L; IQR 2.3 – 4.3) than participants in the population-based group (2.8; 2.1 – 3.8; $P < 2.2E-16$). Age- and sex-specific values for fasting GLP-1 concentrations are illustrated with 2.5, 50, and 97.5 percentile curves for the obesity clinic and the population-based groups (Suppl. Fig. 1) (46). BMI SDS, WC, and BF% were significant predictors of fasting GLP-1 concentrations in boys and girls (Suppl. Fig. 2) (46).

Fasting total GLP-1 as a predictor of cardiometabolic risk factors

GLP-1 concentrations positively associated with BMI SDS, fasting serum insulin_{log}, HOMA-IR_{log}, serum hs-CRP_{log}, serum C-peptide_{log}, plasma TG_{log}, DBP SDS, SBP SDS, plasma ALT_{log}, BF%_{log} and whole blood HbA1c_{log}, adjusted for age, sex, and BMI SDS, except for BMI SDS and BF% which were adjusted for age and sex only (Fig. 1, Suppl. Table 1) (46). GLP-1 did not significantly associate with WC_{log}, plasma glucose_{log}, plasma HDL-C_{log} and plasma LDL-C_{log}, adjusted for age, sex, and BMI SDS.

When assessing the interaction between GLP-1 and study group, GLP-1 concentrations in the obesity clinic group positively associated with HOMA-IR_{log}, insulin_{log}, C-peptide_{log}, hs-CRP_{log}, SBP SDS, TG_{log}, ALT_{log} and WC_{log}, compared to the population-based group, which instead negatively associated with glucose_{log} and positively associated with HDL-C_{log} (all interaction $P < 0.05$), adjusted for group, age, sex, and BMI SDS (Suppl. Table 1) (46).

To examine the role of insulin in relation to GLP-1 as a predictor of CMR factors, the above mentioned models were further adjusted for fasting insulin values (Suppl. Table 3) (46). GLP-1 remained positively associated with hs-CRP_{\log} , BMI SDS, TG_{\log} , DBP SDS and ALT_{\log} . GLP-1 was positively associated with HDL-C_{\log} and negatively associated with $\text{blood glucose}_{\log}$. The relationship between GLP-1 as a predictor of BF\%_{\log} , SBP SDS and HbA1c_{\log} was attenuated, in addition to WC_{\log} and LDL-C_{\log} remaining non-significant.

Fasting total GLP-1 as a predictor of cardiometabolic risk features

A 1-SD increase in total GLP-1 associated with a higher incidence of insulin resistance (OR 1.59), dyslipidemia (OR 1.16), increased ALT (OR 1.14), hyperglycemia (OR 1.12) and hypertension (OR 1.12), adjusted for age, sex, and BMI SDS (Fig. 2, Suppl. Table 2) (46).

When assessing the interaction between GLP-1 and study group, a 1-SD increase in total GLP-1 in the obesity clinic group was associated with a higher incidence of insulin resistance, compared to the population-based group (interaction $P = 0.006$), adjusted for group, age, sex, and BMI SDS (Suppl. Table 2) (46).

To examine the role of insulin in relation to GLP-1 as a predictor of CMR features, the models were further adjusted for fasting insulin concentrations. The risk for dyslipidemia, increased ALT and hyperglycemia were attenuated, whereas the higher incidence for hypertension remained significant (Suppl. Table 4) (46).

Discussion

The current study reports three main findings that were opposite to and hence disproved the proposed hypothesis. First, we demonstrated patients in the obesity clinic group had higher fasting total GLP-1 concentrations than participants in the population-based group. Higher BMI SDS, WC, and BF% predicted higher GLP-1 concentrations in both girls and boys. Second, GLP-1 concentrations positively associated with BMI SDS, insulin, HOMA-IR, hs-CRP, C-peptide, TG, DBP SDS, SBP SDS, ALT, BF%, and HbA1c. GLP-1 remained a significant predictor to some but not all cardiometabolic risk factors, when adjusted for fasting insulin. Third, a 1-SD increase in GLP-1 concentrations associated with a greater incidence of insulin resistance, dyslipidemia, increased ALT, hyperglycemia, and hypertension. When controlling for fasting insulin concentrations, GLP-1 no longer remained a significant predictor of cardiometabolic risk features, with the exception of hypertension.

The observation of elevated fasting GLP-1 together with overweight/obesity, and the more pronounced cardiometabolic risk outcomes in childhood obesity is intriguing. It may be an essential feature of obesity and require further investigation to elucidate the mechanisms. One possibility is to assume a primary role of the higher fasting GLP-1, which may increase circulating insulin, potentially contributing to insulin resistance, and in turn enhancing cardiometabolic risk among patients with overweight/obesity (47). Increased adiposity as a result of high insulin has been established through several well-known mechanisms, including increased lipogenesis, reduced lipolysis and stimulation of adipogenesis (48). According to the adipose expandability theory (49), lipotoxicity induced when the adipose tissue cannot expand any further, may induce low-grade systemic inflammation and hepatosteatosis (47). Mice fed a high-fat diet for 16 weeks displayed a 2-fold increase in basal GLP-1 secretion from colonic cultures (50). Moreover, IL-6 released from white adipose tissue is thought to stimulate L-cell and α -cell GLP-1

production and secretion, subsequently enhancing insulin secretion (51-53), however some studies showed lack of replication to this effect (54,55). In the present study, we observed a significant positive association between fasting total GLP-1 as a predictor of both hs-CRP and ALT in children and adolescents with overweight/obesity, surrogate markers of systemic inflammation and hepatosteatosis (35,56). In fact, a recent study found fasting total GLP-1 to be among the top 20 predictors of MRI-image-derived liver fat content in a machine learning and validation model, comprised of 185 different omics and clinical input variables in the IMI DIRECT prospective cohort study (57).

Furthermore, the microenvironment in the gut is thought to be an important factor for L-cell function. The microbial composition in the small and large intestine are considerably different. Microbial cells are in direct contact with the epithelium and L-cells in the small intestine, whereas the mucus layer separates cells from the microbiota in the colon (58). Obesity is associated with intestinal dysbiosis, including impaired mucus production and thickness, as well as decreased expression of tight junction proteins (59), which may provide the gut microbiota more direct contact with colonic L-cells when in an obesogenic state. Various endogenous factors influenced by the gut microbiota, such as short chain fatty acids and bile acids have been shown to alter GLP-1 secretion (1). For example, ingestion of propionate ester, a stimulator of GLP-1 release from the colonic L-cells via free fatty acid receptor 2 (FFAR2), reduced energy intake and weight gain in humans (60). Rectal administration of the bile acid, taurocholate in obese T2D patients increased active GLP-1 in a dose-dependent manner and decreased blood glucose and food intake, likely occurring via the G-protein-coupled bile acid receptor 1, GPBAR1 (61).

It remains unclear how fasting GLP-1 concentrations under physiological conditions differ from that of pharmacological treatment. GLP-1 receptor agonists (GLP1-RA) are well established as a treatment for T2D, by selectively activating GLP-1 receptor signalling to potentiate glucose-dependent insulin

secretion, which achieves not only glycaemic control, but also reduced risk for cardiovascular disease, with the mechanism most likely acting through reduced body weight and BP, lowered LDL-C and glucose, as well as suppression of low grade inflammation, vasodilation, and natriuresis (62,63). GLP1-RA have also emerged as therapeutic strategies for pediatric obesity, and several randomized placebo-controlled trials have been conducted, where reductions in BMI were achieved, however significant heterogeneity in weight loss response along with gastrointestinal adverse effects were observed, similar to what is seen in adult populations (64).

Our study has both strength and limitations. The obesity clinic and population-based groups entail a much larger and more extensive pediatric study than previously reported (24-26). In addition to our analysis of fasting GLP-1 concentrations in a total of 4312 individuals, we have investigated associations with quantitative cardiometabolic risk factors. Individuals from both groups were recruited from the same geographical area. Individuals with diseases or intake of medications known to influence GLP-1 concentrations were excluded. Other strengths include standardized blood sample collection and samples assayed for GLP-1 in duplicate, minimizing variation that may be caused by non-biological factors. We used a well-documented and validated assay with high specificity and sensitivity for analyzing plasma samples for GLP-1 (36). We measured total GLP-1 (*i.e.* sum of both the intact and metabolite forms). Measurement of total GLP-1 provides better information about the secretion of the hormone and its effects, compared to measurements of intact (or active) GLP-1, because of the extensive degradation of the latter and longer survival of the metabolites in circulation (65). The limitations of the current study affect the mechanistic interpretation and the broader applicability. This includes the cross-sectional rather than longitudinal study design, not allowing elucidation of the time sequence in the development of these associations. Single measurements of fasting GLP-1 for each participant does not allow adjustments in the natural meal-related diurnal or day to day variation.

Conclusions and Future Directions

Fasting plasma total GLP-1 concentrations were higher in children and adolescents with overweight/obesity than in a population-based reference. Elevated fasting GLP-1 concentrations in children and adolescents are positively associated with cardiometabolic risk factors, including higher HOMA-IR, fasting values of insulin, hs-CRP, C-peptide, triglycerides, ALT and HbA1c, SDS of diastolic and systolic blood pressure, BMI SDS and BF%. This elevation in GLP-1 concentrations associates directly with increased odds for exhibiting cardiometabolic risk features such as insulin resistance and to a lesser degree, dyslipidemia, increased ALT, hyperglycemia and hypertension. The associations with markers of dysmetabolism appear to be in part dependent on fasting insulin concentrations.

Significant findings should be confirmed in replication studies. Future studies should be aimed at examining the mechanisms in longitudinal settings, including lipotoxicity and role of additional inflammatory factors contributing to associations of GLP-1 with childhood overweight/obesity. Genetic studies should be performed, including Mendelian randomization to investigate the possible causal role of fasting GLP-1 in the development of insulin resistance, overweight/obesity, and enhanced cardiometabolic risk profiles.

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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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Table 1. Descriptive information on the obesity clinic and population-based groups.

Characteristic	n	Obesity clinic group	n	Population-based group	P-value
Age, years	1978	11.9 [9.7 , 14.2]	2334	11.7 [9.2 , 14.6]	0.62
Sex, boys (%)	1978	922 (46.6)	2334	942 (40.4)	4.1E-04
Fasting plasma total GLP-1, pmol/L	1978	3.3 [2.3 , 4.3]	2334	2.8 [2.1 , 3.8]	< 2.2E-16
<i>Anthropometrics</i>					
Height, cm	1978	155 [143 , 166]	2334	153 [137 , 166]	2.6E-05
Weight, kg	1978	63.9 [49.0 , 82.6]	2334	42.3 [30.9 , 56.3]	< 2.2E-16
BMI SDS	1978	2.87 [2.45 , 3.31]	2334	0.30 [-0.41 , 1.01]	< 2.2E-16
WC, cm	1906	91.0 [82.0 , 102]	2314	65.0 [60.0 , 73.0]	< 2.2E-16
BF%	1754	43.5 [40.1 , 46.9]	291	26.9 [21.9 , 33.6]	< 2.2E-16
<i>CMR features</i>					
Insulin resistance, n (%)	1886	886 (47.0)	2290	226 (9.9)	< 2.2E-16
Dyslipidemia, n (%)	1920	451 (23.5)	2269	144 (6.3)	< 2.2E-16
Increased ALT, n (%)	1933	636 (32.9)	2277	270 (11.9)	< 2.2E-16
Hyperglycemia, n (%)	1893	340 (18.0)	2251	177 (7.9)	< 2.2E-16
Hypertension, n (%)	1905	310 (16.3)	2232	124 (5.6)	< 2.2E-16

Continuous values presented as median [interquartile range] and categorical variables presented as frequencies (percent; %). *P*-values were determined by Wilcoxon rank-sum tests for continuous data and chi-squared tests for categorical data. *Insulin resistance*: was defined as HOMA-

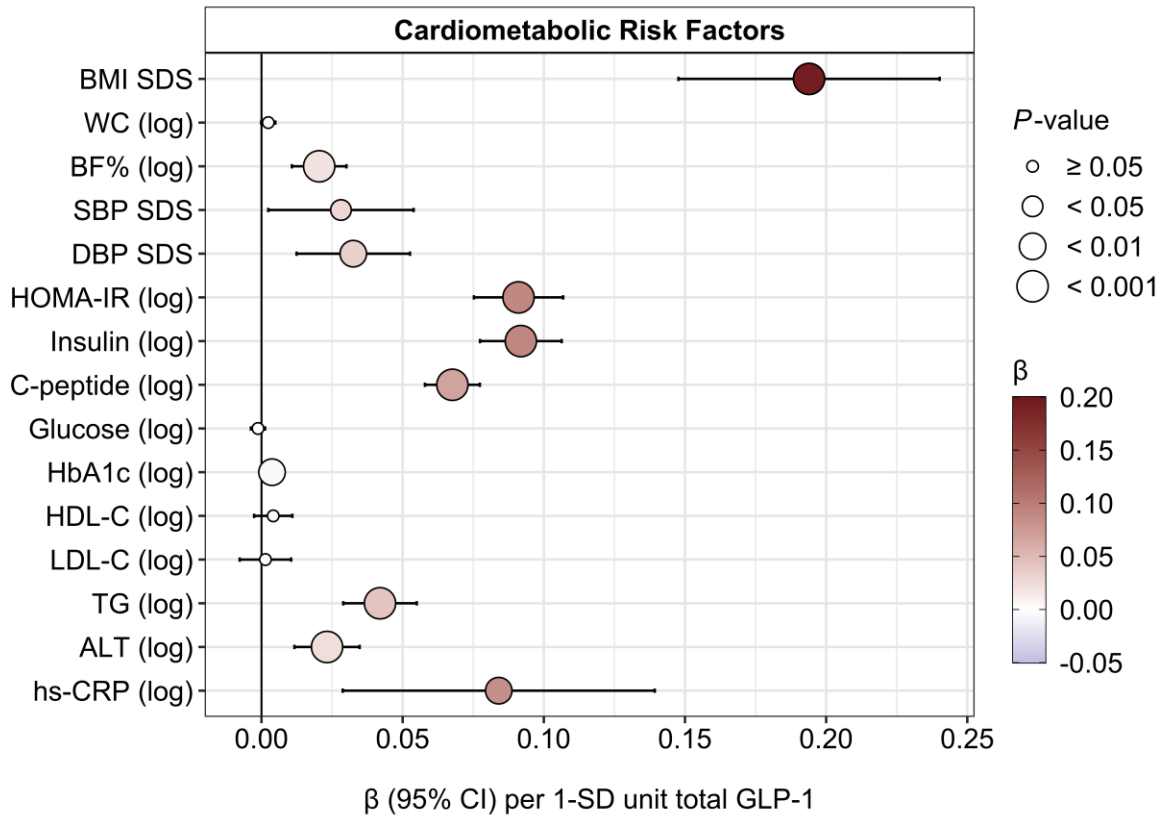
IR values above the 90th percentile for age and sex. *Dyslipidemia*: defined as values beyond the 95th percentile, corresponding to: total cholesterol above 5.2 mM (200 mg/dL), LDL-C above 3.4 mM (130 mg/dL), HDL-C below 0.9 mM (35 mg/dL), and/or triglycerides above 1.7 mM (150 mg/dL). *Increased ALT*: defined as ALT above 24.5 U/L in girls and ALT above 31.5 U/L in boys, which was determined to be the optimal cut-off for identifying hepatic steatosis (liver fat content of >5% measured by proton magnetic resonance spectroscopy in 458 children and adolescents). *Hyperglycemia*: defined as fasting blood glucose between 5.6-6.9 mmol/L and/or HbA1c between 39-47 mmol/mol. *Hypertension*: defined as a systolic and/or diastolic blood pressure above the 95th percentile for age and sex. *Abbreviations*: BF%, body fat percent; BMI SDS, body mass index standard deviation score; CMR, cardiometabolic risk; GLP-1, glucagon-like peptide-1; WC, waist circumference.

Figure 1. Estimated regression β -effects (95% confidence intervals) for associations of fasting plasma total GLP-1 as a predictor of cardiometabolic risk factors. The obesity clinic and population-based groups were pooled to estimate the effect sizes for CMR factors. Formula for linear regression in R: $\text{glm}(\text{CMR factor} \sim \text{fasting plasma total GLP-1 (SD-units)} + \text{age} + \text{sex} + \text{BMI SDS})$. BMI SDS and BF% were not adjusted for BMI SDS. Bonferroni-Holm correction for multiple testing corresponds to $P < 0.003$. *Abbreviations:* ALT, plasma alanine aminotransferase; BF%, body fat percent; BMI SDS, body mass index standard deviation score; serum C-peptide; CMR, cardiometabolic risk; DBP SDS, diastolic blood pressure; plasma glucose; HbA1c, whole blood hemoglobin A1C; HDL-C, plasma high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance; hs-CRP, serum high-sensitivity C-reactive protein; serum insulin; LDL-C, plasma low-density lipoprotein cholesterol; SBP SDS, systolic blood pressure; TG, plasma triglycerides; WC, waist circumference.

Figure 2. Estimated odds ratios (95% confidence intervals) for associations of fasting plasma total GLP-1 as a predictor of cardiometabolic risk features. The obesity clinic and population-based groups were pooled to estimate the effect sizes for CMR features. Formula for interaction logistic regression in R: $\text{glm}(\text{CMR feature (0/1)} \sim \text{fasting plasma total GLP-1 (SD-units)} + \text{age} + \text{sex} + \text{BMI SDS})$. Bonferroni-Holm correction for multiple testing corresponds to $P < 0.01$. *Abbreviations:* ALT, alanine aminotransferase; CMR, cardiometabolic risk.

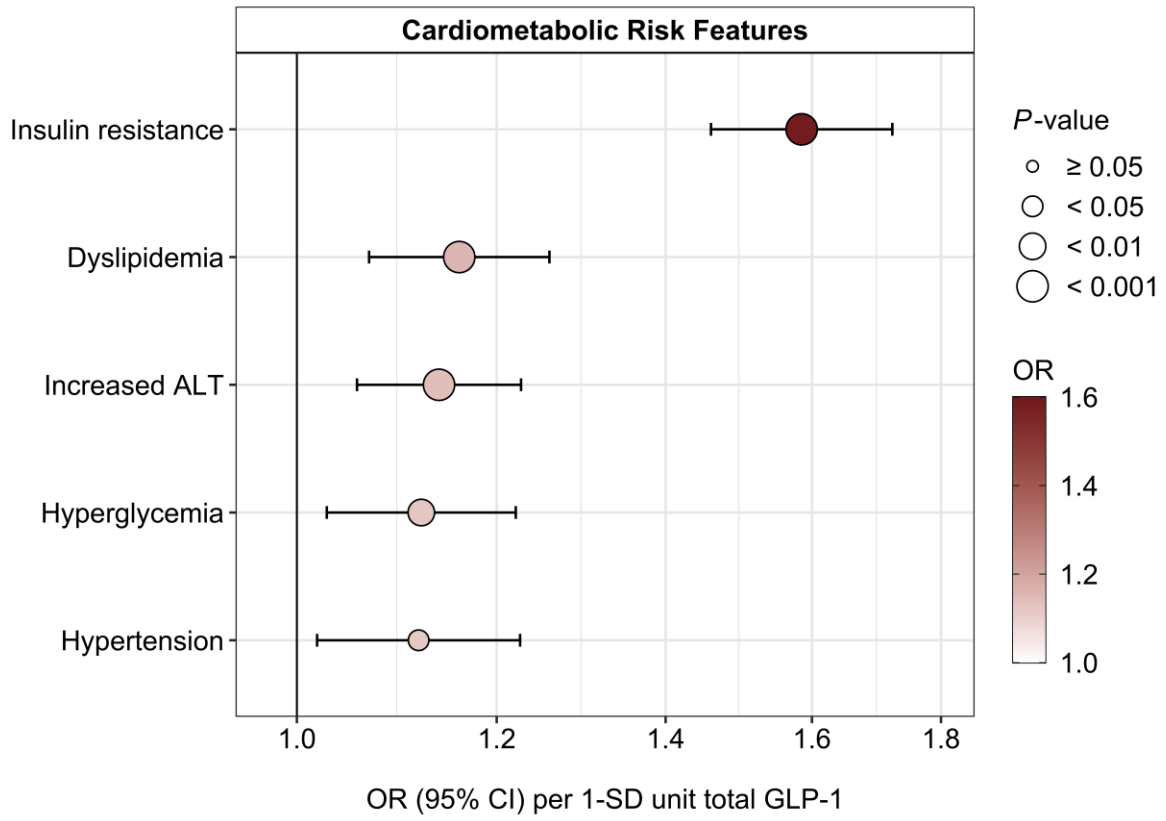
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Figure 1



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Figure 2



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