Title: Women with isolated fasting hyperglycemia in pregnancy are at a higher genetic risk for type 2 diabetes

Short running title: Genetics of GDM diagnostic criteria

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Abstract

Using genetic scores for fasting plasma glucose (FPG GS) and Type 2 diabetes (T2D GS), we investigated whether different diagnostic criteria for gestational diabetes (GDM) have different implications for genetic susceptibility to later T2D in women from the the Hyperglycemia and Adverse Pregnancy Outcome (HAPO) and Atlantic Diabetes in Pregnancy (DIP) studies. To investigate different features of WHO (1999) and IADPSG (2010) criteria for diagnosing GDM, cases were divided into three subgroups: (i) FPG ≥5.1 mmol/L only, n=261; (ii) 2-hour glucose post 75 g oral glucose load ≥7.8 mmol/L only, n=305); and (iii) both FPG ≥5.1 mmol/L and 2-hour glucose ≥7.8 mmol/L, n=135. We compared the FPG and T2D genetic scores of these groups with controls (n=3,083). GDM as defined by fasting hyperglycemia was associated with a higher genetic score for both FPG and T2D, whether or not 2-hour glucose was also raised. GDM defined only by 2-hour hyperglycemia, was associated with a higher T2D GS, but not a higher FPG GS. The T2D GS was similar whether the fasting glucose alone, 2-hour glucose alone, or both fasting and 2-hour glucose were raised. Thus, the newest criteria identify women with a genetic predisposition to T2D who may not have been identified by previous criteria.

Gestational diabetes mellitus (GDM) has been variably defined since criteria were first developed over 50 years ago (1). The World Health Organization (WHO) introduced diagnostic criteria for GDM in 1999, based on criteria for overt diabetes in the general population with a fasting plasma glucose (FPG) ≥7.0 mmol/L or impaired glucose tolerance with a 2-hour glucose post 75 g oral glucose tolerance test (OGTT) ≥7.8 mmol/L, measured between 24 and 28 weeks gestation (2). However, lesser degrees of maternal fasting hyperglycemia have long been associated with a higher risk for adverse perinatal outcomes (3), so a FPG ≥6.1 mmol/L (indicative of impaired fasting glycemia in the non-pregnant population (4)) was also integrated into the WHO criteria.

The Hyperglycemia and Adverse Pregnancy Outcome (HAPO) Study (5) followed 23,316 women who underwent a 2-hour OGTT between 24 and 32 weeks gestation throughout pregnancy and found a continuous association between maternal glucose values and adverse perinatal outcomes, including birth weight ≥90th centile (large for gestational age, LGA) and primary cesarean section. In 2010, the International Association of Diabetes and Pregnancy Study Groups (IADPSG) determined cut-off values equivalent to 1.75 times the odds for adverse pregnancy outcomes at mean glucose values, resulting in diagnostic thresholds for FPG ≥5.1 mmol/L, 1-hour glucose ≥10 mmol/L and 2-hour glucose ≥8.5 mmol/L (6).

WHO adopted the recommendations of IADPSG in 2013 (2), which has resulted in a higher number of cases identified as GDM due to the lower FPG threshold (estimated up to 17.8% prevalence of GDM for IADPSG 2010 criteria (6) vs 9.4% prevalence for WHO 1999 criteria (7)). The HAPO Follow-Up Study found that women diagnosed by the newer IADPSG criteria have a higher risk of developing disorders of glucose metabolism, including T2D, 10 years after the episode of GDM (8). However, for new cases of GDM diagnosed according to the lower FPG threshold, it is not known if there is a genetic predisposition to

T2D, or if alternatively, the underlying genetic predisposition is to raised FPG without a genetic predisposition to T2D.

Genome wide association study (GWAS) data from large population-based studies have identified multiple loci associated with FPG (9) and T2D (10), and various loci associated with fasting hyperglycemia and T2D in the general population have also been associated with GDM (11–13). Specific to the IADPSG 2010 criteria, single nucleotide polymorphisms (SNPs) at the *GCK* and *TCF7L2* loci were shown to be associated with FPG and 2-hour glucose levels post-OGTT in women with GDM (14).

We used a genetic score (GS) for FPG (FPG GS) or T2D (T2D GS) (consisting of previously-identified loci (9,15)) to test the hypothesis that there are different genetic risks for fasting hyperglycemia and T2D depending on the criteria used to diagnose GDM.

Research design and methods

Study population

Women of European ancestry with singleton pregnancies and without known pre-existing diabetes from the Hyperglycemia and Adverse Pregnancy Outcome (HAPO) Study (5) (n=2,665) and Atlantic Diabetes in Pregnancy (DIP) Study (16) (n=1,119) were included. The HAPO study was an observational, multi-centre study (N=23,316 participants from 15 centres) to which women were recruited during pregnancy if they were over 18 years of age (5). The 2,665 European-ancestry participants included in the current study were those with genotype data available on selected SNPs (see below). The DIP study had a case-control design: approximately three genotyped control participants without GDM (defined initially as a maternal FPG <5.6 mmol/L and/or 2-hour glucose post oral glucose load <7.8 mmol/L) were available for every genotyped case participant included in our analyses. Women who were unblinded due to being diagnosed with GDM by pre-existing criteria used at the time of the studies were not excluded from this analysis.

Sample collection and clinical characteristics

The study methods used in HAPO and DIP have been described in detail previously (5,7,16–18). Maternal FPG in mmol/L was measured prior to a standard 2-hour OGTT with 75 g of glucose between 24 and 32 weeks in HAPO and 24 and 28 weeks in DIP. Information on maternal age, pre-pregnancy body mass index (BMI) and systolic blood pressure (SBP, in mmHg) was collected at the OGTT appointment. Clinical characteristics of participants in HAPO and DIP with and without GDM were different (women in DIP were older, had a higher BMI and higher SBP, all P <0.01), hence clinical characteristics have been presented separately.

GDM diagnostic criteria subgroups

We used the IADPSG 2010 and WHO 1999 cut-offs to define fasting and 2-hour hyperglycemia (Supplemental Table 1). Thus, in the current study, women diagnosed with GDM were divided into fasting hyperglycemia only (FPG ≥5.1 mmol/L and 2-hour glucose post 75 g oral glucose load <7.8 mmol/L, n=261), elevated 2-hour glucose only (FPG <5.1 mmol/L and 2-hour glucose post 75 g oral glucose load ≥7.8 mmol/L, n=305) and both (FPG ≥5.1 mmol/L and 2-hour glucose post 75 g oral glucose load ≥7.8 mmol/L, n=135) subgroups (Figure 1). Women without GDM were defined as having FPG <5.1 mmol/L and 2-hour glucose post 75 g oral glucose load <7.8 mmol/L (n=3,083).

Genotyping

Genotyping of individual SNPs in DNA samples from both the DIP and HAPO studies was carried out at LGC Genomics (Hoddesdon, UK; https://www.lgcgroup.com), using the PCR-based KASP™ genotyping assay. We first selected 41 SNPs that had been previously associated with T2D, and 16 SNPs associated with fasting glucose in non-pregnant individuals, for genotyping in the DIP study. Overlap between the T2D and FPG SNPs meant that 7 FPG loci were also in the list of T2D loci. The median genotyping call rate in the DIP samples was 0.992 (range 0.981-0.996), and there was >99% concordance between duplicate samples (8% of total genotyped samples were duplicates). We excluded one FPG SNP and one T2D SNP that showed deviation from Hardy-Weinberg Equilibrium (Bonferroni-corrected P <0.05). For details of included and excluded SNPs and their sources, see Supplemental Table 2.

In the HAPO study, we selected SNPs from the same 16 FPG and 41 T2D loci for genotyping in women of European ancestry with DNA available. The selection and genotyping of SNPs in the HAPO study was performed at different times from that in the DIP study. Owing to the differing availability of published GWAS results at these times, the

genotyped SNPs differed between HAPO and DIP at 9 of the associated loci. The HAPO SNPs at the 9 loci were generally well correlated with those genotyped in DIP (r^2 >0.7, apart from at the *ADAMTS9* locus where r^2 = 0.45). The median genotyping call rate in the HAPO samples was 0.984 (range 0.955-0.991), and the mean concordance between duplicate samples was >98.5% (at least 1% of samples were duplicated). We excluded 1 SNP that showed deviation from Hardy-Weinberg Equilibrium in the HAPO study (Bonferroni-corrected *P* <0.05; see Supplemental Table 3). After exclusion of SNPs that showed deviation from Hardy-Weinberg equilibrium and one SNP from the T2D score whose main effect was on BMI (rs11642841 (*FTO* locus) (19)), a total of 15 SNPs at FPG-associated loci and 38 SNPs at T2D-associated loci were available in both studies for analysis.

Generating a genetic score for FPG and T2D

Weighted genetic scores for FPG (FPG GS) and T2D (T2D GS) were generated using the 15 SNPs and 38 SNPs, respectively. The GSs were calculated by taking the sum of the number of FPG-raising or T2D risk alleles (0, 1 or 2) for each SNP, multiplied by its corresponding beta value (effect size) for association with FPG or T2D, divided by the sum of all beta values and multiplied by the total number of SNPs analyzed (see Supplemental Figure 1 for formula). Where more than 6 SNPs were not called, the individual was excluded from the analysis.

Statistical analyses

Analysis of clinical characteristics

Clinical characteristics were compared between participants with and without GDM in HAPO and DIP using unpaired *t*-tests for normally distributed data and the Wilcoxon Rank-Sum test for non-normally distributed data. *P* values were corrected for 18 comparisons using the Bonferroni method.

Analysis of associations between FPG GS or T2D GS with glucose levels and GDM

Associations of the FPG GS or T2D GS with FPG and 2-hour glucose in women with and without GDM (cases and controls) were analyzed using linear regression in HAPO (which was a representative sample of European participants from the whole study cohort). Means for FPG GS and T2D GS in women with and without GDM were compared using unpaired *t*-tests in each study cohort separately, as the genetic scores were higher overall in DIP. *P* values were corrected for 16 comparisons using the Bonferroni method. We excluded women with a 1-hour glucose value ≥10 mmol/L from the control group (n=43 in HAPO and n=69 in DIP) in additional sensitivity analyses, as these women would have been diagnosed with GDM according to the IADPSG 2010 criteria.

Statistical software

All statistical analyses were performed using Stata version 14.0 (StataCorp LP, College Station, TX, USA). *P* values ≤0.05 were considered to indicate evidence of association, unless otherwise stated.

Ethics approval

Ethics approval was obtained from the Northwestern University Office for the Protection of Research Participants for HAPO. The HAPO study protocol was approved by the institutional review board at each field center and all participants gave written, informed consent. Ethics approval was obtained from the local Galway University Hospital Research Ethics Committee for ATLANTIC DIP and all participants gave written, informed consent.

Results

Clinical characteristics in women with and without GDM

Clinical characteristics for women with and without GDM (diagnosed according to fasting hyperglycaemia (FPG ≥5.1 mmol/L) and/or 2-hour hyperglycemia (glucose value two hours post 75 g oral glucose load ≥7.8 mmol/L) criteria) are summarized in Tables 1 and 2 for HAPO and DIP, respectively. Women with a FPG ≥5.1 mmol/L (on its own or with 2-hour hyperglycemia) had a higher pre-pregnancy BMI than women without GDM in HAPO and DIP (P <0.01). In HAPO we observed a higher SBP for women diagnosed with GDM by a FPG ≥5.1 mmol/L only compared with controls (P <0.01), whereas in DIP there was a higher SBP for women diagnosed by both fasting and 2-hour hyperglycemia criteria compared with controls (P <0.01). All P values remained <0.01 after Bonferroni correction.

FPG and 2-hour glucose are correlated with FPG and T2D GS in pregnant women with and without GDM

FPG in women with and without GDM (cases and controls combined) was positively correlated with FPG GS in HAPO (beta coefficient 0.028 mmol/L (95% CI 0.023-0.032 mmol/L) per one unit higher FPG GS, P <0.01). FPG was also correlated with the T2D GS, but to a lesser extent (beta coefficient 0.008 mmol/L (95% CI 0.004-0.011 mmol/L) per one unit higher T2D GS, P <0.01). These associations remained present when controlling for the 2-hour glucose values; for FPG the beta coefficient was 0.025 mmol/L (95% CI 0.021-0.030 mmol/L) per one unit higher FPG GS (P <0.01) and 0.005 mmol/L (95% CI 0.001-0.008 mmol/L) per one unit higher T2D GS (P <0.01). The 2-hour glucose values were positively correlated with both FPG GS and T2D GS (beta coefficient 0.032 mmol/L (95% CI 0.016-0.048 mmol/L) per one unit higher FPG GS, P <0.001 and beta coefficient 0.034 mmol/L (95% CI CI 0.022-0.045 mmol/L) per one unit higher T2D GS, P <0.001). The association

between 2-hour glucose values and T2D GS remained when taking FPG values into account (beta coefficient 0.027 mmol/L (95% CI 0.016-0.038 mmol/L) per one unit higher T2D GS, *P* <0.001). However, the association with FPG GS was not present when controlling for FPG (beta coefficient 0.007 mmol/L (95% CI -0.009-0.022 mmol/L) per one unit higher FPG GS, *P* =0.41).

Women diagnosed with GDM by fasting criteria have a higher FPG GS

We observed a higher FPG GS in women diagnosed with GDM by fasting hyperglycemia only and by both fasting and 2-hour criteria, compared with controls (Figure 2A). In HAPO, the mean FPG GS in controls was 15.5 (95% CI 15.4-15.6), whilst in women with a FPG ≥5.1 mmol/L only it was 16.9 (95% CI 16.5-17.3, *P* value for comparison with controls <0.01) and in women with both a FPG ≥5.1 mmol/L and 2-hour glucose ≥7.8 mmol/L it was 17.14 (95% CI 16.26-18.02, *P* value for comparison with controls <0.01). In DIP mean FPG GS in controls was 15.8 (95% CI 15.7-16.0), whilst in women with a FPG ≥5.1 mmol/L only it was 17.2 (95% CI 16.6-17.9, *P* value for comparison with controls <0.01) and in women with both a FPG ≥5.1 mmol/L and 2-hour glucose ≥7.8 mmol/L it was 17.5 (95% CI 16.8-18.2), *P* value for comparison with controls <0.01). All *P* values remained <0.01 after Bonferroni correction. In contrast, women diagnosed with GDM by 2-hour only criteria did not have a higher FPG GS (mean FPG GS was 15.9 (95% CI 15.5-16.3) in HAPO and 15.7 (95% CI 15.2-16.2) in DIP, *P* value for comparison with controls >0.05 in both studies). Associations remained after excluding women with a 1-hour glucose ≥10 mmol/L in the control group (Supplemental Tables 3a and 3b).

Women diagnosed with GDM by fasting or 2-hour criteria have a higher T2D GS than controls

The T2D GS was higher than controls in women with fasting or 2-hour hyperglycemia in HAPO (Figure 2B): mean T2D GS in controls was 41.2 (95% CI 41.0-41.4), whilst in women with a FPG ≥5.1 mmol/L only it was 42.2 (95% CI 41.6-42.9, *P* value for comparison with controls <0.01) and in women with a 2-hour glucose ≥7.8 mmol/L only it was 42.3 (95% CI 41.7-42.9, *P* value for comparison with controls <0.01). In women with both, mean T2D GS was 42.3 (95% CI 41.1-43.6, *P* value for comparison with controls <0.05). In DIP, mean T2D GS in controls was 41.8 (95% CI 41.5-42.1), whilst in women with an isolated fasting hyperglycemia it was 43.2 (95% CI 42.2-44.1, *P* value for comparison with controls <0.01), in women with an isolated 2-hour hyperglycemia it was 42.7 (95% CI 42.0-43.5, *P* value for comparison with controls <0.05) and in women with both a FPG ≥5.1 mmol/L and 2-hour glucose ≥7.8 mmol/L it was 43.6 (95% CI 42.7-44.6, *P* value for comparison with controls <0.01). All *P* values survived correction at α=0.05 except for women with both fasting and 2-hour hyperglycemia in HAPO, and women with isolated fasting or 2-hour hyperglycemia in DIP.

If participants diagnosed with GDM by a raised 2-hour glucose were divided by the WHO 1999 (≥7.8 and <8.5 mmol/L) and IADPSG 2010 (≥8.5 mmol/L only) thresholds, a higher T2D GS was observed only in women with a 2-hour glucose ≥8.5 mmol/L; in HAPO (Figure 3A), women with a 2-hour glucose ≥7.8 mmol/L and <8.5 mmol/L only mean T2D GS was 41.6 (95% CI 40.8-42.3), whereas in women with a 2-hour glucose ≥8.5 mmol/L only mean T2D GS was 43.1 (95% CI 42.2-44.0, *P* value for comparison with controls <0.01). In DIP (Figure 3B), mean T2D GS in women with a 2-hour glucose ≥7.8 mmol/L and <8.5 mmol/L was 42.2 (95% CI 41.3-43.1) and mean T2D GS in women with a 2-hour glucose ≥8.5 mmol/L was 43.4 (95% CI 42.2-44.6, *P* value for comparison with controls

<0.01, but >0.05 with Bonferroni correction). The associations seen for the T2D GS were similar when women with a 1-hour glucose ≥10 mmol/L in the control group were excluded (Supplemental Tables 3a and 3b).

Conclusions

In this study of 3,784 pregnant women of European ancestry, we have shown that women diagnosed with GDM with a FPG ≥5.1 mmol/L alone have higher genetic scores for both FPG and T2D. These associations were similar for women who also had a glucose ≥7.8 mmol/L at two hours during a standard OGTT, but for women with 2-hour hyperglycemia on its own, there was no genetic predisposition to raised fasting glucose.

We found that FPG in pregnant women both with and without GDM was correlated with a FPG GS which was generated using SNPs identified in a non-pregnant population (11). The 2-hour glucose values were also correlated with FPG GS, but this could be explained by the association between FPG and 2-hour glucose levels, whereas the association between 2-hour glycaemia and T2D GS was independent of FPG. FPG was correlated with T2D GS, although to a lesser extent than with the FPG GS, which would be expected as there are loci within the T2D GS which also raise fasting glucose (e.g. *GCK*, *MTNR1B*) (9). Thus, the observation that the FPG GS was not higher in women with a 2-hour glucose ≥7.8 mmol/L alone was expected. However, we hypothesized that women with isolated fasting hyperglycaemia might have a genetically higher FPG, without a genetic predisposition to T2D. Despite FPG being less correlated with the T2D GS than 2-hour glycemia, the T2D GS was still associated with the fasting hyperglycemia only criteria, suggesting that these women may possess genetic risk factors for T2D independent of fasting and 2-hour glucose levels. We observed the highest BMIs in women diagnosed with GDM by fasting hyperglycemia only or both criteria, which is consistent with previous research showing that women diagnosed with GDM by IADPSG 2010 criteria were more overweight than those diagnosed by WHO 1996 criteria (7,20). However, the associations with FPG GS and T2D GS are not driven by BMI, suggesting that women with fasting hyperglycemia in pregnancy are likely to have both a metabolic and genetic predisposition to T2D.

Women diagnosed with GDM with 2-hour glucose levels ≥7.8 mmol/L had a higher T2D GS than controls in HAPO and DIP. Since the lower FPG threshold as part of the IADPSG 2010 criteria was associated with a higher genetic risk for T2D, we considered whether the higher 2-hour glucose level may miss women who are also at a higher genetic risk for T2D. But, an association between 2-hour glucose and the T2D GS between 7.8 mmol/L and 8.5 mmol/L was not observed, whereas a higher T2D GS in women with a 2-hour glucose ≥8.5 mmol/L was present, suggesting that the higher risk is driven by those women with a 2-hour glucose above the higher threshold of 8.5 mmol/L. Impaired glucose tolerance and elevated 2-hour glucose levels during an OGTT have been demonstrated in normal pregnancy (21). Thus, values between 7.8 and 8.5 mmol/L may be reflective of a normal physiological response to pregnancy rather than impaired glucose tolerance. On the other hand, FPG levels decrease in pregnancy (22), so, although a threshold of 5.1 mmol/L may not be considered abnormal outside of pregnancy, it represents hyperglycemia within pregnancy.

This work specifically examining the genetic risk of T2D in women diagnosed with GDM according to different criteria supports the results from the recent HAPO Follow-Up Study (8) which showed that women diagnosed with GDM post-hoc according to IADPSG 2010 criteria had a higher risk for T2D 10 to 14 years after pregnancy. Thus, having established that pregnant women with fasting hyperglycemia alone have a higher clinical and genetic risk for T2D compared with women without GDM, the implications for clinical care need to be considered. At present, women at risk for GDM are tested with a 2-hour OGTT, but there may be no additional value in knowing the 2-hour glucose value once it has been established that the FPG is high. The HAPO study showed a continuous association between 2-hour glucose values and adverse pregnancy outcomes (5), but there is no evidence to suggest that both a raised FPG and raised 2-hour glucose confers any additional risk for

pregnancy complications or genetic risk for T2D. In the longer-term, using the lower, IADPSG 2010 FPG threshold for identifying GDM will result in more cases diagnosed, but these women would be an important target for long-term follow-up. The Diabetes Prevention Program (DPP) (23) trial found that lifestyle intervention or metformin treatment reduced risk of progression to T2D in women with impaired glucose tolerance and a history of GDM (according to relevant criteria at time of diagnosis), but a genetic risk score for T2D did not influence treatment response (24). It is not known whether this would be different for women specifically diagnosed by IADPSG 2010 criteria, but it is likely that these women would benefit from monitoring after pregnancy.

There are limitations of this study that are important to consider. Overall, a small number of cases of GDM were analyzed, so with greater numbers there may have been clearer differences in FPG and T2D GS between the different diagnostic groups. For example, women with both a high FPG and 2-hour glucose, which is likely to represent a greater disturbance in glycemic control, may actually have a higher T2D GS than women with isolated fasting hyperglycemia alone. Furthermore, we did not study 1-hour glucose values separately, so women with an isolated raised 1-hour glucose value (i.e. normal fasting and 2-hour glucose) meeting the diagnostic threshold for GDM according to IADPSG 2010 criteria were included in the control group. However, these women made up a very small proportion of the control group (n=43 in HAPO and n=69 in DIP) and exclusion of these women with a raised 1-hour glucose alone did not influence the observed associations (Supplemental Table 3). It is not known whether women with a raised 1-hour glucose in addition to a raised fasting and/or 2-hour glucose have a higher genetic risk for T2D; we did not have the power in this study to investigate this, but it may be an important area of future research.

Finally, we studied women from two different studies, where there were notable differences in clinical characteristics, even for women without GDM. This likely represents differences in the background population as a whole, and there were similar patterns amongst the different diagnostic groups. Additionally, the FPG and T2D GS were consistently higher in DIP than in HAPO. This is likely to reflect differences in SNPs used to generate the genetic scores and possibly a slighter higher genetic disposition to a raised FPG and T2D in DIP. Therefore, the results of these analyses are likely to be applicable to women of European ancestry, but further larger-scale studies, including analysis of women with diverse ancestry, will be needed to confirm the associations identified in this study.

In conclusion, women diagnosed with GDM with a FPG ≥5.1 mmol/L alone are at a higher genetic risk for raised FPG and T2D. Women with an isolated 2-hour glucose ≥8.5 mmol/L during a standard OGTT also have a higher genetic risk for T2D than women with normal glucose in pregnancy, but this may not be true for women with values between 7.8 mmol/L and 8.5 mmol/L. Overall, the IADPSG 2010 criteria identify an important group of women at risk for adverse pregnancy outcomes as well as a higher risk for developing future T2D (8). This study has confirmed that this is partly due to genetic predisposition. Knowing that these women also have a higher genetic risk for fasting hyperglycemia and T2D, genetic testing could be a novel tool to identify women at high risk for GDM at an early stage of pregnancy, helping to target screening and early intervention. Future work should focus on the importance of genetic risk, alongside other clinical risk factors, in predicting GDM.

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Author contributions

A.E.H. carried out analyses, wrote the manuscript, reviewed and edited the manuscript and contributed to the discussion. G.M.H. was involved in the original HAPO analyses, reviewed and edited the manuscript and contributed to the discussion. A.M.E. was involved in the original DIP analyses, reviewed and edited the manuscript and contributed to the discussion. K.A.P. researched data, reviewed and edited the manuscript and contributed to the discussion. D.M.S. was involved in the original HAPO analyses, reviewed and edited the manuscript and contributed to the discussion. L.P.L. led the collection and preparation of the HAPO samples for genotyping, reviewed and edited the manuscript and contributed to the discussion. W.L.L. was involved in the original data acquisition and analysis in HAPO, reviewed and edited the manuscript and contributed to the discussion. F.P.D. was involved in the original data acquisition and analysis in DIP, reviewed and edited the manuscript and contributed to the discussion. A.T.H. was involved in the original conception and design of EFSOCH, researched data, reviewed and edited the manuscript and contributed to the discussion. R.M.F. researched data, wrote the manuscript, reviewed and edited the manuscript and contributed to the discussion.

Guarantor statement

W.L.L. (HAPO), F.D.P. (DIP) and A.T.H. are the guarantors of this work, have full access to the data and take responsibility for the integrity of results and accuracy of data analysis presented.

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Conflict of interest statement

The authors confirm no conflicts of interest in relation to this work.

Prior publication

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References

1. O'SULLIVAN JB. Criteria for the oral glucose tolerance test in pregnancy. Diabetes. 1964;13:278–285.

2. WHO Diagnostic criteria and classification of hyperglycaemia first detected in pregnancy Available from http://www.who.int/diabetes/publications/Hyperglycaemia_In_Pregnancy/en/. Accessed 13 June 2018.

3. Sermer M, Naylor CD, Gare DJ, Kenshole AB, Ritchie JWK, Farine D, et al. Impact of increasing carbohydrate intolerance on maternal-fetal outcomes in 3637 women without gestational diabetes: The Toronto tri-hospital gestational diabetes project. Am J Obstet Gynecol. 1995 Jul 1;173(1):146–56.

4. World Health Organization, International Diabetes Federation. Definition and diagnosis of diabetes mellitus and intermediate hyperglycaemia: report of a WHO/IDF consultation. Available from http://www.who.int/diabetes/publications/diagnosis_diabetes2006/en. Accessed 28 August 2018./

5. Hyperglycemia and Adverse Pregnancy Outcomes. N Engl J Med. 2008 May 8;358(19):1991–2002.

6. International Association of Diabetes and Pregnancy Study Groups Recommendations on the Diagnosis and Classification of Hyperglycemia in Pregnancy. Diabetes Care. 2010 Mar;33(3):676–82.

7. O'Sullivan EP, Avalos G, O'Reilly M, Dennedy MC, Gaffney G, Dunne F, et al. Atlantic Diabetes in Pregnancy (DIP): the prevalence and outcomes of gestational diabetes mellitus using new diagnostic criteria. Diabetologia. 2011 Jul;54(7):1670–5.

8. Bellamy L, Casas J-P, Hingorani AD, Williams D. Type 2 diabetes mellitus after gestational diabetes: a systematic review and meta-analysis. Lancet. 2009 May 23;373(9677):1773–9.

9. Dupuis J, Langenberg C, Prokopenko I, Saxena R, Soranzo N, Jackson AU, et al. New genetic loci implicated in fasting glucose homeostasis and their impact on type 2 diabetes risk. Nat Genet. 2010 Feb;42(2):105–16.

10. Scott RA, Lagou V, Welch RP, Wheeler E, Montasser ME, Luan J, et al. Large-scale association analyses identify new loci influencing glycemic traits and provide insight into the underlying biological pathways. Nat Genet. 2012 Sep;44(9):991–1005.

11. Wu L, Cui L, Tam WH, Ma RCW, Wang CC. Genetic variants associated with gestational diabetes mellitus: a meta-analysis and subgroup analysis. Scientific Reports. 2016 Sep 29;6(1):30539.

12. Ding M, Chavarro J, Olsen S, Lin Y, Ley SH, Bao W, et al. Genetic variants of gestational diabetes mellitus: a study of 112 SNPs among 8722 women in two independent populations. Diabetologia. 2018 Aug 12;61(8):1758–68.

13. Ekelund M, Shaat N, Almgren P, Anderberg E, Landin-Olsson M, Lyssenko V, et al. Genetic prediction of postpartum diabetes in women with gestational diabetes mellitus. Diabetes Res Clin Pract. 2012 Sep 1;97(3):394–8.

14. Freathy RM, Hayes MG, Urbanek M, Lowe LP, Lee H, Ackerman C, et al. Hyperglycemia and Adverse Pregnancy Outcome (HAPO) study: common genetic variants in GCK and TCF7L2 are associated with fasting and postchallenge glucose levels in pregnancy and with the new consensus definition of gestational diabetes mellitus from the International Association of Diabetes and Pregnancy Study Groups. Diabetes. 2010 Oct;59(10):2682–9.

15. Owens LA, O'Sullivan EP, Kirwan B, Avalos G, Gaffney G, Dunne F. ATLANTIC DIP: The Impact of Obesity on Pregnancy Outcome in Glucose-Tolerant Women. Diabetes Care. 2010 Mar;33(3):577–9.

16. The Hyperglycemia and Adverse Pregnancy Outcome (HAPO) Study. Int J Gynecol Obstet. 2002 Jul 1;78(1):69–77.

17. HAPO Study Cooperative Research Group, Nesbitt GS, Smye M, Sheridan B, Lappin TRJ, Trimble ER. Integration of local and central laboratory functions in a worldwide multicentre study: Experience from the Hyperglycemia and Adverse Pregnancy Outcome (HAPO) Study. Clin Trials. 2006;3(4):397–407.

18. Zhang G, Karns R, Narancic NS, Sun G, Cheng H, Missoni S, et al. Common SNPs in FTO gene are associated with obesity related anthropometric traits in an island population from the eastern Adriatic coast of Croatia. PLoS ONE. 2010 Apr 28;5(4):e10375.

19. Harreiter J, Simmons D, Desoye G, Corcoy R, Adelantado JM, Devlieger R, et al. IADPSG and WHO 2013 Gestational Diabetes Mellitus Criteria Identify Obese Women With Marked Insulin Resistance in Early Pregnancy. Diabetes Care. 2016 Jul 1;39(7):e90–2.

20. Catalano PM, Tyzbir ED, Wolfe RR, Calles J, Roman NM, Amini SB, et al. Carbohydrate metabolism during pregnancy in control subjects and women with gestational diabetes. Am J Physiol. 1993 Jan;264(1):E60-67.

21. Lind T. Metabolic changes in pregnancy relevant to diabetes mellitus. Postgrad Med J. 1979 May;55(643):353–7.

22. Lowe WL, Scholtens DM, Lowe LP, Kuang A, Nodzenski M, Talbot O, et al. Association of Gestational Diabetes With Maternal Disorders of Glucose Metabolism and Childhood Adiposity. JAMA. 2018 11;320(10):1005–16.

23. Ratner RE, Christophi CA, Metzger BE, Dabelea D, Bennett PH, Pi-Sunyer X, et al. Prevention of diabetes in women with a history of gestational diabetes: effects of metformin and lifestyle interventions. J Clin Endocrinol Metab. 2008 Dec;93(12):4774–9.

24. Sullivan SD, Jablonski KA, Florez JC, Dabelea D, Franks PW, Dagogo-Jack S, et al. Genetic Risk of Progression to Type 2 Diabetes and Response to Intensive Lifestyle or

Metformin in Prediabetic Women With and Without a History of Gestational Diabetes Mellitus. Diabetes Care. 2014 Apr;37(4):909–11.

Tables

Table 1. Clinical characteristics for participants diagnosed with GDM by the different criteria in HAPO.

HAPO	**Controls with normal glucose**	**FPG ≥5.1 mmol/L only**	**2-hour glucose* ≥7.8 mmol/L only**	**Both (FPG ≥5.1 mmol/L and 2-hour glucose* ≥7.8 mmol/L)**
Median maternal age in years (IQR)	31 (26-34) n=2,238	31 (27-35) n=183	32 (28-36)† n=188	32 (29-36) n=56
Median pre-pregnancy BMI (IQR)	22.9 (21.0-26.0) n=2,086	27.4 (23.9-34.2)† n=157	23.9 (21.3-27.9) n=179	28.0 (23.8-32.6)† n=50
Median SBP in mmHg (IQR)	108 (102-114) n=2,238	113 (105-119)† n=183	108 (101-115) n=188	111 (104-117) n=56

BMI, body mass index; FPG, fasting plasma glucose; GDM, gestational diabetes; HAPO, Hyperglycemia and Pregnancy Outcome Study; SBP, systolic blood pressure.

*The 2-hour glucose level refers to the glucose level measured at 2 hours following a 75 g oral glucose load as part of an oral glucose tolerance test.

† *P* value <0.01 (remained <0.01 after Bonferroni correction).

Table 2. Clinical characteristics for women diagnosed with GDM by the different criteria in DIP.

DIP	**Controls with normal glucose**	**FPG ≥5.1 mmol/L only**	**2-hour glucose* ≥7.8 mmol/L only**	**Both (FPG ≥5.1 mmol/L and 2-hour glucose* ≥7.8 mmol/L)**
Median maternal age in years (IQR)	32 (29-36) n=512	34 (30-37) n=54	34 (30-38) n=77	34 (30-36) n=54
Median pre-pregnancy BMI (IQR)	25.6 (23.5-29.4) n=434	31.6 (28.0-38.3)† n=43	27 (24.5-30.8) n=77	33.6 (30.0-37.6)† n=46
Median SBP in mmHg (IQR)	117 (108-124) n=443	119 (110-130) n=30	120 (110-130) n=55	126 (115-137)† n=33

BMI, body mass index; DIP, Atlantic Diabetes in Pregnancy; FPG, fasting plasma glucose; GDM, gestational diabetes; SBP, systolic blood pressure.

*The 2-hour glucose level refers to the glucose level measured at 2 hours following a 75 g oral glucose load as part of an oral glucose tolerance test.

†*P* value <0.01 (remained <0.01 after Bonferroni correction).

Figure legends

Figure 1. Distribution of participants diagnosed with GDM by different criteria in HAPO and DIP. The 2-hour glucose level refers to the glucose level measured at 2 hours following a 75 g oral glucose load as part of an oral glucose tolerance test. DIP; Atlantic Diabetes in Pregnancy; FPG, fasting plasma glucose; GDM, gestational diabetes; HAPO; Hyperglycemia and Adverse Pregnancy Outcome Study.

Figure 2. Plots showing mean FPG GS (A) or T2D GS (B) in each GDM diagnostic category in HAPO and DIP. Black circles = HAPO, white squares = DIP. The 2-hour glucose level refers to the glucose level measured at 2 hours following a 75 g oral glucose load as part of an oral glucose tolerance test. Error bars show 95% confidence intervals. Analyses were performed in each study cohort separately due to the genetic scores being higher overall in DIP.

**P* value for comparison between cases and controls <0.05 ***P* value for comparison between cases and controls <0.01. All *P* values survived Bonferonni correction at α=0.05 except for the T2D GS in women with both fasting and 2-hour hyperglycemia in HAPO, and T2D GS in women with isolated fasting or 2-hour hyperglycemia in DIP.

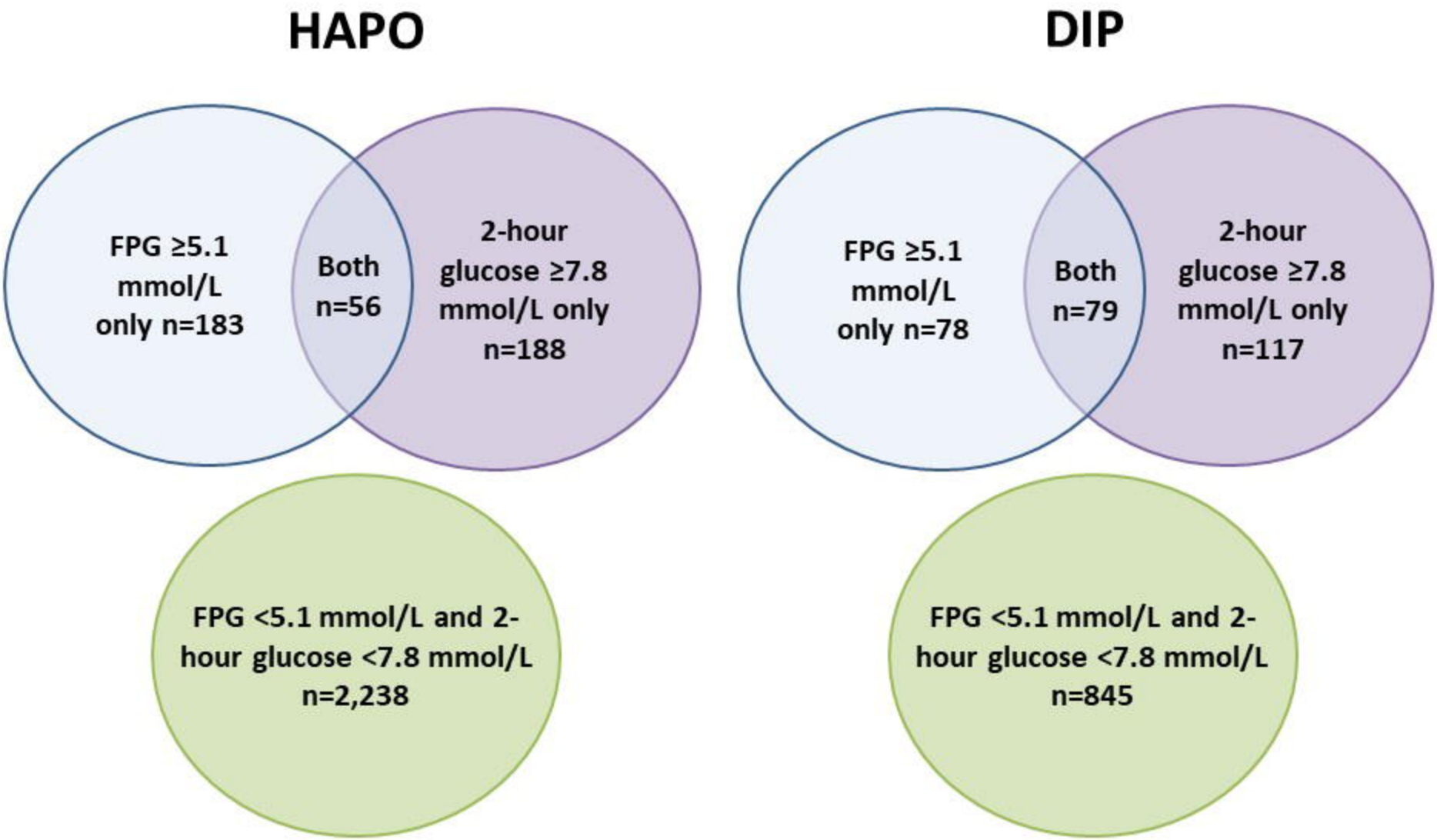
DIP; Atlantic Diabetes in Pregnancy; GDM, gestational diabetes; GS, genetic risk score; FPG, fasting plasma glucose; HAPO; Hyperglycemia and Adverse Pregnancy Outcome Study; OR, odds ratio; T2D, type 2 diabetes.

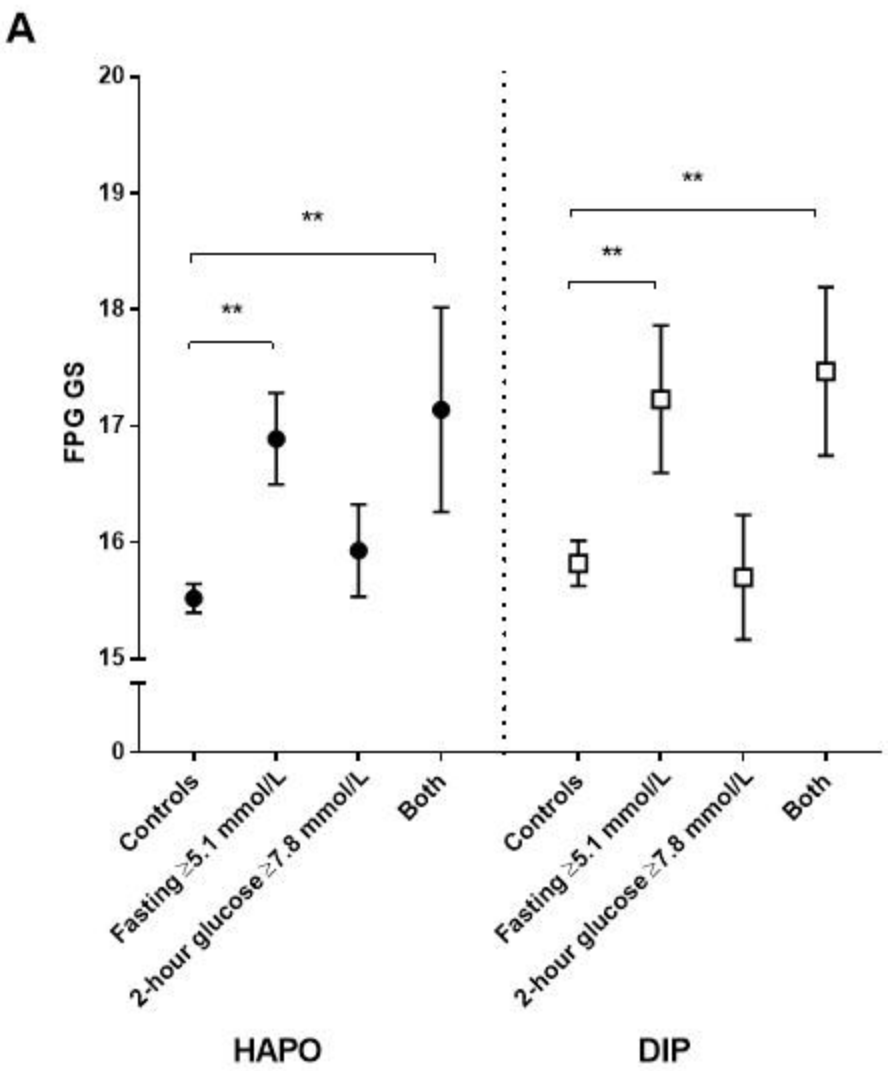
Figure 3. Plot showing mean T2D GS in women with a raised 2-hour glucose alone divided by WHO 1999 (≥7.8 mmol/L and <8.5 mmol/L) and IADPSG 2010 (≥8.5 mmol/L) thresholds in HAPO and DIP. Black circles = HAPO, white squares = DIP. The 2-hour glucose level refers to the glucose level measured at 2 hours following a 75 g oral glucose load as part of an oral glucose tolerance test. Error bars show 95% confidence

intervals. Analyses were performed in each study cohort separately due to the genetic scores being higher overall in DIP.

**P value for comparison between cases and controls <0.01. The P value for women with an isolated 2-hour glucose ≥8.5 mmol/L in DIP did not survive Bonferroni correction at α=0.05.

DIP; Atlantic Diabetes in Pregnancy; GDM, gestational diabetes; GS, genetic risk score; HAPO; Hyperglycemia and Adverse Pregnancy Outcome Study; IADPSG: International Association of Diabetes and Pregnancy Study Groups; OR, odds ratio; T2D, type 2 diabetes; WHO, World Health Organization.


HAPO
FPG ≥5.1 mmol/L only n=183
Both n=56
2-hour glucose ≥7.8 mmol/L only n=188
FPG <5.1 mmol/L and 2-hour glucose <7.8 mmol/L n=2,238
DIP
FPG ≥5.1 mmol/L only n=78
Both n=79
2-hour glucose ≥7.8 mmol/L only n=117
FPG <5.1 mmol/L and 2-hour glucose <7.8 mmol/L n=845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Fasting ≥5.1 mmol/L
2-hour glucose ≥7.8 mmol/L
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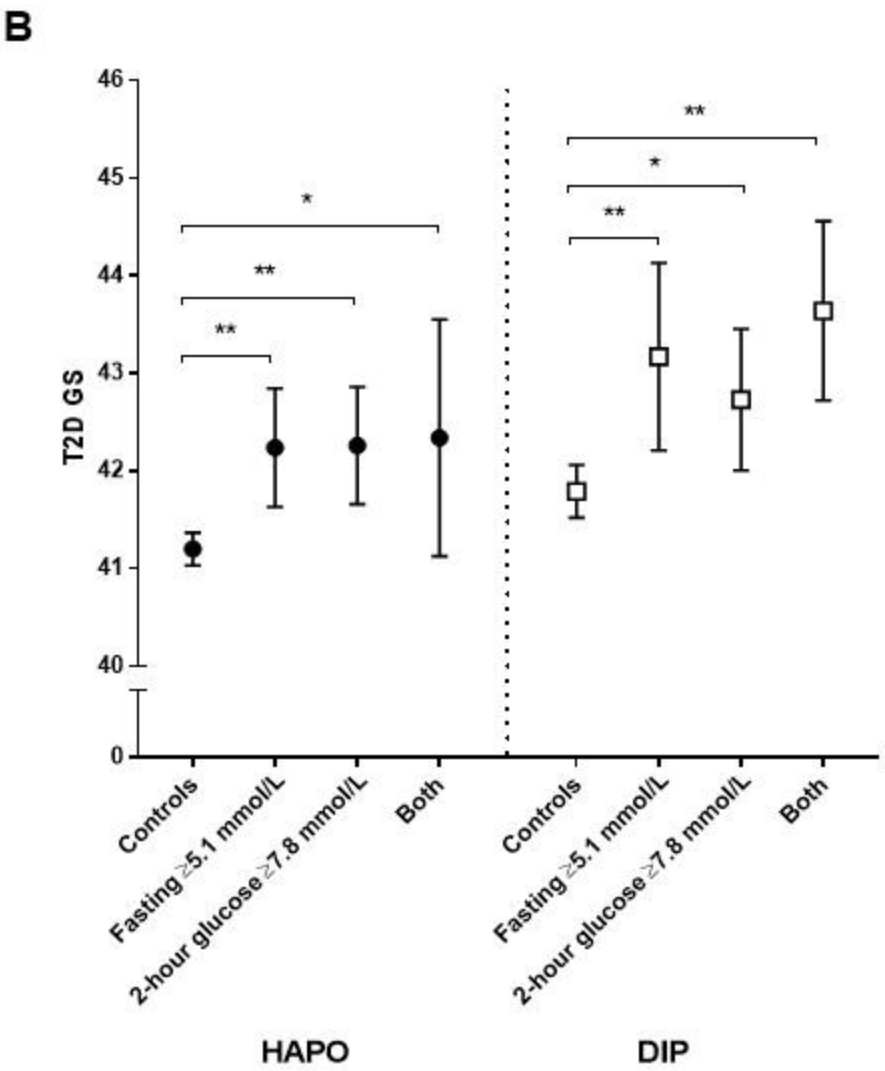
HAPO
DIP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2-hour glucose ≥7.8 mmol/L
Both
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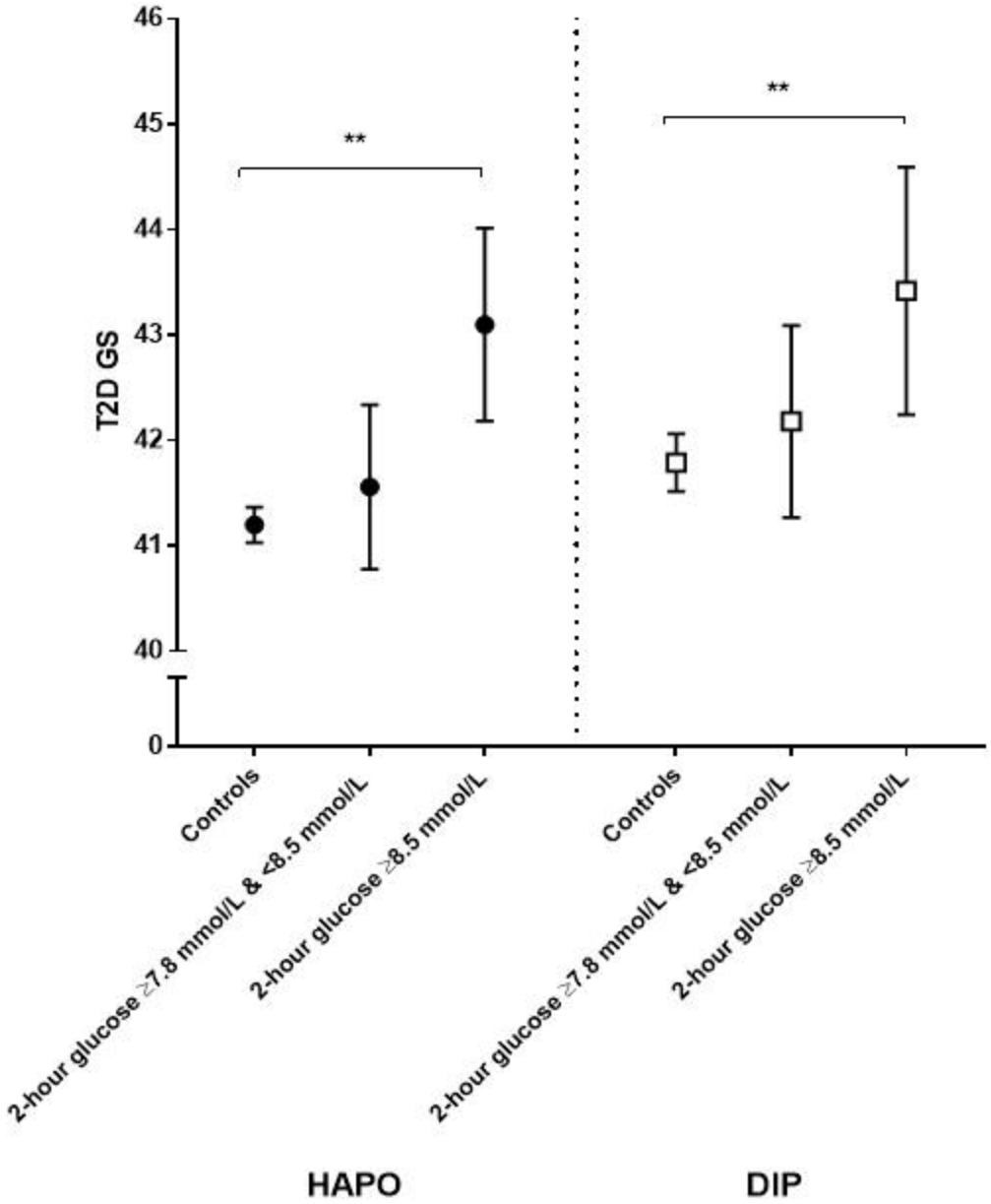
Fasting ≥5.1 mmol/L
2-hour glucose ≥7.8 mmol/L
Both
HAPO
DIP


T2D GS
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Controls
2-hour glucose ≥7.8 mmol/L & <8.5 mmol/L
2-hour glucose ≥8.5 mmol/L
Controls
2-hour glucose ≥7.8 mmol/L & <8.5 mmol/L
2-hour glucose ≥8.5 mmol/L
HAPO
DIP