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Glucagon-like peptide-1 secretion in people with *versus* without type 2 diabetes: a systematic review and meta-analysis of cross-sectional studies

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ABSTRACT

Aims/Hypothesis: The aim of this systematic review was to synthesise the study findings on whether GLP-1 secretion in response to a meal tolerance test is affected by the presence of type 2 diabetes (T2D). The influence of putative moderators such as age, sex, meal type, meal form, and assay type were also explored.
Methods: A literature search identified 32 relevant studies. The sample mean and SD for fasting GLP-1_{TOTAL} and GLP-1_{TOTAL} iAUC were extracted and used to calculate between-group standardised mean differences (SMD), which were meta-analysed using a random-effects model to derive pooled estimates of Hedges' g and 95 % prediction intervals (PI).
Results: Pooled across 18 studies, the overall SMD in GLP-1_{TOTAL} iAUC between individuals with T2D ($n = 270$, 1047 ± 930 pmol·L^{-1}·min) and individuals without T2D ($n = 402$, 1204 ± 937 pmol·L^{-1}·min) was very small, not statistically significant and heterogenous across studies ($g = -0.15$, $p = 0.43$, PI: −1.53, 1.23). Subgroup analyses demonstrated an effect of assay type whereby Hedges' g for GLP-1 iAUC was greater in individuals with, *versus* those without T2D when using ELISA or Mesoscale ($g = 0.67$ [moderate], $p = 0.009$), but not when using RIA ($g = -0.30$ [small], $p = 0.10$). Pooled across 30 studies, the SMD in fasting GLP-1_{TOTAL} between individuals with T2D ($n = 580$, 16.2 ± 6.9 pmol·L^{-1}) *versus* individuals without T2D ($n = 1363$, 12.4 ± 5.7 pmol·L^{-1}) was small and heterogenous between studies ($g = 0.24$, $p = 0.21$, PI: −1.55, 2.02).
Conclusions: Differences in fasting GLP-1_{TOTAL} and GLP-1_{TOTAL} iAUC between individuals with, *versus* those without T2D were generally small and inconsistent between studies. Factors influencing study heterogeneity such as small sample sizes and poor matching of groups may help to explain the wide prediction intervals observed. Considerations to improve comparisons of GLP-1 secretion in T2D and potential mediating factors more important than T2D diagnosis *per se* are outlined. PROSPERO ID: CRD42020195612.

1. Introduction

The 'incretin effect' is the amplification of insulin secretion observed following oral ingestion *versus* intravenous (isoglycaemic) glucose administration [1]. This effect is mainly mediated by the incretin hormones, GIP and GLP-1. GIP and GLP-1 are peptide hormones secreted by intestinal K- and L-cells, respectively [2]. These hormones both stimulate glucose-dependent insulin secretion, while GLP-1 also reduces the rate of gastric emptying and suppresses food intake [3,4]. In individuals with type 2 diabetes (T2D), the incretin effect is, in spite of a preservation of GIP secretion, reduced in comparison to individuals without T2D, which contributes to reduced glucose tolerance [5]. This reduction could be due to either 1) lower GLP-1 release following meals, and/or 2) reduced beta-cell sensitivity to GIP and/or GLP-1. Unlike GIP,

Abbreviations: BMI, Body mass index; CI, Confidence interval; ELISA, Enzyme-linked immunosorbent assay; GIP, Glucose-dependent insulinotropic peptide; GLP-1, Glucagon-like peptide-1; iAUC, Incremental area under the curve; MMTT, Mixed meal tolerance test; MPGF, Major proglucagon fragment; OGTT, Oral glucose tolerance test; PI, Prediction interval; RIA, Radioimmunoassay; SD, Standard deviation; SGLT-1, sodium-glucose transporter protein-1; SMD, Standardised Mean Difference; T, Tau; T2D, Type 2 diabetes; tAUC, Total area under the curve.

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the insulinotropic action of GLP-1 is partially preserved in individual's with T2D [6,7] although its potency is clearly impaired [8]. Nevertheless, reduced GLP-1 secretion could account for some of the reduced incretin effect observed amongst individuals with T2D.

Despite the plausibility of the above notion, there have been conflicting findings suggesting that GLP-1 secretion is reduced [9,10], similar [11], and even increased [12] in individuals with T2D compared to individuals without T2D. The authors of two previous meta-analyses concluded that there was no evidence of reduced GLP-1 secretion in individuals with T2D *versus* matched individuals without T2D [13,14]. Despite these conclusions, there is still general uncertainty in the literature, especially after further relevant data have been published since these meta-analyses were undertaken. There have also been recent calls for meta-analyses to incorporate considerations of prediction intervals [15] and double-counting of control groups from multiple treatment groups being present in some studies [16]. The following sections will outline the new aspects of this meta-analysis including fasting GLP-1, meal type and form, and assay type.

In contrast to postprandial GLP-1, fasting GLP-1 concentrations have yet to be analysed in a systematic review. Fasting GLP-1 concentrations have been demonstrated to be greater in adolescent and adult men and women with overweight/obesity compared to lean individuals [17,18], with fasting GLP-1 concentrations positively associated with BMI, fasting insulinemia, and HOMA-IR [17]. If individuals with T2D also display greater fasting GLP-1 concentrations, this may lead to de-sensitisation of GLP-1 releasing L-cells, leading to lower meal-induced GLP-1 release [19]. This could also have important implications for the use of total area under the curve (tAUC) to measure meal-induced GLP-1 release, since this may be influenced to a major extent by variations in the fasting concentrations.

Such variability in the literature regarding GLP-1 secretion in individuals with T2D may also suggest the presence of moderator variables. One such variable may be related to meal type, given the wide array of signalling pathways involved in GLP-1 secretion. Glucose-mediated GLP-1 secretion is not known to be dependent on receptor activation, but appears to depend on absorption *via* SGLT-1 [20–22], whereas fat- and protein- mediated GLP-1 secretion relies primarily on the activation of a number of nutrient sensors and transporters expressed in enteroendocrine cells [23–25].

Another important factor is the measurement of GLP-1 which is not straightforward due to its rapid and extensive degradation in the circulation, the low concentrations, and the specificity required of assays to measure the relevant GLP-1 isoforms [2]. GLP-1_{TOTAL}, comprising of the intact form and the primary metabolite, is therefore a more appropriate measure compared to the rapidly degraded intact GLP-1 moiety for estimations of secretion. GLP-1_{TOTAL} can be measured using single antibody assays with antibodies specific for the amidated C-terminus [26]. Because of the importance of antisera directed against specific GLP-1 epitopes, the majority of studies employ a well-defined radioimmunoassay [27]. Commercially available antisera and assay kits are often poorly characterised which limits the certainty of which GLP-1 isoforms are detected [28]. As a result, commercially available assays tend to exhibit varying specificity [29], which suggests the type of assay may influence the quantified difference in GLP-1 secretion between individuals with and without T2D. A particular problem applies to the use of the sandwich ELISA which, despite its potentially superior sensitivity, depends on two binding epitopes of the molecule. Given the extensive degradation of GLP-1 from both termini, this almost invariably affects the binding of the antibodies, which is why the assays exhibit serious variability in specificity for the various isoforms [2]. Another potential problem is sample pre-treatment. Some assays [9,30] use plasma extraction to remove potentially interfering substances. This pre-treatment approach may markedly influence both fasting levels and meal responses [2].

The aim of the present review was to synthesise the published literature in order to determine whether GLP-1 secretion is reduced in individuals with *versus* without T2D in response to oral glucose and/or mixed meal ingestion, and whether there are any differences in fasting GLP-1 concentrations between study samples. The putative roles of potential mediating factors (*e.g.* meal composition and form, and assay type) were also explored.

2. Methods

This systematic review and meta-analysis was undertaken in alignment with the PRISMA (Preferred Reporting Items for Systematic Review and Meta-analyses) guidelines [31], and was prospectively registered with PROSPERO (CRD42020195612).

2.1. Literature search

Eligible studies were identified by electronic and manual searches in literature references. Electronic searches were conducted *via* the Web of Science, PubMed, Cochrane Library, and Embase from July 2020 through November 2022. The search terms included ("glucagon-like peptide-1" OR "GLP-1") AND ("secretion" OR "release") AND ("type 2 diabetes") AND ("human*" OR "men" OR "man" OR "women") AND ("OGTT" OR "MMTT" OR "protein" OR "fat" OR "carbohydrate" OR "meal" OR "postprandial"). Eligible studies were assessed independently by two authors according to inclusion criteria. There were no restrictions of the publication date of studies, however studies were required to be in English or able to be translated.

2.2. Inclusion and exclusion criteria

In general, clinical trials measuring GLP-1_{TOTAL} responses to a test meal in individuals with T2D *versus* individuals without T2D were required. For inclusion, studies had to meet the following requirements:

- participants were over the age of 18
- participants did not have >1 comorbidity unrelated to metabolic syndrome
- Individuals with T2D were clinically diagnosed with fasting glucose and/or 2-h glucose >7.0 and >11.1 mmol·L^{-1}, respectively (American Diabetes Association, 2015)

Furthermore, studies were excluded if they:

- used non-specific assays which cross-react with the major proglucagon fragment
- used intact GLP-1 measurements only
- were not peer reviewed
- did not have enough data

All studies were required to involve measurements of either fasting plasma GLP-1 concentrations, or plasma GLP-1_{TOTAL} iAUC in response to a meal challenge in individuals clinically diagnosed with T2D and individuals without T2D. Studies that employed an intravenous infusion in parallel with a test meal were considered eligible if they included a placebo infusion condition and studies involving weight-loss surgery were included if they employed a test meal for a group with T2D and a matched control group prior to surgery. Two researchers (JW and SC) independently assessed studies for inclusion and any disagreements were resolved by a third reviewer (JG). Potential studies that could be included based on their title or abstract were retrieved in full-text and reviewed against the inclusion/exclusion criteria independently by JW and SC. Similarly, JG was consulted in the event of any disputes. In total, 32 studies met criteria for inclusion and were included in this systematic review and meta-analysis (Fig. 1).

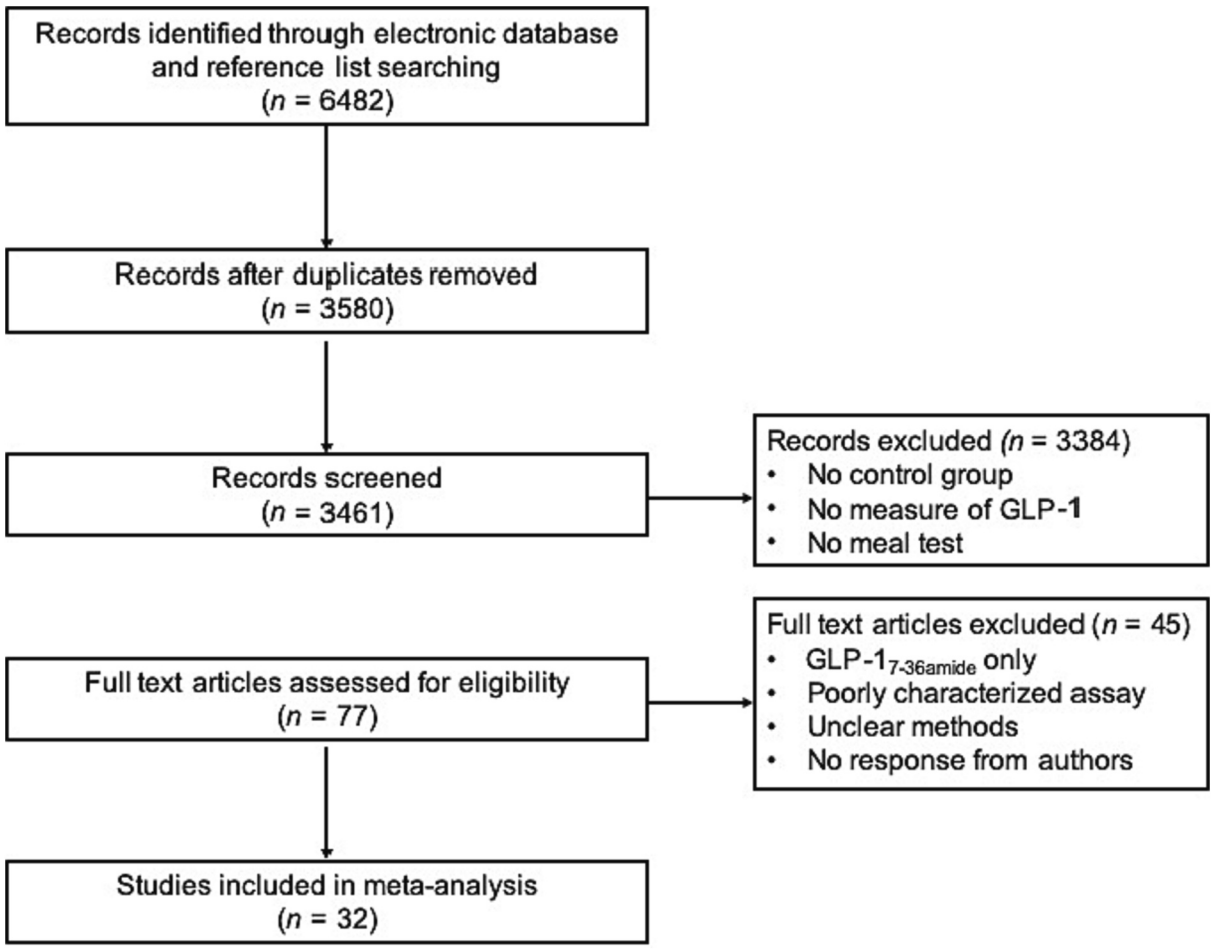

Fig. 1. PRISMA flow diagram of the study screening and selection process.

2.3. Data extraction

From eligible published studies, data (mean and SD) were extracted independently by two researchers (JW and SC). Data were extracted into a standardised spreadsheet, which included characteristics of articles, outcome data, and conditions for the Cochrane collaboration's tool for assessing risk of bias [32]. The participant characteristics, assay employed, meal characteristics, fasting plasma glucose and GLP-1, and plasma GLP-1_{TOTAL} iAUC were collected. Where it was not possible to extract this information, authors were contacted for clarification.

Where GLP-1_{TOTAL} values were reported in $pg{\cdot}ml^{-1}$, values were converted to $pmol{\cdot}L^{-1}$ by dividing by 4. When values were only presented in figure form, the figure was digitised and the means and SD/SE were measured manually to the scale provided on the figure. SEs were converted to SDs using $SD = SE \sqrt{n}$.

2.4. Assessment of risk of bias in included studies

The Cochrane risk of bias tool was implemented to assess internal validity of eligible studies [32] independently by two researchers (JW and SC). Each study was assessed in the following 6 domains: incomplete outcome data, selective reporting, group similarity at baseline, intention to treat analysis, timing of outcome assessments, and other bias *e.g.* conflicts of interest, sample size. Each domain was judged to be either high, low or unclear (if insufficient detail was provided) risk. Disagreements were resolved by a third reviewer (JG). The following domains were not deemed appropriate for the research designs of included studies (because the outcome measure is not subject to conscious bias): random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, and co-intervention and compliance bias and were omitted from the risk of bias (Fig. 2).

2.5. Statistical analysis

Missing standard deviations were estimated from reported standard errors or confidence intervals according to Cochrane Handbook guidelines. [33] Reported mean intervention effects (quantified using the Hedges g standardised mean difference) were pooled in a random effects meta-analysis using Stata 16 software (StataCorp LLC, TX, USA). Random effects meta-analysis models, rather than a fixed effects models, were selected *a priori* based on the philosophical absence of a consistent "true effect" across the studies [34]. A random effects model was selected as it was more realistic and appropriate to assume that the true effect could vary from study to study. Nevertheless, fixed effects sensitivity analyses were undertaken as these can help interpret the seriousness of any small study effects (publication bias) that are detected [33].

Data entry was carried out for all results by JW & SC and peer-reviewed by GA. Study effect sizes were weighted using the inverse variance approach. The restricted maximum likelihood approach was adopted with the Knapp-Hartung modification to standard errors applied [35]. Ninety-five percent confidence intervals (CIs) and prediction intervals (PI) were calculated for group differences in GLP-1 iAUC and fasting GLP-1. The PI is better aligned to the selection of a random effects model than a CI. A PI quantifies the likely range in which a new study's effect size will fall into, assuming that this future study is of a similar nature to those meta-analysed [15]. A meta-analysis was

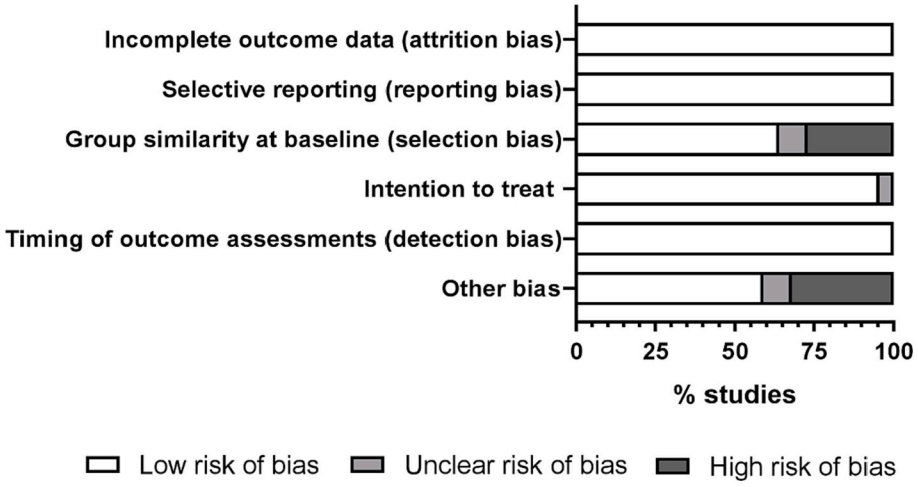

Fig. 2. Judgement about each risk of bias domain presented as a percentage across all included studies.

undertaken only where there were at least five studies to ensure minimally acceptable statistical power and relatively precise inferences [36].

I-squared and tau statistics were used to quantify between-study heterogeneity in the meta-analyses. Tau (T) is the estimated standard deviation of underlying true effects across studies [34].

"Double counting" is an important consideration in any meta-

Table 1
Study characteristics.

Reference	Outcomes	Sample size		Meal test	Meal dose (kcal)	Duration of test (min)	Assay
		T2D (n = 628)	C (n = 1409)				
Plourde et al. 2014 [39]	Fasting, iAUC	9	9	MMTT[b]	295	180	ELISA
Alsalim et al. 2018 [40]	Fasting	12	12	–	–	400	ELISA
Nauck et al. 2016 [41]	iAUC	32	29	MMTT[b]	541	240	RIA
Faerch et al. 2015 [10]	Fasting	163	774	–	–	120	RIA
Kozawa et al. 2010 [42]	Fasting	4	5	–	–	180	RIA
Muscelli et al. 2008 [43]	Fasting	10	24	–	–	180	RIA
Bagger et al. 2011 [44]	Fasting	8	8	–	–	240	RIA
Alsalim et al. 2016 [45]	Fasting	18	18	–	–	300	RIA
Jørgensen et al. 2012 [46]	Fasting, iAUC	13	12	MMTT[a]	300	240	RIA
Knop et al. 2007a [47]	Fasting, iAUC	8	8	OGTT[a]	200	240	RIA
Vilsbøll et al., 2001 [48]	Fasting	12	12	–	–	180	RIA
Sonne et al. 2013 [49]	Fasting, iAUC	15	15	OGTT[a]	300	240	RIA
				Low Fat MMTT[a]	500		
				Mod Fat MMTT[a]	500		
				High Fat MMTT[a]	500		
Toft-Nielsen et al. 2001 [9]	Fasting, iAUC	54	33	MMTT[b]	538	240	RIA
Theodorakis et al. 2006 [50]	Fasting	17	36	–	–	120	RIA
Højberg et al. 2008 [51]	Fasting, iAUC	9	9	MMTT[b]	538	240	RIA
Yabe et al. 2015 [52]	Fasting	28	54	–	–	120	RIA
Ryskjaer et al. 2006 [53]	Fasting, iAUC	8	8	MMTT[b]	566	180	RIA
Astiarraga et al. 2018 [54]	Fasting	13	10	–	–	180	ELISA
Martinussen et al. 2015 [55]	Fasting, iAUC	10	12	MMTT[a]	300	180	RIA
Tricò et al. 2015 [56]	Fasting, iAUC	10	12	OGTT[a]	300	120	Mesoscale[c]
Alssema et al. 2013 [57]	Fasting, iAUC	20	163	OGTT[a]	300	120	RIA
				MMTT[b]	833		
Lund et al. 2015 [58]	Fasting, iAUC	15	18	OGTT[a]	100	120	RIA
Ruetten et al. 2018 [59]	Fasting, iAUC	22	23	MMTT[b]	470	120	RIA
Knop et al., 2007b [60]	Fasting, iAUC	10	10	OGTT[a]	200	240	RIA
Rohde et al. 2017 [61]	iAUC	9	10	MMTT[a]	525	240	RIA
Vaag et al., 1996 [62]	Fasting, iAUC	12	13	OGTT[a]	300	180	RIA
Ahrén et al., 2000 [63]	Fasting	6	6	–	–	120	RIA
Kuwata et al. 2016 [64]	Fasting	12	10	–	–	240	Mesoscale[c]
St-Jean et al., 2017 [65]	Fasting	8	9	–	–	360	ELISA
Yan et al. 2014 [66]	Fasting, iAUC	10	10	MMTT[a]	75	180	ELISA
				MMTT[a]	150		
				MMTT[a]	300		
Purnell et al. 2018 [67]	Fasting	40	22	–	–	240	Mesoscale[c]
Greenfield et al. 2009 [68]	Fasting, iAUC	4	8	OGTT[a]	300	120	RIA

T2D, Type 2 Diabetes, C, Control group, OGTT, Oral glucose tolerance test, MMTT, Mixed meal tolerance test, RIA, Radioimmunoassay, ELISA, enzyme-linked immunosorbent assay, iAUC, incremental area under the curve.

[a] Liquid meal.

[b] Mixed meal.

[c] Mesoscale provides sandwich immunoassays that measure GLP-1 by electrochemiluminescence.

analysis and refers to problems due to including the same study, or groups from the same study, more than once in the meta-analysis, leading to spurious statistical precision [16]. We addressed this problem by pooling multiple study groups from the same study using the approach reported in the Cochrane Handbook [33].

In the absence of definitive information about the clinical relevance of a unit change in GLP-1 secretion on morbidity and mortality [37] we used thresholds of standardised mean difference to interpret the magnitude of any pooled effects. These thresholds were interpreted as follows; very small <0.2, small 0.2–0.3, moderate 0.4–0.8, and large >0.8 [38]. A two-tailed p value of ≤0.05 was deemed statistically significant.

Sub-group analyses were undertaken to explore whether meal type, meal form and assay type influenced study effect sizes. A random effects multivariable meta-regression was undertaken to explore the influence of fasting glucose, meal dose, age and BMI on study effect sizes.

3. Results

3.1. Study selection

A summary of data searching and extraction is provided in Fig. 1. In total, 6011 records were identified, of which 3285 records remained following the removal of duplicates. Following title and abstract screening, 72 full texts were retrieved and evaluated against the inclusion criteria. Consequently, a further 40 studies were excluded and, therefore, 32 studies remained for analysis in our review. The various study characteristics of included studies are presented in Table 1.

3.2. Methodological quality

The risk of bias for all studies was generally low across studies (Fig. 2). However, the areas of greatest risk of bias appeared to be in the domains for group matching and other biases (low sample sizes). The most common bias appeared to be inequalities in sex, age, and BMI between study groups. Notably, a large number of studies (~33 % of whole sample of studies) recruited <10 participants for each group which decreases the precision for estimate of effect size in each of these studies. While the small samples sizes and poor matching are problematic for the individual studies, these are precisely the limitations that can be addressed by collating conceptually similar studies in a meta-analysis such as the present one.

3.3. GLP-1 iAUC

Data from 270 individuals with T2D and 402 individuals without T2D were pooled from 18 studies with 25 datasets (pooled to avoid double counting of studies). Following the random-effects meta-analysis, we found that the pooled standardised mean difference in GLP-1 iAUC between individuals with and without T2D was not statistically significant ($p = 0.43$) and very small in magnitude (according to our selected thresholds), and the prediction interval for this pooled mean difference was very wide (Fig. 3; $g = -0.15$ [very small], PI [−1.53, 1.23]). The mean absolute values of GLP-1 iAUC were 1047 ± 930 and 1204 ± 938 pmol·L^{-1}·min for individuals with and without T2D, respectively. A regression-based Egger test did not show any clear evidence of small study effects (Fig. 4; $p = 0.33$). Subgroup analyses (See supplementary file) demonstrated no meaningful or statistically significant sub-group effects in GLP-1 iAUC when data were separated by meal type ($p = 0.39$) or meal form ($p = 0.72$). However, there was a large effect of assay type, whereby GLP-1 iAUC was greater in individuals with T2D compared to individuals without T2D when using ELISA or Meso-scale ($g = 0.67$ [moderate], $p = 0.009$, 95 % CI [0.41, 0.93])., but not when using RIA ($g = -0.30$ [small], $p = 0.10$, 95 % CI [−0.72, 0.12]). The difference between these two effect sizes was statistically significant ($p = 0.002$). A random-effects multivariable meta-regression of GLP-1

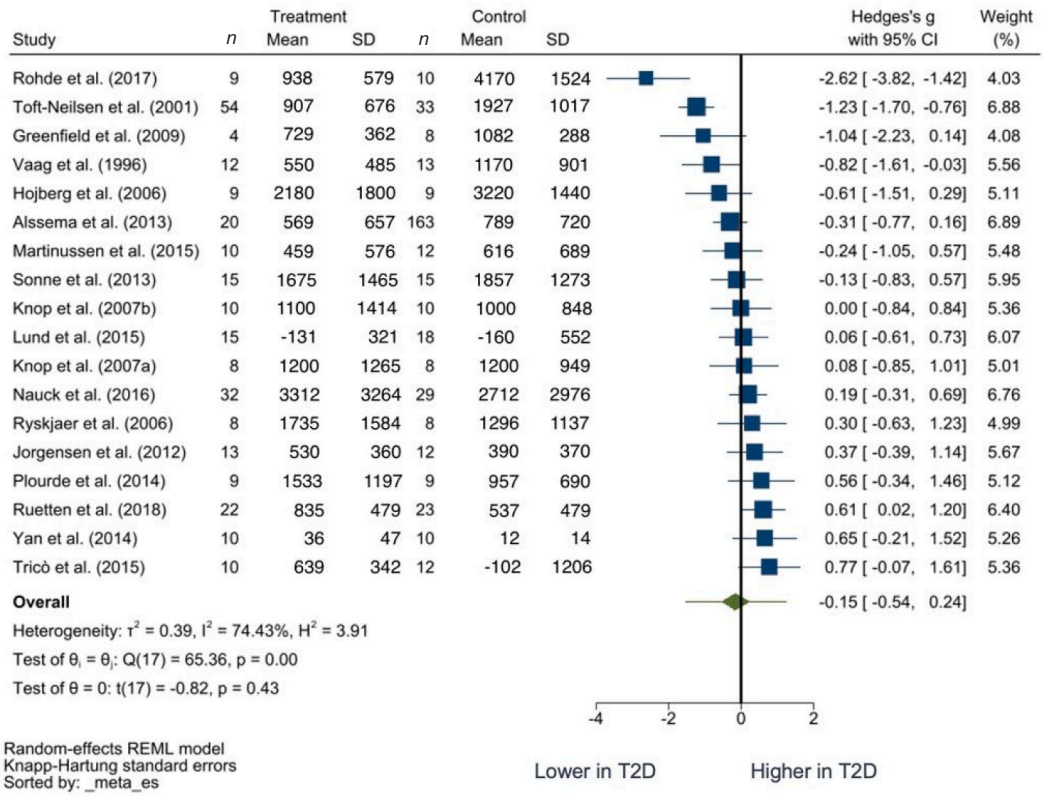

Fig. 3. Forest plot of GLP-1 iAUC (pmol·L^{-1}·min) for individuals with T2D (treatment) and individuals without T2D (control). A negative standardised mean difference is indicative of mean GLP-1 iAUC being lower in T2D than non-T2D.

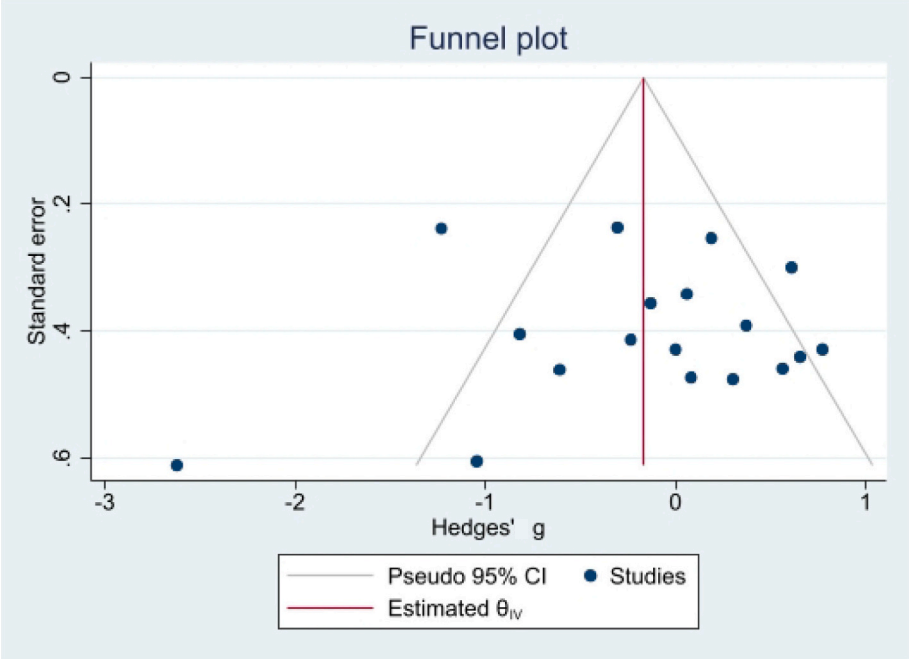

Fig. 4. Funnel plot for studies that presented GLP-1 iAUC. A negative Hedges' g is indicative of mean GLP-1 iAUC being higher in T2D than non-T2D.

iAUC suggested that BMI, age, fasting glucose, and meal dose did not influence the GLP-1 iAUC effect sizes (all $p > 0.05$, See Supplementary File). Heterogeneity between studies was high ($I^2 = 74.4$ %; $T^2 = 0.39$). We undertook a sensitivity analysis whereby a fixed effects model was selected. The pooled effect size from this model was a similar −0.17 (95%CI: −0.33 to 0.002, $p = 0.052$.

3.4. Fasting GLP-1

Data from 580 individuals with T2D and 1363 individuals without T2D were pooled from 30 studies. This random-effects meta-analysis demonstrated that the standardised mean difference in fasting GLP-1 between individuals with T2D *versus* individuals without T2D was not statistically significant ($p = 0.21$) and small in magnitude (according to our selected thresholds). Again, the prediction interval was wide indicating that a future study of the same nature could result in large effect sizes in either direction (Fig. 5; $g = 0.24$ [small], PI [−1.55, 2.02]). Mean fasting GLP-1 was 16.2 ± 6.9 and 12.4 ± 5.7 pmol·L^{-1} for individuals with and without T2D, respectively. A regression-based Egger test did show some evidence of publication bias due to asymmetry of small studies where one very large negative effect size ($g = -3.9$) was reported (Fig. 6; $p = 0.08$). Subgroup analyses demonstrated no differences in fasting GLP-1 between groups when accounting for assay type ($p > 0.05$). Random-effects meta-regression of fasting GLP-1 suggested that BMI, age, and fasting glucose did not affect the fasting GLP-1 outcome. Heterogeneity between studies was high ($I^2 = 87.7$ %; $T^2 = 0.73$).

Although Muscelli et al. (2008) reported that their data were presented as mean and standard deviation (SD), in one of their Figure legends they reported that mean and standard error (SE) were presented. It was, therefore, not wholly certain whether the SDs we extracted for the fasting GLP-1 data were, in fact, standard errors (SE). Therefore, a sensitivity analysis was conducted on the basis that error bars in the relevant Figure represented SEs rather than SDs. We then calculated the SD using the equation $=SE\sqrt{n}$. After this was applied, the meta-analysis pooled SMD was found to be 0.25 (PI: −0.28, 0.78), which was similar to the original estimate. We also undertook a sensitivity analysis whereby a fixed effects model was selected. The effect size from this model was a similar 0.26 (95%CI: 0.15 to 0.37, $P < 0.0001$). This similarity indicates that the small study effects we detected for fasting GLP-1 are not serious [33].

4. Discussion

The findings from this systematic review demonstrate that the standardised mean differences in postprandial GLP-1 secretion and fasting GLP-1 between individuals with *versus* without T2D are very small to small when compared with traditional thresholds for standardised effects. Furthermore, this analysis revealed that studies utilising ELISA or sandwich immunoassays tended to report higher GLP-1 iAUC in individuals with T2D compared to individuals without T2D, which was not apparent with studies utilising RIAs. Importantly, it is difficult to make judgement about the biological relevance of the pooled standardised effect sizes partly because of the absence of robust "anchors" to morbidity risk and partly because of the wide prediction intervals obtained for the effect sizes.

Similar to previous meta-analyses, in the present study, there was no clear difference in plasma GLP-1 iAUC between individuals with and without T2D [13,14]. Despite this, some of the largest single studies to date have reported lower GLP-1 secretion in individuals with *versus* without T2D [9,10]. Large heterogeneity between studies resulting in positive, neutral, and negative effect sizes may partly explain why differences in SMDs between groups were very small to small. Additionally, important moderating variables such as BMI and visceral fat content may vary largely within groups. These differences within and between studies present a challenge for determining the most important factors influencing GLP-1 secretion. The present analysis suggests that diagnosis of type 2 diabetes is not associated with large differences in GLP-1 secretion, and thus these other moderating variables may be more important than diabetes diagnosis *per se*.

There were no clear differentiating effects of meal type *i.e.*, OGTT or MMTT, or meal form *i.e.*, liquid or mixed, on GLP-1 iAUC. Given the diversity of intestinal receptors/transporters that are specific to different nutrients it was hypothesised that a potential reduction in GLP-1 secretion in individuals with T2D may occur as a result of receptor/transporter resistance to a specific macronutrient(s), thereby manifesting in different responses based on whether an OGTT or MMTT was administered. While these results do not support this hypothesis, there have not been enough studies to isolate the specific effects of different

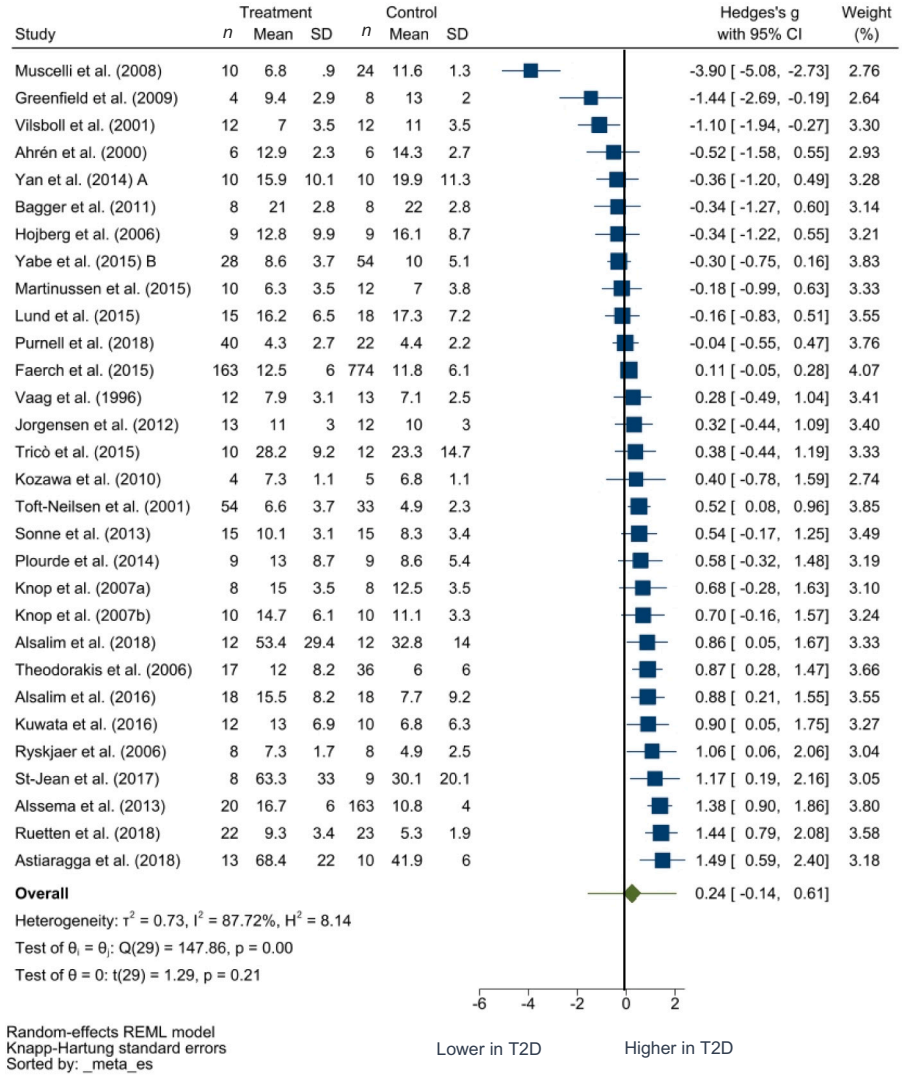

Fig. 5. Forest plot of fasting GLP-1 (pmol·L^{-1}) for individuals with T2D (treatment) and individuals without T2D (control). A negative standardised mean difference is indicative of mean fasting GLP-1 being lower in T2D than non-T2D.

macronutrients on GLP-1 release in T2D and it is likely that MMTTs incorporating a combination of macronutrients may have masked any specific nutrient resistance that may have influenced GLP-1 secretion. In 33 participants of normal weight, overweight and obesity, GLP-1, oxyntomodulin and glicentin were increased to a greater extent following lipid *versus* glucose ingestion, with variation in size of response between these peptides following the ingestion of different macronutrients [69]. Moreover, It was recently demonstrated that the exaggerated GLP-1 response to meal intake after gastric bypass operations is mainly due to the effect of carbohydrates [70]. While rate of gastric emptying in the instance of gastric bypass may account for an exaggerated GLP-1 response, a recent paper suggested high levels of inter-individual variation for GLP-1 secretion following the ingestion/administration of different macronutrients, independent of gastric emptying [71]. Thus, individual food items may affect GLP-1 (and other peptides) secretion differentially and this may be impacted by certain metabolic conditions including T2D.

One factor that did appear to influence the estimates of GLP-1 secretion was the assay type employed. As mentioned in the introduction, reliable measurement of GLP-1 is difficult due to the existence of a number of additional preproglucagon (GCG) -derived fragments and GLP-1 moieties which can cross-react with the measurement of the intended form of GLP-1 [2,26]. A well-defined RIA previously described by [27] was utilised by the majority of studies, while only 5 out of 25 studies utilised ELISA or sandwich immunoassay (Mesoscale) to measure GLP-1 iAUC. Interestingly, the studies using ELISA/sandwich immunoassay demonstrated greater GLP-1 iAUC in individuals with T2D *versus* those without T2D, whereas no such difference was apparent in studies that measured GLP-1 by RIA. Previous research comparing the specificity of many commercially available assays suggests the ability of the mesoscale immunoassay to detect non-active forms is incomplete and the recovery of non-amidated forms when using the Merck Millipore ELISA is variable [29]. One limitation of the assay type sub-analyses is that it was only possible to group assays into RIA's or ELISA/mesoscale. The findings from [29] suggest that the reliability and accuracy of ELISAs and RIAs can vary by manufacturer/antibody selection. These findings suggest that choice of assay is an important consideration and should be based on the research question and conditions of the study, but unless there is greater consistency of assay use between studies it will remain difficult to compare findings between studies. It is possible that new, more accurate, methods for measuring GLP-1, including highly specific ELISAs and methods based on mass spectrometry, will become more widely available in the not too distant future. A recent sandwich ELISA recognising both GLP-1$_{9\text{-}36NH2}$ and GLP-1$_{9\text{-}37}$ has been developed and validated [72], and a new ELISA assay (from Ansh) has been reported to show specificity to a number of proglucagon-derived

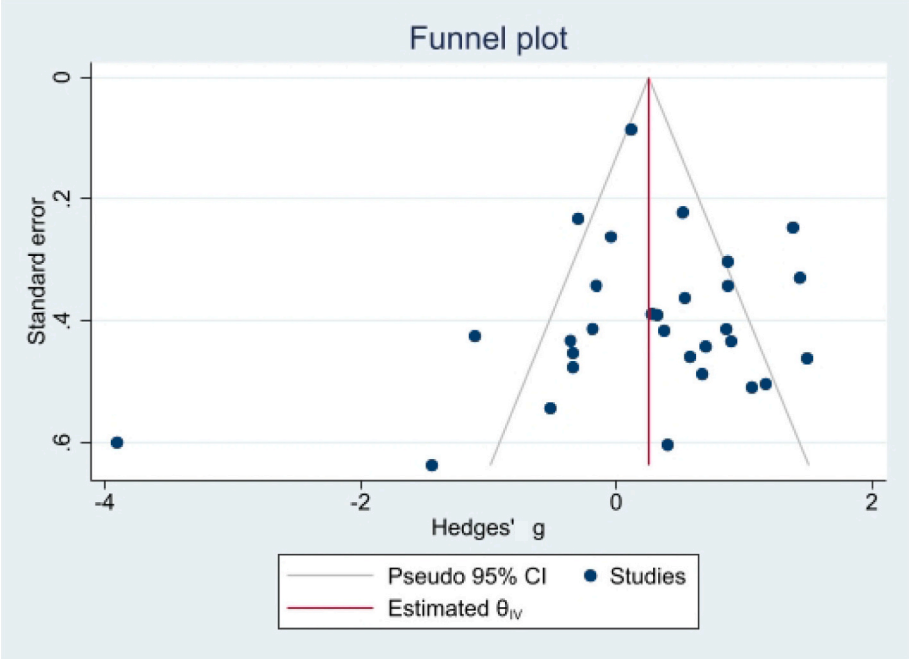

Fig. 6. Funnel plot for studies that presented fasting GLP-1. A negative Hedges' g is indicative of mean fasting GLP-1 being lower in T2D than non-T2D.

peptides including oxyntomodulin, glicentin, glucagon and MPGF [73].

To our knowledge this is the first systematic review to investigate fasting GLP-1 in studies involving individuals with and without T2D. The findings of the present study suggest that differences in fasting GLP-1 between these two groups are small (Hedges' g). Unlike for GLP-1 iAUC, the assay type had no clear influence on fasting GLP-1. This suggests that assay type may make more of a difference at higher concentrations of GLP-1, and/or that assays are not sensitive or accurate enough to determine the low circulating fasting concentrations regardless of the presence of T2D or not. The prediction interval generated suggests that any future study comparing fasting GLP-1 between individuals with and without T2D could find greater, equal, or lower fasting GLP-1 in individuals with *versus* without T2D. Nevertheless, the Egger's test demonstrated evidence of publication bias where there was an asymmetry of effect sizes reported by included studies. This asymmetry related to the existence of a few large negative effect sizes. The majority of studies reported positive effect sizes indicating that fasting GLP-1 was greater in individuals with T2D compared to individuals without T2D (16.0 ± 6.8 and 12.3 ± 5.7 pmol·L^{-1}, respectively) which is in agreement with the mean fasting glucose for individuals with and without T2D. However, the positive effect sizes were mostly small and large prediction intervals suggest uncertainty in future studies comparing GLP-1 concentrations in individuals with T2D compared to without T2D.

The studies included in the present systematic review and meta-analyses were highly heterogenous according to the I^2 and T^2 statistic, and the wide prediction intervals. Despite all included studies being conceptually similar, large between-study differences in specific methodologies are a limitation for the present systematic review and may explain why there is such conflicting evidence in the literature. Interestingly, similarly high study heterogeneity was reported in a recent meta-analysis which showed a variable impairment in the incretin effect in T2D [74]. Taken together, this highlights the importance of acknowledging possible sources of study heterogeneity in the GLP-1 related literature, and these are addressed below. Firstly, a number of included studies were at high risk of bias due to small sample sizes and/or differences between groups at baseline. It could be possible that these studies were underpowered to detect a genuine effect and that subsequent variability in these studies contributed to large PIs. Additional inconsistency may come from meal ingestion. Most commonly, an OGTT or MMTT is ingested to measure GLP-1 responses, however within each type of test meal there is large variability in terms of caloric dose and/or macronutrient composition of the meal consumed. Therefore, grouping studies into those that fed an OGTT and/or MMTT may not be a truly matched comparison. Another confounding factor is the varying relationship between the meal size and the body mass of the participants. It cannot be excluded that identical meals will variably affect GLP-1 responses in individuals with large differences in body mass. In general, GLP-1 responses have been smaller in people with overweight [10], and weight loss has been reported to restore responses [74]. Thus, overweight may be an important factor for reduced GLP-1 responses in patients with T2DM, most of whom are also overweight/obese [2].

Within the literature, there is also poor consistency for reporting GLP-1 secretion. In the present meta-analyses, a number of studies were excluded as the units provided for AUC could not be interpreted. Units may differ based on whether AUCs are time-averaged and also by the length of the postprandial period. To avoid uncertainty and improve the comparability of studies it would be helpful for authors to report their data clearly and thoroughly. For example, authors could report AUC for each segment of the postprandial period separately to allow greater comparison and insight between studies. Tools such as the Time Series Response Analyser [75] would streamline this process. Further issues surround the grouping of individuals with impaired glucose tolerance and T2D. This makes it difficult to separate the physiology of GLP-1 secretion in individuals with a clinical diagnosis of T2D and those with deteriorating glucose control. Indeed, a study with a comparatively large group of people with T2D and controls also included a group with impaired glucose tolerance, and their GLP-1 responses were positioned between those of the controls and individuals with T2D [9]. Lastly, studies are generally poor at describing whether diabetes medication is withheld during data collection, and if so, for how long. This is particularly important considering some common medication, such as metformin, has been shown to increase GLP-1 secretion [76,77], and could therefore influence some of the effect sizes reported. Based on the thorough review of studies reporting GLP-1 secretion in T2D, considerations for the comparability of research studies and recommended solutions are provided in Table 2.

In conclusion, differences in fasting GLP-1 and GLP-1 iAUC between

Table 2
Considerations for GLP-1 literature and recommended solutions.

Issue within literature	Consequence of this issue	Recommended solution
Unclear/inconsistent use of AUC and appropriate units	Difficulty comparing across studies, introduces risk of misinterpretation	Comprehensive reporting of method and units
Use of tAUC only, to measure meal-induced GLP-1 release	Total area under the curve is not the most suitable measure for postprandial change in GLP-1 secretion as it does not consider the change relative to baseline. This also introduces the possibility of overestimating GLP-1 release in groups with higher fasting GLP-1 relative to the comparator group(s).	Both measures add value. Consider the use of both total and incremental area under the curve when reporting postprandial responses
Inconsistent postprandial period	There is evidence of early and/or late phase GLP-1 secretion differences in T2D and inappropriate postprandial sampling protocols (*i.e.* too short, too infrequent) may limit full characterisation of postprandial GLP-1 responses.	While the length of the postprandial period may differ dependent on context, a standard approach could be to sample at least every 30 min, and report AUC data for each segment.
Selection of assay	Circulating concentrations of GLP-1 are in the low picomolar range. The existence of a number of preproglucagon fragments which can cross-react with the intended measure of GLP-1	Carefully consider research aims, choose a well-defined assay that includes key information on the use of antisera and the forms of GLP-1 captured.
Measure of active/ inactive GLP-1	Rapid degradation in the circulation. Concentrations potentially below limit of detection of assays	Measure total GLP-1, which incorporates the intact form and metabolite.
Grouping of T2D with individuals with impaired glucose tolerance	If GLP-1 secretion is influenced by degree of glucose control, it is likely that data will be variable. Difficult to identify a true effect in individuals with T2D.	Group by T2D classification or if possible, treat glucose tolerance as a continuous variable.
Poor reporting of medication	Information regarding the use of medication and whether medication is withheld during data collection is inconsistent. Certain medication *e.g.* Metformin, can increase GLP-1 secretion, which can confound the true effect of meal-induced GLP-1 release in T2D.	Authors should clearly report the type of medication individuals are using, the dose/frequency, and also whether or not they withhold medication during data collection, and if so, for how long.
Poor reporting of meal characteristics	Level of detail (such as type, form and composition) provided for ingested meals is often inconsistent. Missing information can introduce risk of misinterpretation.	Authors should clearly report the type of meal fed *i.e.* OGTT, MMTT, the type and form of food ingested, as well as the macronutrient composition and calorie content.

individuals with T2D and individuals without T2D were small and inconsistent. This suggests that reduced beta-cell responsiveness to GIP and/or GLP-1 as opposed to reduced GLP-1 secretion may provide a better explanation for the reduced incretin effect in T2D. When studies employed either ELISA or sandwich immunoassay (Mesoscale), GLP-1 iAUC was higher in individuals with T2D compared to individuals without T2D. This demonstrates the difficulty surrounding GLP-1 measurement and the importance of using a well-defined assay. Evidence of high heterogeneity between studies, small sample sizes, and poor matching of groups may help to explain the wide prediction intervals and conflicting evidence in the literature surrounding GLP-1 secretion in T2D. Recommendations for improving comparisons for GLP-1 secretion between individuals with and without T2D were outlined, as well as potential mediators more important than T2D diagnosis *per se*.

Declaration of competing interest

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