Title: Women diagnosed with gestational diabetes according to the newest WHO criteria have a raised genetic risk for type 2 diabetes

Authors: Alice E Hughes[1,2], M. Geoffrey Hayes[3], Aoife M Egan[4], Kashyap A Patel[1,2], Denise M Scholtens[3], Lynn P Lowe[3], William L Lowe Jr[3], Fidelma P Dunne[5], Andrew T Hattersley[1,2,6] and Rachel M Freathy[1].

Affiliations: [1]Institute of Biomedical and Clinical Science, University of Exeter Medical School, University of Exeter, Exeter, U.K.

[2]Royal Devon and Exeter NHS Foundation Trust, Exeter, U.K.

[3]Northwestern University Feinberg School of Medicine, Chicago, U.S.A.

[4]Division of Endocrinology, Diabetes and Metabolism, Mayo Clinic School of Medicine, Rochester, MN, U.S.A.

[5]Galway Diabetes Research Centre and Saolta Hospital Group, National University of Ireland, Galway, Ireland

[6]National Institute for Health Research Exeter Clinical Research Facility, Exeter, U.K.

Corresponding author: Dr Rachel M Freathy, Institute of Biomedical and Clinical Science, University of Exeter Medical School, University of Exeter, RILD Building, Royal Devon and Exeter Hospital, Barrack Road, Exeter, EX2 5DW, U.K., R.Freathy@exeter.ac.uk, +44 (0)1392 408238. ORCID iD: 0000-0003-4152-2238

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Abstract

Objective

Using genetic scores for fasting plasma glucose (FPG GS) and type 2 diabetes (T2D GS), we investigated whether the different fasting, 1-hour and 2-hour glucose thresholds from the WHO 2013 criteria for gestational diabetes (GDM) have different implications for genetic susceptibility to raised fasting glucose and type 2 diabetes in women from the Hyperglycemia and Adverse Pregnancy Outcome (HAPO) and Atlantic Diabetes in Pregnancy (DIP) studies.

Research Design and Methods

Cases were divided into three subgroups: (i) FPG ≥5.1 mmol/L only, n=222; (ii) 1-hour glucose post 75 g oral glucose load ≥10 mmol/L only, n=154 (iii) 2-hour glucose ≥8.5 mmol/L only, n=73); and (iv) both FPG ≥5.1 mmol/L and either of a 1-hour glucose ≥10 mmol/L or 2-hour glucose ≥8.5 mmol/L, n=172. We compared the FPG and T2D GS of these groups with controls (n=3,091) in HAPO and DIP separately.

Results

In HAPO and DIP, the mean FPG GS in women with a FPG ≥5.1 mmol/L, either on its own or with 1-hour glucose ≥10 mmol/L or 2-hour glucose ≥8.5 mmol/L, was higher than controls (all P <0.01). Mean T2D GS in women with a raised FPG alone or with either a raised 1-hour or 2-hour glucose was higher than controls (all P <0.05). GDM defined by 1-hour or 2-hour hyperglycaemia only was also associated with a higher T2D GS than controls (all P <0.05).

Conclusions

The WHO 2013 criteria for GDM identify women with a genetic predisposition to type 2 diabetes as well as a risk for adverse pregnancy outcomes.

What is already known about this subject?

The WHO 2013 diagnostic criteria for gestational diabetes (GDM) include measures of fasting, 1-hour and 2-hour glucose and the thresholds for diagnosis were chosen for their similar risks for adverse pregnancy outcomes. The HAPO Follow-Up Study showed that these women are at risk for developing type 2 diabetes, but, it is not known whether the different measurements of glycaemia have different impacts on genetic risk for raised fasting plasma glucose or type 2 diabetes.

What are the new findings?

- Women with fasting hyperglycaemia (≥5.1 mmol/L) on its own, or with either a raised 1-hour (≥10 mmol/L) or 2-hour glucose (≥8.5 mmol/L) have a higher genetic risk for raised fasting plasma glucose and type 2 diabetes.
- Women diagnosed with gestational diabetes due to a raised 2-hour glucose on its own have a higher genetic risk for type 2 diabetes, but not for fasting hyperglycaemia.

How might these results change the focus of research or clinical practice?

The newest WHO 2013 criteria for diagnosing gestational diabetes identify women with a genetic predisposition to type 2 diabetes, which could provide novel information for predicting gestational diabetes and targeting of long-term follow-up.

Introduction

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 hyperglycaemia have long been associated with a higher risk for adverse perinatal outcomes [3], so a FPG ≥6.1 mmol/L (indicative of impaired fasting glycaemia 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 caesarean 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]). Whilst these thresholds were chosen for their Obstetric risks, the HAPO Follow-Up Study found that women diagnosed by the newer criteria have a higher risk of developing disorders of glucose metabolism, including T2D, 10 years after the episode of GDM [8].

However, it is not known whether the underlying genetic predisposition to fasting hyperglycaemia and type 2 diabetes varies depending on how the diagnosis of GDM is met.

Genome wide association study (GWAS) data from large population-based studies have identified multiple loci associated with FPG [9] and type 2 diabetes [10], and various loci associated with fasting hyperglycaemia and type 2 diabetes in the general population have also been associated with GDM [11–13]. Specific to the WHO 2013 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 may be different genetic risks for fasting hyperglycaemia and type 2 diabetes 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*=2,628) and Atlantic Diabetes in Pregnancy (DIP) study [16] (*n*=1,084) 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 diabetes or 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 WHO 2013 cut-offs (previously IADPSG 2010) to define fasting and 2-hour hyperglycaemia. Thus, in the current study, women diagnosed with GDM were divided into fasting hyperglycemia only (FPG ≥5.1 mmol/L and 1-hour and 2-hour glucose post 75 g oral glucose load <10 mmol/L and <8.5 mmol/L, respectively, n=222), elevated 1-hour glucose only (1-hour glucose ≥10 mmol/l, FPG <5.1 mmol/L and 2-hour glucose <8.5 mmol/l, n=154), elevated 2-hour glucose only (2-hour glucose ≥8.5 mmol/L, FPG <5.1 mmol/L and 1-hour glucose <10 mmol/L , n=73) and both (FPG ≥5.1 mmol/L and either a 1-hour glucose ≥10 mmol/L or 2-hour glucose ≥8.5 mmol/L, or both, n=172) subgroups (Figure 1). Women without GDM were defined as having FPG <5.1 mmol/L, 1-hour glucose <10 mmol/L and 2-hour glucose <7.8 mmol/L (n=3,091). The distributions of the women in the different groups and in each of the study cohorts are shown in Figure 1.

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^{TM} genotyping assay. We first selected 41 SNPs that had been previously associated with type 2 diabetes, and 16 SNPs associated with fasting glucose in non-pregnant individuals, for genotyping in the DIP study. Overlap between the type 2 diabetes and FPG SNPs meant that 7 FPG loci were also in the list of type 2 diabetes 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 type 2 diabetes SNP that showed deviation from Hardy-Weinberg Equilibrium (Bonferroni-corrected *P* value <0.05). For details of included and excluded SNPs and their sources, see Supplementary Table 1.

In the HAPO study, we selected SNPs from the same 16 FPG and 41 type 2 diabetes 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* value <0.05; see Supplementary Table 1). After exclusion of SNPs that showed deviation from Hardy-Weinberg equilibrium and one SNP from the type 2 diabetes score whose main effect was on BMI (rs11642841 (*FTO* locus) [19]), a total of 15 SNPs at FPG-associated loci and 38 SNPs at type 2 diabetes-associated loci were available in both studies for analysis.

Generating a genetic score for FPG and type 2 diabetes

Weighted genetic scores for FPG (FPG GS) and type 2 diabetes (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 type 2 diabetes risk alleles (0, 1 or 2) for each SNP, multiplied by its corresponding beta value (effect size) for association with FPG or type 2 diabetes, divided by the sum of all beta values and multiplied by the total number of SNPs analyzed (see Supplementary Figure 1 for formula). GS were generated for participants with complete data only.

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 24 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, 1-hour 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) and *P* values corrected for 12 comparisons using the Bonferroni method. 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 Bonferroni corrected for 16 comparisons.

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. Uncorrected *P* values are presented unless the association weakens, where the Bonferroni corrected *P* value is also given.

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 are summarized in Tables 1a and 1b for HAPO and DIP, respectively. Women with a FPG ≥5.1 mmol/L (on its own or with either 1-hour or 2-hour hyperglycaemia) had a higher pre-pregnancy BMI than women without GDM in HAPO and DIP (*P* values <0.001). Women with both fasting and either 1-hour or 2-hour hyperglycaemia were older compared with controls in HAPO (*P* value <0.001 and <0.05 after Bonferroni correction). In HAPO we observed a higher SBP for women diagnosed with GDM by a FPG ≥5.1 mmol/L only compared with controls (*P* value <0.001) and they had a higher SBP when either their 1-hour or 2-hour glucose was also raised, but the *P* value was >0.05 after Bonferroni correction. In DIP there was a higher SBP for women diagnosed by both fasting and either 1-hour or 2-hour hyperglycemia criteria compared with controls (*P* value <0.01 and <0.05 after Bonferroni correction).

FPG, 1-hour and 2-hour glucose are associated with FPG and T2D GS in pregnant women with and without GDM

FPG, 1-hour and 2-hour glucose values were associated with the fasting and type 2 diabetes genetic scores in HAPO (Table 2). Adjusting for the different measures of glucose tolerance suggested that these associations were not independent of one another.

Women diagnosed with GDM by fasting glucose 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 either 1-hour or 2-hour criteria, compared with controls (Figure 2A, all *P* values for comparison with control group <0.05 after Bonferroni correction). There was also evidence that women with a raised 1-hour glucose only also had a higher FPG GS in

HAPO (*P* value for comparison with controls <0.01 but >0.05 with Bonferroni correction), but this was not as strong in DIP (*P* value =0.05). In contrast, women diagnosed with GDM by 2-hour only criteria did not have a higher FPG GS overall (*P* values for comparison with controls >0.05 in both studies).

Women diagnosed with GDM by fasting, 1-hour or 2-hour criteria have a higher T2D GS than controls

The T2D GS was higher than controls in women with fasting, 1-hour or 2-hour hyperglycaemia in HAPO and DIP (Figure 2B): all *P* values for comparison with controls were <0.05 after correction except for the fasting and 1-hour only groups.

Conclusions

In this study of 3,712 pregnant women of European ancestry, we have shown that women diagnosed with GDM according to the WHO 2013 criteria have a raised genetic risk for type 2 diabetes. A genetic predisposition to a higher FPG was present for women who met the fasting glucose criteria (and 1-hour glucose criteria in HAPO), but was not present for women who met the 2-hour criteria.

We found that FPG in pregnant women both with and without GDM was positively associated with a FPG GS which was generated using SNPs identified in a non-pregnant population [9]. The 1-hour and 2-hour glucose values were also correlated with the FPG GS, but this could potentially be explained by their association with FPG, since this association was not as strong once this was taken into account. Thus, the observation that the FPG GS was not higher in women diagnosed with GDM due to a 2-hour glucose ≥8.5 mmol/L alone was expected.

FPG was associated with the T2D GS, which would be expected, as there are loci within the T2D GS which also raise fasting glucose (e.g. *GCK*, *MTNR1B*) [9]. The *ADCY5* locus has also been found to be associated with 2-hour glucose values [20]. Thus, the observation of a higher T2D GS in women meeting fasting or 2-hour criteria is not surprising. A GWAS for 1-hour glucose values was not available at the time of writing (a 1-hour glucose threshold is not part of most criteria for diabetes outside of pregnancy so is infrequently measured), but since the T2D GS was associated with 1-hour glucose values as well, it is likely that this explains the higher T2D GS seen in the women meeting this criterion, and will contribute to the higher T2D GS seen in women with a raised fasting and either a raised 1-hour or 2-hour glucose. However, it is important to note that the relationships between the T2D GS and the different glucose categories did not appear to be independent of one another, and again, although women meeting the diagnosis for GDM in one category may not meet the

thresholds for GDM in other categories, they are likely to have a degree of fasting and postprandial hyperglycaemia which will contribute to their higher genetic risk for type 2 diabetes compared with women without GDM.

One might expect that women with both fasting and postprandial hyperglycaemia would have the highest genetic risk for type 2 diabetes, but we did not observe this for the T2D GS in women with both a FPG ≥5.1 mmol/L and either of a 1-hour glucose ≥10 mmol/L or 2-hour glucose ≥8.5 mmol/L. The T2D GS in that group was similar to women with a raised 2-hour glucose alone in HAPO and DIP. On the whole, the relationship between GDM and a higher T2D GS was clearer for women with a raised 2-hour glucose or a combination of raised fasting and 1-hour or 2-hour glucose, than for women with an isolated fasting or 1-hour hyperglycaemia. Studies with greater statistical power will be needed to confirm whether genetic risk of T2D is heterogeneous across the diagnostic criteria.

This work specifically examining the genetic risk of type 2 diabetes in women diagnosed with GDM according to different criteria supports the results from the recent HAPO Follow-Up Study [21] which showed that women diagnosed with GDM post-hoc according to WHO 2013 criteria had a higher risk for type 2 diabetes 10 to 14 years after pregnancy. 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 the WHO 2013 criteria were more overweight than those diagnosed by WHO 1996 criteria [7,22]. However, the associations seen for GDM with FPG GS and T2D GS are not driven by BMI (the genetic variants included within the scores do not primarily affect FPG and T2D risk because of an effect on BMI), suggesting that women with fasting hyperglycaemia in pregnancy are likely to have both BMI-related metabolic factors and a genetic predisposition contributing to type 2 diabetes risk. In the longer-term, although using the lower FPG threshold for identifying GDM will result in more cases

diagnosed, these women will 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 type 2 diabetes 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 type 2 diabetes did not influence treatment response [24]. It is not known whether this would be different for women specifically diagnosed by WHO 2013 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. The small number of cases of GDM included has been mentioned and could explain why there were not clear differences in T2D GS seen between the different diagnostic categories. We also studied women from two different studies, where there were notable differences in clinical characteristics, even for women without GDM. 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 type 2 diabetes in DIP. However, there were remarkably similar patterns for the genetic score associations amongst the different diagnostic groups in both studies. 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 according to the newest WHO 2013 criteria have a higher genetic risk for type 2 diabetes compared with women without GDM. Overall, the criteria identify an important group of women at risk for adverse pregnancy outcomes as well as a higher risk for developing future type 2 diabetes [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 type 2 diabetes, 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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Contributors

AEH carried out analyses, wrote the manuscript, reviewed and edited the manuscript and contributed to the discussion. GMH was involved in the original HAPO analyses, reviewed and edited the manuscript and contributed to the discussion. AME was involved in the original DIP analyses, reviewed and edited the manuscript and contributed to the discussion. KAP researched data, reviewed and edited the manuscript and contributed to the discussion. DMS was involved in the original HAPO analyses, reviewed and edited the manuscript and contributed to the discussion. LPL led the collection and preparation of the HAPO samples for genotyping, reviewed and edited the manuscript and contributed to the discussion. WLL was involved in the original data acquisition and analysis in HAPO, reviewed and edited the manuscript and contributed to the discussion. FPD was involved in the original data acquisition and analysis in DIP, reviewed and edited the manuscript and contributed to the discussion. ATH researched data, reviewed and edited the manuscript and contributed to the discussion. RMF researched data, wrote the manuscript, reviewed and edited the manuscript and contributed to the discussion. WLL (HAPO), FDP (DIP) and RMF are the guarantors of this work, accept full responsibility for the work and/or the conduct of the study, had access to the data, and controlled the decision to publish.

ORCID iDs

Alice E Hughes https://orcid.org/0000-0003-1352-4447

Rachel M Freathy https://orcid.org/0000-0003-4152-2238

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Competing interests

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

Patient consent for publication

Not required.

Data availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Prior publication

Parts of this work were presented at the European Association for the Study of Diabetes

Annual Meeting, Stockholm, Sweden, 14-18 September 2015, the Royal College of Obstetricians and Gynaecologists Annual Academic Meeting, 8-9 February 2018,the East of England Deanery Registrar Prize Meeting, 15 June 2018 and the European Association for the Study of Diabetes Diabetic Pregnancy Study Group Annual Meeting, Graz, Austria, 5-8 September 2019. A previous version of the manuscript was posted as a preprint on bioRxiv, 8th April 2020 (doi: https://doi.org/10.1101/671057).

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Tables

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

HAPO	**Controls with normal glucose**	**FPG ≥5.1 mmol/L only**	**1-hr glucose[a] ≥10 mmol/L only**	**2-hr glucose[a] ≥8.5 mmol/L only**	**Both (FPG ≥5.1 mmol/L and either 1-hr glucose[a] ≥10 mmol/L or 2-hr glucose[a] ≥8.5 mmol/L)**
Median FPG in mmol/L (IQR)	4.5 (4.3-4.7) n=2,275	5.2 (5.1-5.3) n=164	4.8 (4.6-4.9) n=66	4.5 (4.3-4.7) n=48	5.3 (5.2-5.5) n=75
Median 1-hr glucose in mmol/L (IQR)	7.1 (6.0-8.0) n=2,275	8.4 (7.6-9.2) n=164	10.4 (10.2-11.0) n=66	9.0 (8.6-9.5) n=48	10.6 (10.0-11.2) n=75
Median 2-hr glucose in mmol/L (IQR)	5.8 (5.1-6.5) n=2,275	6.6 (6.0-7.1) n=164	7.4 (6.6-7.9) n=66	8.9 (8.6-9.1) n=48	7.9 (7.1-8.9) n=75
Median maternal age in years (IQR)	31 (26-34) n=2,275	31 (27-35) n=164	31 (27-35) n=66	32 (27-34) n=48	32 (29-36)** n=75
Median pre-pregnancy BMI (IQR)	22.9 (21.0-26.1) n=2,125	27.5 (23.8-33.1)*** n=142	24.4 (21.2-27.9) n=59	23.0 (20.1-25.1) n=45	28.0 (23.8-35.2)*** n=65
Median SBP in mmHg (IQR)	108 (102-114) n=2,275	113 (106-119)*** n=164	110 (103-118) n=66	104 (100-116) n=48	110 (103-118)* n=75

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

[a]The 1-hr and 2-hr glucose level refers to the glucose level measured at 1 and 2 hours, respectively, following a 75 g oral glucose load as part of an oral glucose tolerance test.

*P value <0.05 for comparison with controls (>0.05 after Bonferroni correction).

**P value <0.001 for comparison with controls (<0.05 after Bonferroni correction).

**P value <0.001 for comparison with controls (remained <0.001 after Bonferroni correction).

Table 1b. Clinical characteristics for women diagnosed with GDM by the different criteria in DIP.

DIP	Controls with normal glucose	FPG ≥5.1 mmol/L only	1-hr glucose[a] ≥10 mmol/L only	2-hr glucose[a] ≥8.5 mmol/L only	Both (FPG ≥5.1 mmol/L and either 1-hr glucose[a] or 2-hr glucose[a] ≥8.5 mmol/L)
Median FPG in mmol/L (IQR)	4.3 (4.1-4.5) n=816	5.3 (5.2-5.5) n=58	4.6 (4.4-4.8) n=88	4.5 (4.2-4.7) n=25	5.5 (5.2-5.9) n=97
Median 1-hr glucose[a] in mmol/L (IQR)	6.6 (5.6-7.7) n=816	8.7 (7.5-9.1) n=58	10.8 (10.2-11.2) n=88	8.6 (8.1-9.1) n=25	11.2 (10.2-12.0) n=97
Median 2-hr glucose[a] in mmol/L (IQR)	5.2 (4.6-6.0) n=816	6.1 (5.5-7.0) n=58	6.9 (5.9-7.8) n=88	8.8 (8.6-9.2) n=25	8.5 (7.5-9.3) n=97
Median maternal age in years (IQR)	32 (29-36) n=521	35 (31-39)* n=35	34 (31-37)* n=69	32 (29-40) n=16	33 (30-36) n=72
Median pre-pregnancy BMI (IQR)	25.4 (23.4-28.8) n=454	31.6 (29.0-38.3)*** n=33	29.6 (25.5-35.7)*** n=56	28.5 (25.5-31.1) n=16	33.5 (28.3-37.6)*** n=55
Median SBP in mmHg (IQR)	117 (108-124) n=437	119 (110-130) n=21	120 (113-130)* n=38	122 (111-134) n=12	120 (115-134)** n=41

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

[a]The 1-hr and 2-hr glucose level refers to the glucose level measured at 1 and 2 hours, respectively, following a 75 g oral glucose load as part of an oral glucose tolerance test.

[*]*P* value <0.01 for comparison with controls (>0.05 after Bonferroni correction).

[**]*P* value <0.01 for comparison with controls (<0.05 after Bonferroni correction).

[***]*P* value <0.001 for comparison with controls (remained <0.001 after Bonferroni correction).

Table 2. Associations for FPG and T2D GS with different measures of glucose tolerance in women with and without diabetes in HAPO[a].

Glucose value	Beta coefficient per one unit higher FPG GS (95% CI)	Beta coefficient per one unit higher FPG GS, with adjustment for other glucose values (95% CI)	Beta coefficient per one unit higher T2D GS (95% CI)	Beta coefficient per one unit higher T2D GS , with adjustment for other glucose values (95% CI)
Fasting	0.028 mmol/L (0.023-0.032 mmol/L)***	0.022 mmol/L (0.018-0.027 mmol/L)***	0.008 mmol/L (0.004-0.011 mmol/L)***	0.003 mmol/L (-0.0004-0.006 mmol/L)
1-hr[b]	0.060 mmol/L (0.040-0.081 mmol/L)***	0.009 mmol/L (-0.007-0.025 mmol/L)	0.051 mmol/L (0.037-0.066 mmol/L)***	0.019 mmol/L (0.008-0.031 mmol/L)**
2-hr[b]	0.032 mmol/L (0.016-0.048 mmol/L)***	0.0003 mmol/L (-0.013-0.013 mmol/L)	0.034 mmol/L (0.022-0.045 mmol/L)***	0.009 mmol/L (0.00001-0.018 mmol/L)

CI, confidence interval; FPG, fasting plasma glucose; GS, genetic score; HAPO, Hyperglycemia and Adverse Pregnancy Outcome Study; T2D, type 2 diabetes

[a]These analyses were performed in HAPO as it was a representative sample of pregnant women of European ancestry.

[b]The 1-hr and 2-hr glucose level refers to the glucose level measured at 1 and 2 hours, respectively, following a 75 g oral glucose load as part of an oral glucose tolerance test.

[**]*P* value <0.001, <0.01 after Bonferroni correction.

[***]*P* value <0.001, remained <0.001 after Bonferroni correction.

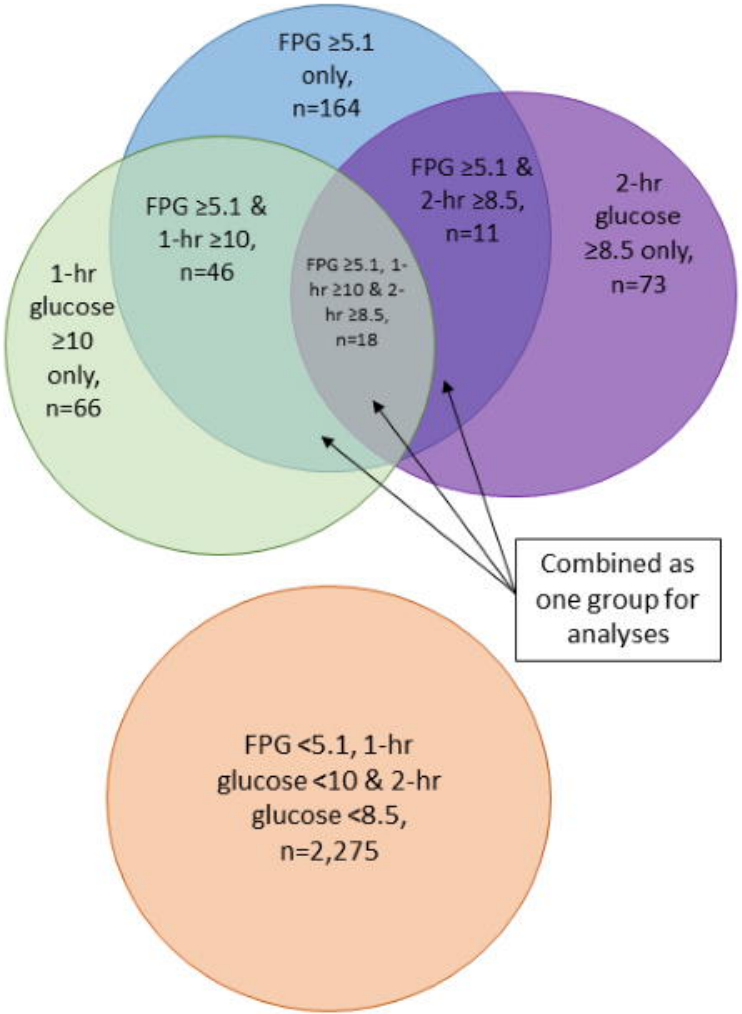
Figure legends

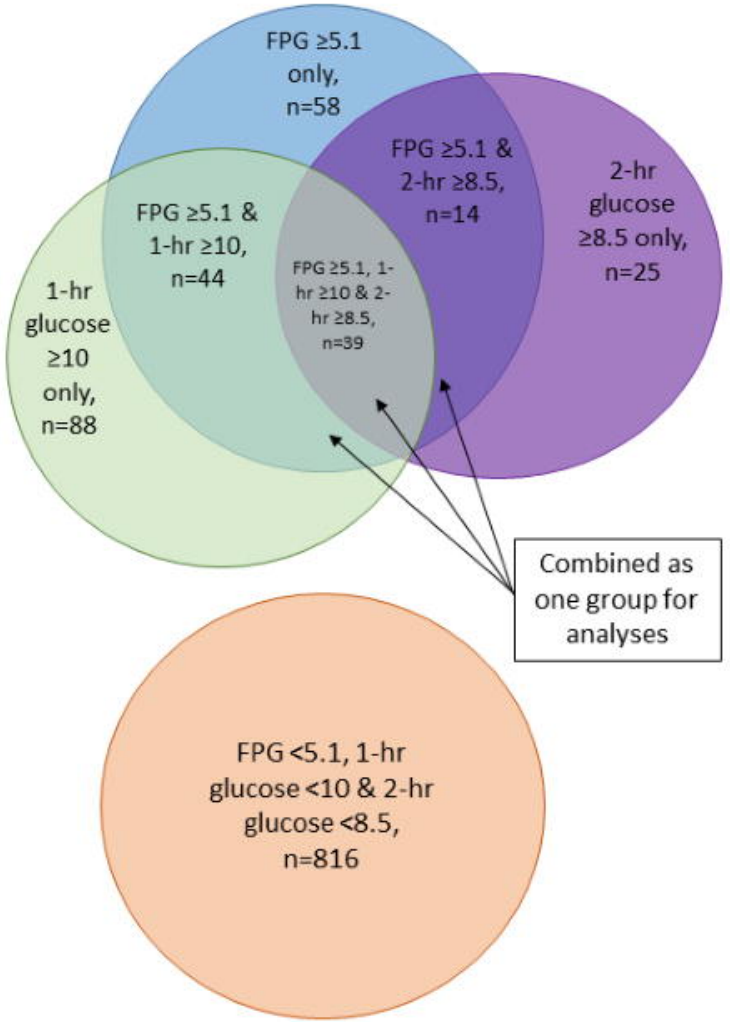
Figure 1. Distribution of participants diagnosed with GDM by different glucose categories in HAPO and DIP. All glucose values are in mmol/L. The 1-hr and 2-hr glucose levels refer to the glucose level measured at 1 and 2 hours, respectively, following a 75 g oral glucose load as part of an oral glucose tolerance test. Women with a FPG ≥5.1 mmol/L and either a 1-hr glucose ≥10 mmol/L or 2-hr glucose ≥8.5 mmol/L, or both, were combined as one group for analyses. DIP; Atlantic Diabetes in Pregnancy; FPG, fasting plasma glucose; GDM, gestational diabetes; HAPO; Hyperglycemia and Adverse Pregnancy Outcome Study.

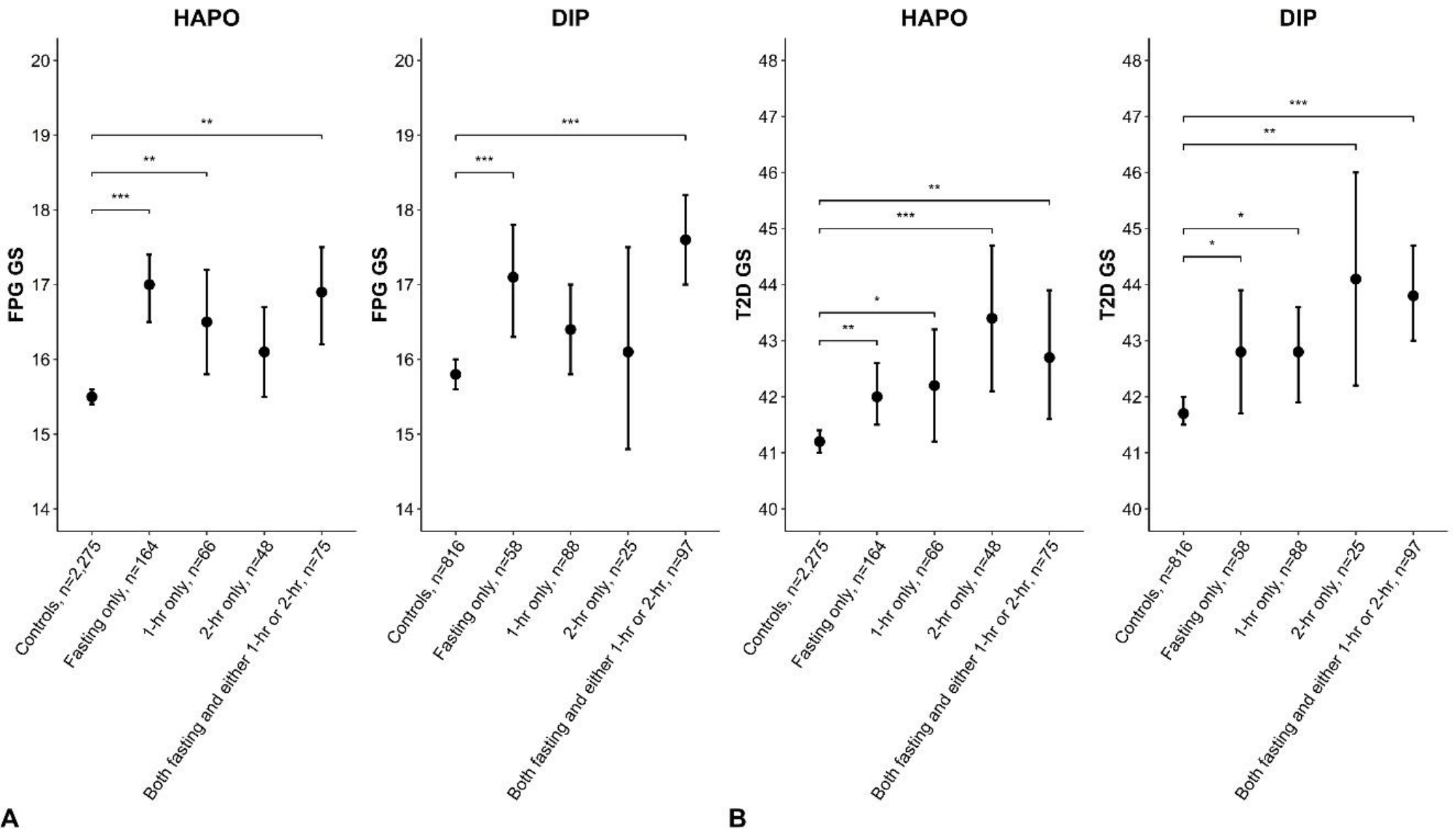
Figure 2. Plots showing mean FPG GS (A) or T2D GS (B) in each GDM glucose diagnostic category in HAPO and DIP. The 1-hr and 2-hr glucose groups refer to glucose levels measured at 1 and 2 hours, respectively, following a 75 g oral glucose load as part of an oral glucose tolerance test. The control group include women with a FPG <5.1 mmol/L, 1-hr glucose <10 mmol/L and 2-hour glucose <8.5 mmol/L. The fasting only group includes women with a FPG ≥5.1 mmol/L, a 1-hr glucose <10 mmol/L and 2-hour glucose <8.5 mmol/L. The 1-hr only group includes women with 1-hr glucose ≥10 mmol/L, FPG <5.1 mmol/L and 2-hr glucose <8.5 mmol/L. The 2-hr only group includes women with a 2-hr glucose ≥8.5 mmol/L, FPG <5.1 mmol/L and 1-hr glucose <10 mmol/L. The remaining group includes women with both a FPG ≥5.1 mmol/L and either a 1-hr glucose ≥10mmol/L or 2-hr glucose ≥8.5 mmol/L, or both. Error bars show 95% confidence intervals.

**P* value for comparison between cases and controls <0.05 ***P* value for comparison between cases and controls <0.01. ****P* value for comparison between cases and controls <0.001. All *P* values survived Bonferroni correction at α=0.05 except for the FPG GS in women with 1-hour hyperglycaemia in HAPO and the T2D GS in women with isolated fasting or 1-hour hyperglycaemia in HAPO and DIP.

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


FPG ≥5.1 only, n=164
FPG ≥5.1 & 1-hr ≥10, n=46
FPG ≥5.1 & 2-hr ≥8.5, n=11
2-hr glucose ≥8.5 only, n=73
1-hr glucose ≥10 only, n=66
FPG ≥5.1, 1-hr ≥10 & 2-hr ≥8.5, n=18
Combined as one group for analyses
FPG <5.1, 1-hr glucose <10 & 2-hr glucose <8.5, n=2,275
HAPO


FPG ≥5.1 only, n=58
FPG ≥5.1 & 1-hr ≥10, n=44
FPG ≥5.1 & 2-hr ≥8.5, n=14
2-hr glucose ≥8.5 only, n=25
1-hr glucose ≥10 only, n=88
FPG ≥5.1, 1-hr ≥10 & 2-hr ≥8.5, n=39
Combined as one group for analyses
FPG <5.1, 1-hr glucose <10 & 2-hr glucose <8.5, n=816
DIP


HAPO
DIP
HAPO
DIP
FPG GS
T2D GS
Controls, n=2,275
Fasting only, n=164
1-hr only, n=66
2-hr only, n=48
Both fasting and either 1-hr or 2-hr, n=75
Controls, n=816
Fasting only, n=58
1-hr only, n=88
2-hr only, n=25
Both fasting and either 1-hr or 2-hr, n=97
A
B