The association of cardiometabolic, diet and lifestyle parameters with plasma glucagon-like peptide-1: An IMI DIRECT study

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Abstract

Context

The role of glucagon-like peptide-1(GLP-1) in Type 2 diabetes (T2D) and obesity is not fully understood.

Objective

We investigate the association of cardiometabolic, diet and lifestyle parameters on fasting and postprandial GLP-1 in people at risk of, or living with, T2D.

Method

We analysed cross-sectional data from the two Innovative Medicines Initiative (IMI) Diabetes Research on Patient Stratification (DIRECT) cohorts, cohort 1(n=2127) individuals at risk of diabetes; cohort 2 (n=789) individuals with new-onset of T2D.

Results

Our multiple regression analysis reveals that fasting total GLP-1 is associated with an insulin resistant phenotype and observe a strong independent relationship with male sex, increased adiposity and liver fat particularly in the prediabetes population. In contrast, we showed that incremental GLP-1 decreases with worsening glycaemia, higher adiposity, liver fat, male sex and reduced insulin sensitivity in the prediabetes cohort. Higher fasting total GLP-1

was associated with a low intake of wholegrain, fruit and vegetables inpeople with prediabetes, and with a high intake of red meat and alcohol in people with diabetes.

Conclusion

These studies provide novel insights into the association between fasting and incremental GLP-1, metabolic traits of diabetes and obesity, and dietary intake and raise intriguing questions regarding the relevance of fasting GLP-1 in the pathophysiology T2D.

Article highlights

Why did we undertake this study?

Evidence from animal studies suggests that fasting GLP-1 may be relevant in the context of insulin resistance and obesity, however very few human population studies have looked at this relationship.

What is specific question(s) we wanted to answer?

What are the cardiometabolic, diet and lifestyle parameters associated with fasting and postprandial total GLP-1 in people at risk of, or living with, T2D.

What did we find?

Fasting total GLP-1 is increased and incremental GLP-1 is decreased with worsening glycaemia, adiposity, liver fat, insulin resistance along with worse diet quality profile and a higher alcohol consumption in individuals at-risk for, and living with T2D.

What are the implications of our findings?

Higher fasting total GLP-1 levels are seen in more insulin resistant phenotypes, independent of obesity status and liver fat, suggesting that insulin sensitivity may be a determinant of fasting GLP-1. In turn fasting GLP-1 may be a biomarker of those at risk of developing T2D, in whom dietary modulation may be an effective therapeutic strategy.

Introduction

The incretin peptide glucagon-like peptide-1 (GLP-1) has multiple metabolic effects including stimulation of glucose-dependent pancreatic insulin secretion, suppression of glucagon release, slowing gastric motility, and increasing satiety(1). GLP-1 is secreted from the enteroendocrine L-cells distributed throughout the intestine. Mechanisms for enteroendocrine GLP-1 secretion involve direct nutrient stimulation of intestinal L-cells, and neuroendocrine and olfactory stimulation has also been reported(2, 3). The role of glucose-stimulated release of

GLP-1 in the development and physiology of type 2 diabetes (T2D) remains controversial (3, 4). A recent large cohort study provided evidence that the postprandial secretion of GLP-1 is reduced in those T2D and obesity (5). In contrast, a meta-analysis, comparing GLP-1 in people with diabetes and weight-matched controls, found the incremental concentrations of GLP-1 did not differ between groups and were unaffected by weight (4).

The half-life of intact GLP-1 is very short, 1-5 mins, due to enzymatic degradation. After subcutaneous administration of GLP-1, the concentration of GLP-1 returns to basal after a few minutes(6). These data indicate that concentrations of GLP-1 return to basal levels relatively quickly and thus it can be inferred that intact GLP-1 plasma levels will be near basal concentrations for the majority of a 24hr period. Surprisingly, very little attention has been paid to fasting total GLP-1 concentrations and the physiological relevance of fasting GLP-1 concentrations remains uncertain. A recent study by Stinson et al. showed elevated fasting total GLP-1 in children was positively associated with adiposity, glycaemic and cardiometabolic markers (7). Similar findings have been reported in animal studies (8-10). However, there is a lack of human research investigating the relationship between fasting GLP-1 and glycaemic homeostasis, obesity, insulin sensitivity, macronutrients and dietary patterns.

We aimed to investigate the associations of fasting total GLP-1 and incremental GLP-1 (calculated as postprandial 60 min total GLP-1 minus fasting total GLP-1) with diet, lifestyle, and cardiometabolic parameters in two deeply phenotyped cohorts from the Innovative Medicines Initiative (IMI) Diabetes Research on Patient Stratification (DIRECT) Consortium (https://directdiabetes.org) (11): Cohort 1, those at risk of T2D; and cohort 2, new-onset T2D. These cohorts allow for comprehensive assessment of the association of GLP-1 with cardiometabolic risk factors such as insulin resistance, obesity, liver fat and lifestyle in adults.

Research Design and Methods

Study design and participants

The IMI DIRECT multicentre study is a European Union Innovative Medicines Initiative project collaborating among investigators from European leading academic institutions and pharmaceutical companies. The overarching objective of the DIRECT study is to discover and validate biomarkers of glycaemic deterioration before and after onset of T2D and has been reported in detail elsewhere (11, 12). DIRECT established two multicentre prospective

cohort studies comprised of adults of Northern European-ancestry; cohort 1 consisted of 2127 participants at risk for diabetes with normal or impaired glucose regulation (see Table 1) and cohort 2 consisted of 789 participants with new onset T2D (512 not treated with any diabetic medication, 273 metformin treated). The cohorts were conducted in seven study centres: Malmö Sweden, Copenhagen Denmark, Exeter UK, Newcastle UK, Dundee UK, Kuopio Finland and Amsterdam, The Netherlands. Study inclusion and exclusion criteria for cohort 1 and 2 are outlined in table S2. Screening examinations including collection of anthropometrics and blood samples were carried out the morning after a 10-hour overnight fast in the DIRECT study centres by trained nurses; metformin was omitted 24 hours prior to the examination. The study protocol has been described in detail elsewhere (11). The IMI DIRECT cohorts only collected GLP-1 biomarkers at baseline, therefore this study is a cross-sectional analysis of the baseline data.

Ethical approval

All participants provided written informed consent and the study protocol was approved by the regional research ethics review boards. The research conformed to the ethical principles for medical research involving human participants outlined in the declaration of Helsinki.

Data collection

Biochemistry assays

Fasting plasma glucose and insulin assays, fasting HbA1c, and fasting blood lipids (cholesterol, triacylglycerol, LDL and HDL-cholesterol) were measured as previously described (12). Each biochemical assay was performed using validated standard methods. Reference samples were included in all procedures to control for inter-assay variation and laboratories regularly participated in international external quality assessment schemes. Methodology is reported elsewhere (11,12). Plasma concentrations of total GLP-1 were assayed using MSD GLP-1 total kit (product code K150JVC; Meso Scale Diagnostics). Blood samples were collected at two different time points (0 and 60 min) during the 75 g frequently sampled oral glucose tolerance test (OGTT) (cohort 1) or mixed meal tolerance test (MMTT) (cohort 2). P800 tubes (Becton Dickinson, Wokingham, UK) were used to provide immediate protection from intrinsic proteolysis. This GLP-1 assay has been validated against alternative GLP-1 assays in-house and by Bak et al(13). The percentage of clinical samples with >20% coefficient of variation (CV) for the total GLP-

1 assay was 1% and 14% respectively, and inter and intra-assay variation was between 6-10% (unpublished in-house data).

Body composition

BMI was calculated as weight in kg divided by height in meters squared (kg/m^2), and waist circumference was measured at the level of the umbilicus at mid-respiration.

Magnetic resonance imaging

Whole body tissue composition was assessed using magnetic resonance imaging (MRI). Multi-echo imaging sequencing was applied to identify liver fat. The methodology has been described in detail elsewhere (14).

Dietary data

Self-reported dietary intake was assessed by 24-hour multi-pass method and a food habit questionnaire, which was filled in by each participant the day before the study visit. Detailed description of the coding and diet analysis protocol are reported elsewhere (15, 16) and in Supplementary Material (17). Investigated nutritional variables are shown in Table 3. Dietary patterns were assessed as concordance with World Health Organisation (WHO) dietary guidelines using the validated 'Healthy Diet Indicator' (HDI) (18).

T_{pred} score

Targeted metabolomic data on fasting plasma blood samples was processed using assay Absolute*IDQ*™ p150 Kit (BIOCRATES Life Sciences, Innsbruck, Austria) quantifying 163 metabolites (amino acids, acylcarnitnes, sugars, glycerophospholipids, sphingolipids) (19). These metabolites were used to build a regression model to develop the predictive metabolomic score, T_{pred}, for assessing healthiness of diets. The dietary metabolic T_{pred} score has previously been demonstrated as an objective measurement for measuring concordance with WHO dietary guidelines (16).

Statistical analysis

The association between fasting and incremental total GLP-1 concentration, glycaemic traits and associated metabolic risk markers were analysed using multivariable generalised linear models, with plasma GLP-1 as the dependent variable. Incremental total GLP-1 was calculated as postprandial 60 min total GLP-1 minus fasting total GLP-1. Cohorts 1 and 2 were analysed separately. In the baseline model, the independent variables were selected based on a backward step-wise regression. Independent variables of significance included in the baseline model were age, sex, BMI, glycaemic status (cohort 1=normal glucose tolerance (NGT), impaired fasting glucose (IFG)/ impaired glucose tolerance (IGT), screen detected diabetes), lipids (fasting triglycerides, low density lipoprotein (LDL)-and high-density lipoprotein (HDL)-cholesterol), study centre, alcohol consumption, and metformin usage (cohort 2 only). Matsuda insulin sensitivity index was derived from OGTT and MMTT data, as previously published(11, 12). To investigate the independent effects of insulin sensitivity on GLP-1 concentrations, the Matsuda index was later added as a covariate to the baseline model. MRI derived fat distribution was available in a subset of cohort 1 (n=770) and cohort 2 (n=480), and was also subsequently added to the baseline model, alone and with Matsuda index.. The variance inflation factor of all model covariates was no greater than 2.

The dietary analysis was conducted on a sub-sample with diet data from cohort 1 (n=648) and cohort 2 (n=1729). The association of dietary intake with fasting and incremental total GLP-1, was analysed using multivariable generalised linear models applying all covariates from our baseline model except for glycaemic status, which was removed in a backwards, stepwise regression.

For this analysis all continuous variables were normally transformed if needed prior to regression e.g. GLP-1 concentration (fasting and incremental), liver fat and alcohol, were log transformed; the reported coefficients were back transformed and presented as percentages. RStudio version 1.2.5033 and SAS version 9.4 (SAS Institute Inc. VX, Cary, NC, USA) were used for the analyses. The statistical significance threshold was set at $p<0.05$.

Results

Baseline characteristics of participants in the DIRECT cohorts.

Table 1 shows descriptive characteristics for the two cohorts in DIRECT; cohort 1 (at risk for T2D) and cohort 2 (diagnosed with T2D). Participants in cohort 1 had a higher percentage of men than in cohort 2, 71 % and 57%, respectively. Age did not differ between cohorts. Cohort 1 had lower adiposity markers (BMI, waist circumference and liver fat percentage), lower measures of glycaemia (fasting glucose, HbA1c), and a better lipid profile compared to cohort 2. Participants in cohort 2 had higher concentrations of fasting total GLP-1 compared to cohort 1 (median 7.4 vs 5.39 pg/ml). In cohort 2, the GLP-1 concentration was higher in metformin treated patients (median 7.92 pg/ml, n=273) compared to non-metformin treated patients (median 7.21 pg/ml, n=512), as previously described by Preiss et al.(20), thus all analyses of cohort 2 were adjusted for metformin usage.

Fasting total GLP-1 association with cardiometabolic traits, sociodemographic and lifestyle parameters.

In univariate analysis, men had a 36.4% (cohort 1) and 33.5% (cohort 2) greater fasting total GLP-1 than women (Table S3(17)). Fasting total GLP-1 increased with increasing glycaemia compared to normoglycaemia, in cohort 1.Those with screen-detected T2D had a 51.6% higher fasting total GLP-1 than those with normal glucose tolerance; a similar picture was seen in those from cohort 2 with established diabetes where fasting total GLP-1 increased with increased HbA1c. Univariately, in both cohorts, fasting total GLP-1 was increased with increasing adiposity (waist circumference and waist-to-hip ratio, but not BMI in cohort 2) and liver fat (Table S3(17)).

In the multivariable baseline model (Figure 1 and Table S4) increased fasting total GLP-1 was associated with glycaemic status IGT, IGF&IGT and SD-T2D(17). Higher fasting total GLP-1 was strongly associated with reduced insulin sensitivity in all models; inclusion of insulin sensitivity to the baseline model (Table S5) removed the association of glycaemic status with GLP-1 concentrations. The association of increased fasting total GLP-1 with obesity and male sex, remained independent of other model covariates, even after including the addition of insulin sensitivity to the base model (Table S5)(17). In an additional multivariable model, increased fasting total GLP-1 was strongly associated with liver fat (Table S6), although the effect size was markedly attenuated when insulin sensitivity was included(Table 2) (17). In this model the main independent determinants of an increased fasting

GLP-1 in cohort 1 were lower insulin sensitivity, increased BMI, higher fasting HDL, triglycerides, liver fat and male sex. In those with T2D (cohort 2) this was limited to lower insulin sensitivity, higher fasting triglycerides and male sex.

Incremental total GLP-1 association with cardiometabolic traits, sociodemographic and lifestyle parameters.

In univariate analysis, men had a 14.8% (cohort 1) and 13.4% (cohort 2) lower incremental GLP-1 than women (Table S3)(17). In both cohorts, incremental GLP-1 increased with increasing age, insulin sensitivity and HDL-Cholesterol, and reduced with increasing adiposity (BMI, waist and liver fat) (Table S3)(17). After adjustments, the incremental GLP-1 decreased with glycaemic status in cohort 1 (10.2% reduction in IGT and 15.5% reduction in screen-detected T2D) (Figure 1). This association was attenuated with the addition of insulin sensitivity to the baseline model (Table S5). The strong association of incremental GLP-1 with insulin sensitivity was independent of BMI in this cohort, with a 5% increase in incremental GLP-1 being associated with a 1 unit increase in Matsuda index . In a multivariate analysis model with liver fat, lower incremental GLP-1 was associated with higher %liver fat independent of BMI and other model parameters (Table S6)(17). When the Matsuda index was added to this model the association between incremental GLP-1 and liver fat was largely attenuated (Table 2). In this final model the main independent determinants of a reduced incremental GLP-1 in cohort 1 were lower age, lower insulin sensitivity, increased BMI, and male sex. In those with T2D (cohort 2) this was limited to lower insulin sensitivity and male sex.

Fasting and incremental total GLP-1 association with dietary intake and dietary patterns.

A less favourable diet profile was associated with a higher fasting total GLP-1 in both cohorts. In cohort 1, a higher fasting total GLP-1 was observed in participants who consumed a diet low in wholegrain (-0.06%, p=0.04), carbohydrate (-0.05%, p=0.006), fruit, and vegetable (-0.01%, p=0.02) (Table 3). Table 3 show that a higher incremental total GLP-1 in cohort 1 was associated with a consumption of higher red meat intake. No other associations were observed between incremental total GLP-1 and dietary intake in cohort 1.

In cohort 2, participants consuming a diet high in red meat (0.06%, p=0.049 and alcohol (0.15%, p=0.0003) were associated with higher fasting total GLP-1 (Table 3). The univariate model (Table S7) showed no associations

between alcohol intake and total GLP-1 but it did show that participants with a better adherence to WHO dietary guidelines were associated with a lower total fasting GLP-1 (-6.7%, p=0.04)(17). No associations were observed for incremental total GLP-1 and dietary intake in cohort 2.

Discussion

This study utilises clinical data from two large deeply phenotyped cohorts from the IMI-DIRECT consortium. Our new detailed analysis shows that increased fasting total GLP-1 is observed with male sex, increased adiposity and liver fat, and decreased insulin sensitivity particularly in the prediabetes population. In contrast we show that incremental GLP-1 decreases with worsening glycaemia and observe strong independent relationships between a lower incremental GLP-1 and higher adiposity, liver fat, male sex and reduced insulin sensitivity in the cohort at risk of T2D. We find that dietary patterns are associated with both fasting total GLP-1 but not incremental total GLP-1. These studies provide novel insights into the relationship between fasting and incremental GLP-1, metabolic traits of diabetes and obesity, and dietary intake and raise intriguing questions regarding the relevance of fasting GLP-1 in the pathophysiology T2D.

Fasting total GLP-1 is increased and incremental GLP-1 is reduced with worsening glycaemia

In the IMI-DIRECT studies we show a strong association of increased fasting total GLP-1 with worse glycaemic status – both in univariate and sex, age and BMI adjusted models. Interestingly, we found men had higher fasting total GLP-1 levels than women and the association of fasting GLP-1 with glycaemia was seen in both men and women. This data is supported by a few small studies reporting increases in fasting GLP-1 in T2D however our analysis of fasting GLP-1 is more extensive than any previous studies(5) (21-23).

Conversly, for incremental GLP-1 we show a reduction with worse glycaemic status in prediabetes (); this result is only seen in the baseline adjusted model and not univariately. The prior literature is conflicting – with smaller studies showing no effect of glycaemia on post-prandial GLP-1 response (3-5, 21, 24). However, the ADDITION-PRO study, which is most similar in scale and design to our studies, reports a similar reduction in incremental total GLP-1 with glycaemic status, but in women not men. This highlights the need to recognise the large differences in

GLP-1 concentrations between men and women and probably explains why the difference in incremental total GLP-1 is only seen inour adjusted model, that includes sex as a covariate.

Fasting total GLP-1 is increased and incremental GLP-1 is reduced with adiposity, liver fat and insulin resistance

We have shown that in both the IMI-DIRECT cohorts, that higher fasting total GLP-1 levels are seen in more insulin resistant phenotypes. The association with insulin sensitivity was independent of obesity status and liver fat, suggesting that insulin sensitivity may be a determinant of fasting GLP-1(25, 26). Adjusting for insulin sensitivity attenuated the effects of glycaemic state on fasting total GLP-1, indicating that differences in fasting GLP-1 across different levels of glycaemia may reflect differences in insulin sensitivity. Causal inference studies would be required to clarify the causal direction of insulin sensitivity with both fasting and incremental GLP-1 secretion.

Fasting total GLP-1 was positively associated with overweight and obesity in the prediabetes cohort, even when adjusted for glycaemic status or insulin sensitivity and liver fat. Our finding is consistent with smaller studies showing higher fasting GLP-1 levels in obese individuals without diabetes (27-29). Interestingly the link between higher liver fat levels and fasting GLP-1 in prediabetes can not be solely explained by increased obesity. Although it is difficult to measure GLP-1 in mice, elevated levels of fasting GLP-1 have also been seen in mouse models of obesity, including high fat diet (HFD) fed mice and ob/ob (obese mutated) mice (8, 30). Of note, an increase in L cell number has been seen by some studies with obesity, mainly involving HFD induced obesity, and this is one explanation for the relationship between raised fasting GLP-1 and obesity in prediabetes(10).

In both the IMI-DIRECT cohorts, the incremental total GLP-1 is associated with increased adiposity, liver fat and insulin resistance. This is in agreement with a twin cohort study showing that in the context of acquired obesity, lower incremental GLP-1 secretion is associated with higher adiposity and decreased insulin sensitivity(31), and the ADDITION-PRO study showing that in people with prediabetes a higher incremental GLP-1 was associated with lower adiposity (BMI and waist circumference) and better insulin sensitivity (5). In our studies, the inclusion of insulin sensitivity to the baseline model abolished the association of glucose tolerance with incremental GLP-1, suggesting that the differences seen cross sectionally by glycaemic status may reflect differences in insulin sensitivity. Inclusion of insulin sensitivity in any of the models was strongly associated with incremental GLP-1

independently of BMI and liver fat; and in the cohort 2, inclusion of insulin sensitivity removed any association of adiposity with GLP-1, suggesting that it is insulin sensitivity per se that is altering the post-prandial rise in GLP-1.

Fasting total GLP-1 is increased with a worse diet quality profile and a higher alcohol consumption

In this study, we profiled nutritional drivers in diets of individuals at risk or living with T2D to investigate if fasting and incremental GLP-1 are partly mediated by dietary intake. We found reduced relationship with fasting total GLP-1 in participants consuming a diet high in carbohydrates, wholegrain, and fruit, and vegetable. Very few studies have investigated the relationship of fasting total GLP-1 and diet. Basolo et al. also showed that fasting GLP-1 concentration was associated with lower carbohydrate intake and increases with overeating in non-diabetic participants(32). However, further studies are needed to fully understand the relationship whether wholegrain food cause fasting GLP-1 decrease or GLP-1 decrease is a compensation. The beneficial effect of fermentable dietary fibre in wholegrain, fruit and vegetables on postprandial GLP-1 regulation in the distal colon and glycaemic control has been established in both animal and human studies (33-35). The short chain fatty acid (SCFA) propionate, produced through fermentation of undigested carbohydrates or dietary fibre by the gut microbiota, has shown to alter the enteroendocrine cells and increase the number of L-cells(35). Understanding how different macronutrients and food groups influence fasting GLP-1 plasma levels and glucose homeostasis is imperative to form effective dietary guidelines in people at risk or living with T2D.

Our study also found that alcohol consumption was associated with a higher fasting total GLP-1 in people with T2D. To our knowledge, the data presented herein is the first to report on the relationship between alcohol consumption and fasting GLP-1 in humans. High alcohol intake is linked with development of T2D and is shown to affect GLP-1 secretion, lipid metabolism, and insulin secretion in people with T2D (36, 37). It is unknown what is the driving mechanism behind its relationship with fasting total GLP-1. Dalgaard et al. showed decreased postprandial GLP-1 in people with T2D after a meal with alcohol, which may be mediated by the interplay between the GLP-1 and lipid metabolism (free fatty acids)(36).

We did not find any significant associations between incremental total GLP-1 and differences in dietary patterns, except for a link with red meat intake in cohort 1. Of note the GLP-1 increments were evaluated at 60min after a

standardised stimulus (oral glucose in cohort 1 and a liquid mixed meal in cohort 2), thus we would not anticipate this relationship reflecting any direct impact of differences in diet on GLP-1 secretion.

Role of fasting GLP-1 in physiology

With accumulating evidence for the association of raised fasting GLP-1 with prediabetes, obesity and insulin sensitivity further research is needed to uncover the underlying mechanism in order to understand the relevance of this association. One possibility is increased basal secretion. In humans, plasma GLP-1 is secreted from L-cells as active GLP-1(7-36)amide before being metabolised by DPP4 to the 'inactive' GLP-1(9-36)amide. In the fasting state most GLP-1 would be expected to be metabolised to the so called 'inactive'form with the total assay reflecting this. As active GLP-1(7-36)amide and inactive GLP-1(9-36)amide are both renally cleared and elevated levels are seen with decreased renal function (2, 38) we included creatinine clearance in our models with no impact on the results (data not shown). Evidence for continuous GLP-1 basal secretion has been demonstrated when fasting GLP-1 levels were lowered with somatostatin, also known for its paracrine regulation of postprandial GLP-1 secretion(39). However, the contribution of this secretion to the fasting total GLP-1, or the role of 'inactive' GLP-1(9-36)amide, is unknown. Interestingly there is evidence to suggest that 'inactive'GLP-1(9-36)amide is an outdated misnoma. Mounting research suggests GLP-1 receptor independent effects of GLP-1(9-36)amide exist that are different to the GLP-1 receptor mediated actions of GLP-1(7-36)amide(40). In T2D the association with metformin is previously described and suggests a possible stimulation of secretion as well as weak DPP4 inhibition by metformin(20). These results suggest that insulin sensitivity may be correlated with raised fasting GLP-1 secretion rather than clearance. Potential mechanisms for increased basal secretion of GLP-1 could also involve altered microbiome influencing macronutrient stimulated signalling in the gut, a direct effect of insulin action on L-cells, or more controversially pancreatic alpha-cell GLP-1 production or even beta-cell GLP-1 resistance in the insulin resistant state(2). Data herein has provided an understanding of the factors associated with the development of fasting GLP-1 in populations with prediabetes, however further analysis is needed to clarifying the directionality of its relationship with insulin sensitivity, for example using mendelian randomisation.

Limitations

There are several commercially available kits for measuring total GLP-1 and this may influence interstudy variability as investigated by Bak et al.(13). Our analysis uses data from the Meso Scale Diagnostics total GLP-1 kit. This kit detects all six isoforms of GLP-1 but it predominantly detects isoform GLP-1(7-36) and thus may underestimate the true circulating values of GLP-1(13). We have, however, established that this assay has no cross reactivity with glucagon (data not shown) which could potentially have confounded our results.

The associations of metabolic traits with fasting GLP-1 were largely the converse of those seen with incremental GLP-1, suggesting that the differences seen with incremental GLP-1 could have been secondary to the alteration in the baseline concentrations, as there was little variation in absolute post-prandial levels across many of these traits. However, adjusting for baseline GLP-1 concentrations had little impact on the incremental associations described above (data not shown). Furthermore, we only measured GLP-1 at two time points – 0 min (fasting) and 60 min (post glucose or liquid mixed meal). This was largely a pragmatic decision due to practicality and cost given the ~3000 participants being studied, but it would have potentially been more informative to include additional time points prior to the 60-min measure to capture peak secretion, and additional time points after to capture GLP-1 clearance.

Limitations to our study design inhibits the direct comparability of post-prandial GLP-1 between our two study populations. Furthermore, our study population of prediabetes (cohort 1) is a mixed population of people at-risk of T2D and healthy. Hence, cohort 1 analyses included glycaemic status. Another important limitation to our study is that the associations between fasting GLP-1 and glycaemic status, insulin sensitivity, obesity and diet do not assess causality, temporality with progression of diabetes, or physiological role of fasting GLP-1 in terms of GLP-1 active(7,36): inactive metabolite(9,36) plasma levels.

Summary

In summary, increased fasting total GLP-1 is associated with less favourable glycaemic, adiposity and cardio-metabolic markers in both individuals at-risk of, and living with, T2D. These associations may be partly driven by a worse dietary pattern low in fruit, vegetables, and wholegrain and high in red meat and alcohol. This is in contrast

to incremental total GLP-1 which is associated with lower adiposity, liver fat and better insulin sensitivity in those at risk of T2D. Future studies are required to investigate the causal and biological mechanisms for these findings, particularly in light of the fasting GLP-1 associations, which may provide insight into the pathophysiological processes in the incretin axis in those at risk of, and with established, diabetes.

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Declarations of interests

The views expressed in this article are those of the author(s) and not necessarily those of the NHS, the NIHR, or the Department of Health. MMcC has served on advisory panels for Pfizer, NovoNordisk and Zoe Global, has received honoraria from Merck, Pfizer, Novo Nordisk and Eli Lilly, and research funding from Abbvie, Astra Zeneca, Boehringer Ingelheim, Eli Lilly, Janssen, Merck, Novo Nordisk, Pfizer, Roche, Sanofi Aventis, Servier, and Takeda. As of June 2019, MMcC and AM are employees of Genentech, and a holder of Roche stock. PWF has received consulting honoraria from Eli Lilly and Novo Nordisk A/S. He has also received research grants from multiple pharmaceutical companies and is a consultant and stock owner in Zoe Global Ltd. He is currently the Scientific Director in Patient Care at the Novo Nordisk Foundation.

Author contributions

GF, EP, MW, RE, and AD formulated the research questions and methodological design; MW, RE and AD were responsible for data analysis. RE and MW were responsible for drafting of the manuscript. GF, EP, MW, RE, and AD contributed to the interpretation of results and final manuscript. MW and AD were responsible for baseline characterisation of plasma GLP-1. RE contributed to the dietary data analysis, coding and quality control. EH, IGP and JMP were responsible for the validation of the metabolomic data extracts used in the analyses and constructing the T_{pred} score. MH, SS, CP and JA were responsible for the metabolomics measurements and analysis. AV and JF were responsible for data analysis and quality checking of the metabolomic dataset. Additionally, study design and coordination were contributed by RWK, JA, Jbel, Jbeu., Sbru, GF, TH, AH, ML, Amar, TJMcD, OP, JMS, HJAT, Amah, MIMcC, HR, MW, EP, MH and IP. PWF, RWK, GNG, TW, Jbel, Jbeu, Sbra, Fde M, IMF, GF, THH, TK, AK, Amar, TJMcD, FR, ELT, AV and Amah contributed to sample assaying, data analysis/processing and/or data quality control procedures. RWK, GNG, IMF, THH, TH, AH, TK, ML, Amar, TJMcD, OP, FR, Jbeu, MW, EP and PWF contributed to quality control and data collection at study centres. All authors contributed to drafting the article and/or revising it critically for important intellectual content. All authors approved the final version of the manuscript. All authors accept responsibility for all aspects of the work insofar as ensuring that questions related to the accuracy or integrity of any part of the article are appropriately investigated and resolved.

Data availability

The clinical and molecular raw data as well as the processed are available under restricted access due to the informed consent given by study participants, the various national ethical approvals for the present study, and the European General Data Protection Regulation (GDPR), individual-level clinical and molecular data cannot be transferred from the centralized IMI-DIRECT repository. Requests for access will be informed on how data can be accessed via the DIRECT secure analysis platform following submission of an appropriate application. The IMI-DIRECT data access policy is available at https://directdiabetes.org.

Supplemental results are available in a repository as detailed in the reference(17).

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Figure 1: Independent effects of age, sex, BMI, glucose tolerance, lipids, alcohol, centre and metformin in the baseline model for total GLP-1

Diagrammatic representation of the multivariable baseline regression model (supplementary table 12). % difference represents percentage changes in total GLP-1 per one unit change in independent variable adjusted for other model covariates: age, sex, BMI, glucose tolerance (cohort 1 only), lipids, alcohol and centre. Black = Fasting Total GLP-1, (cohort 1, n=2226; cohort 2, n=739). Blue= Incremental Total GLP-1 (cohort 1, n=2207; cohort 2, n=739). Figure A. represents data from Cohort 1 = participants with a range of prediabetes glucose tolerances, including: Impaired fasting glucose (IFG), Impaired glucose tolerance (IGT), both IFG and IGT (IFG&IGT) and those with screen detected Type 2 Diabetes (SD-DM). Figure B. represents data from Cohort 2 = participants with Type 2 diabetes. Reference groups = Normal glucose tolerance for IFG, IGT, IFG&IGT, SD-DM. Normal weight for obesity and overweight, No alcohol intake for occasional and regular alcohol status.

Table 1: Study population baseline characteristics in the IMI-DIRECT cohorts

Abbreviations: Cohort 1, participants at risk for diabetes; cohort 2, participants with recently diagnosed type 2 diabetes; T_{pred}, metabolic profile score; HDI, Healthy Diet Indicator (World Health Organisation diet score); HDL, high density lipoprotein; LDL, low density lipoprotein; HbA1c, glycated haemoglobin. [a] Values are unadjusted means (standard deviation) or n (%) except †, which are medians (interquartile range). *Sample size for cohort 1 n=1785, for cohort 2 n=688.

Table 2. Total GLP-1 independent association with cardiometabolic traits, sociodemographic and lifestyle parameters adjusted for age, sex, BMI, glucose tolerance, lipids, alcohol, centre, metformin, liver fat and insulin sensitivity

[a] Multivariable linear regression model % difference represents percentage changes in fasted total GLP-1 per one unit change in independent variable adjusted for other listed model covariates age, sex, BMI, glucose tolerance(cohort 1 only), lipids, alcohol, centre, metformin (cohort 2 only) and liver fat. Ref= reference group, Impaired fasting glucose (IFG), Impaired glucose tolerance (IGT), Impaired fasting glucose and Impaired glucose tolerance (IFG&IGT), Screen detected- diabetes mellitus (SD-DM). BMI categories, normal weight, overweight and obese.

Table 3: Total GLP-1 association with dietary intake adjusted for age, sex, BMI, alcohol, lipids, centre and metformin

[a] Multivariable linear regression model % difference represents the % difference in total GLP-1 per one-unit change in nutritional variable adjusted for age, sex, BMI, study centre, and metformin (cohort 2 only). Abbreviation: Cohort 1; participants at risk for diabetes, cohort 2; participants with diabetes type 2, NSP, non-starch polysaccharides, HDI, Healthy Diet Indicator World Health Organisation diet score; T_{pred}, metabolic profile score. §Not adjusted for alcohol.

Table 1: Study population baseline characteristics in the IMI-DIRECT cohorts

	Cohort 1 (n =2226)		Cohort 2 (n =789)	
	Mean [a] or n	SD or %	Mean [a] or n	SD or %
Sex (male) %	1383	71.7	448	57.1
Age (years)	62.0	6.5	62.0	8.1
Adiposity traits				
Body mass index (kg/m^2)	28.0	4.0	30.6	5.0
Weight (kg)	84.9	13.4	89.4	16.9
Waist circumference (cm)	99.7	10.9	103.2	12.2
Liver fat (%) †	3.3	5	6.1	9.2
Diet quality*				
T_{pred} metabolic score [range -3.5, 3.5]	-0.7	0.8	-0.5	0.8
HDI diet score [range 0, 12]	4.4	2.7	4.7	2.6
Daily energy intake (kcal)	1987	666.5	1816.8	629.6
Alcohol %*				
No alcohol	1410	78.9	510.0	74.1
Within UK guidelines	235	13.2	87.0	12.6
Above UK guidelines	140	7.8	91.0	13.2
Cigarette smoking %				
Never	933	48.5	374.0	49.5
Former	733	38.1	280.0	37.0
Current	258	13.4	102.0	12.5
GLP-1				
Fasting total GLP-1 (pg/ml) †	5.39	4.6	7.4	7.03
Incremental 60 min total GLP-1 (pg/ml) †	13.17	9.59	16.1	11.08
Cardiometabolic traits				
Matsuda index	4.91	3.07	2.97	2.22
Fasting glucose (mmol/L)			7.1	1.4

Normal glucose tolerance (NGT) (mmol/L), n=1539	5.4	0.3		
Impaired fasting glucose (IFG) (mmol/L), n=335	6.4	0.2		
Impaired glucose tolerance (IGT) (mmol/L), n=178	5.5	0.4		
IFG&IGT, n=109	6.4	0.2		
Screen detected-diabetes mellitus (SD-DM), n=88	6.9	0.9		
Fasting insulin (pmol/L)	67.8	48.6	106.1	70.2
HbA1c % (mmol/mol)	5.5 (37.2)	0.28 (3.1)	6.4 (46.5)	0.53 (5.8)
Fasting triglycerides (mmol/L)	1.4	0.6	1.5	0.8
Fasting LDL-cholesterol (mmol/L)	3.3	0.9	3.4	0.9
Fasting HDL-cholesterol (mmol/L)	1.3	0.3	1.2	0.4

Abbreviations: Cohort 1, participants at risk for diabetes; cohort 2, participants with recently diagnosed type 2 diabetes; T_{pred}, metabolic profile score; HDI, Healthy Diet Indicator (World Health Organisation diet score); HDL, high density lipoprotein; LDL, low density lipoprotein;

HbA1c, glycated haemoglobin. [a] Values are unadjusted means (standard deviation) or n (%) except †, which are medians (interquartile range)

*Sample size for cohort 1 n=1785, for cohort 2 n=688.

Table 2. Total GLP-1 independent association with cardiometabolic traits, sociodemographic and lifestyle parameters adjusted for age, sex, BMI, glucose tolerance, lipids, alcohol, centre, metformin, liver fat and insulin sensitivity

Variable	Fasted total GLP-1						Incremental total GLP-1					
	Cohort 1 (n=770)			Cohort 2 (n=480)			Cohort 1 (n=770)			Cohort 2 (n=480)		
	% difference[a]	95% CI	*p* value	% difference[a]	95% CI	*p* value	% difference[a]	95% CI	*p* value	% difference[a]	95% CI	*p* value
Glycaemic & cardiometabolic traits												
Normal glucose tolerance (NGT) (mmol/L)	Ref						Ref					
Impaired fasting glucose (IFG) (mmol/L)	-7.04	-16.0, 2.84	0.15				6.48	-4.02, 18.1	0.24			
Impaired glucose tolerance (IGT) (mmol/L)	10.8	-3.26, 27.0	0.13				-8.15	-20.1, 5.56	0.23			
IFG & IGT	-5.06	-20.0, 12.7	0.55				-7.95	-22.8, 9.71	0.35			
SD- DM	5.26	-12.8, 27.0	0.59				10.6	-8.73, 34.1	0.30			
Fasting LDL (mmol/L)	-2.16	-6.09, 1.95	0.29	-5.92	-12.4, 1.05	0.09	0.97	-3.20, 5.31	0.65	0.76	-4.51, 6.31	0.75
Fasting HDL (mmol/L)	17.4	4.10, 12.8	0.008	9.85	-8.96, 32.6	0.33	8.42	-4.13, 22.6	0.20	15.9	0.71. 33.5	0.04
Fasting Triglycerides (mmol/L)	11.6	5.56, 19.1	0.001	11.1	2.10, 20.9	0.01	-3.29	-9.51, 3.37	0.32	3.71	-2.68, 10.5	0.26
Matsuda Index	-6.17	-7.57, 4.75	<.0001	-7.39	-10.2, -4.54	<.0001	4.32	2.72, 5.94	<.0001	4.52	2.17, 6.92	0.0002
Adiposity traits												
Normal weight (BMI<25 kg/m^2)	Ref						Ref			Ref		
Obesity (BMI 25-30 kg/m^2)	15.6	1.93, 31.2	0.02	5.50	-16.6, 33.4	0.65	-18.2	-28.1, -6.84	0.002	-5.34	-20.6, 12.9	0.54

Overweight (BMI >30 kg/m^2)	6.06	-3.79, 16.9	0.23	4.09	-16.0, 29.0	0.71	-10.1	-18.6, -0.59	0.04	-10.4	-23.7, 5.28	0.18
Liver fat (%)	0.07	0.02, 0.12	0.006	0.02	-0.07, 0.11	0.62	-0.07	-0.12, -0.02	0.010	-0.02	-0.09, 0.05	0.54
Sociodemographic & lifestyle factors												
Sex- Male	29.4	14.4, 46.3	<.0001	33.7	16.0, 54.1	<.0001	-31.2	-39.4, -21.9	<.0001	-15.5	-24.1, -5.97	0.001
Age (years)	0.21	-0.36, 0.77	0.47	-0.05	-0.86, 0.76	0.90	1.23	0.64, 1.82	<.0001	0.36	-0.25, 0.97	0.25
Alcohol- no alcohol	Ref						Ref			Ref		
Alcohol status -occasional	-5.93	-18.0, 7.97	0.38	-3.05	-20.5, 18.2	0.76	-3.46	-16.2, 11.2	0.62	-6.96	-19.9, 8.06	0.34
Alcohol status- regular	-7.17	-17.5, 4.50	0.22	-7.05	-22.6, 11.6	0.43	2.20	-9.47, 15.4	0.72	-6.96	-18.9, 6.80	0.30
Metformin (cohort 2 only)				14.3	-2.00, 33.2	0.09				-6.97	-17.1, 4.42	0.22

[a] Multivariable linear regression model % difference represents percentage changes in fasted total GLP-1 per one unit change in independent variable adjusted for other listed model covariates age, sex, BMI, glucose tolerance(cohort 1 only), lipids, alcohol, centre, metformin (cohort 2 only) and liver fat. Ref= reference group, Impaired fasting glucose (IFG), Impaired glucose tolerance (IGT), Impaired fasting glucose and Impaired glucose tolerance (IFG&IGT), Screen detected- diabetes mellitus (SD-DM). BMI categories, normal weight, overweight and obese.

Table 3: Total GLP-1 association with dietary intake adjusted for age, sex, BMI, alcohol, lipids, centre and metformin

	Fasted total GLP-1						**Incremental total GLP-1**					
	Cohort 1 (n=1729)			**Cohort 2 (n=648)**			**Cohort 1 (n=1729)**			**Cohort 2 (n=648)**		
	% difference [a]	95% CI	*P* value	% difference [a]	95% CI	*P* value	% difference [a]	95% CI	*P* value	% difference [a]	95% CI	*P* value
Total fat (g)	0.02	-0.06, 0.11	0.53	0.12	-0.04, 0.27	0.14	0.07	-0.005, 0.14	0.07	-0.04	-0.16, 0.08	0.49
Saturated fat (g)	0.01	-0.20, 0.17	0.89	0.11	-0.26, 0.48	0.57	0.15	-0.012, 0.31	0.07	-0.07	-0.34, 0.20	0.58
Protein (g)	-0.04	-0.12, 0.04	0.29	0.05	-0.11, 0.21	0.51	0.006	-0.061, 0.073	0.85	0.08	-0.03, 0.20	0.17
Carbohydrate (g)	-0.05	-0.08, -0.01	0.006	0.01	-0.08, 0.05	0.68	-0.006	-0.04, 0.02	0.70	0.01	-0.04, 0.05	0.81
Fibre NSP (g)	-0.34	-0.75, 0.07	0.11	-0.44	-1.10, 0.39	0.26	0.18	-0.18, 0.54	0.34	0.06	-0.61, 0.50	0.84

Wholegrain (g)	-0.06	-0.12, -0.002	0.04	-0.02	-0.15, 0.11	0.73	0.003	-0.05, 0.05	0.91	0.03	-0.06, 0.13	0.46
Fruit & vegetable (g)	-0.01	-0.02, -0.003	0.02	-0.02	-0.04, 0.002	0.07	0.006	-0.003, 0.02	0.21	-0.009	-0.02, 0.006	0.23
Red meat (g)	0.01	-0.02, 0.04	0.44	0.06	-0.002, 0.12	0.05	0.03	0.0001, 0.05	0.04	0.02	-0.02, 0.06	0.50
Mean energy (kcal)	-0.003	-0.007, 0.002	0.26	0.003	-0.006, 0.01	0.54	0.002	-0.003, 0.005	0.45	0.0004	-0.006, 0.006	0.99
Alcohol (g) §	-0.21	-5.30, 4.88	0.93	0.15	0.07, 0.23	0.0003	0.006	-0.03 0.04	0.78	-0.02	-0.08, 0.06	0.67
HDI diet score	-0.47	-1.56, 0.63	0.40	-0.92	-3.02, 1.16	0.39	0.05	-0.87, 1,01	0.92	0.14	-1.37, 1.65	0.89
T_{pred} metabolic score	0.73	-2.84, 4.29	0.7	-2.10	-9.32, 5.14	0.57	-0.75	-3.9, 2.39	0.64	-1.13	-2.35, 4.09	0.67

[a] Multivariable linear regression model % difference represents the % difference in total GLP-1 per one-unit change in nutritional variable adjusted for age, sex, BMI, study centre, and metformin (cohort 2 only). Abbreviation: Cohort 1; participants at risk for diabetes, cohort 2; participants with diabetes type 2, NSP, non-starch polysaccharides, HDI, Healthy Diet Indicator World Health Organisation diet score; T_{pred}, metabolic profile score. §Not adjusted for alcohol.