All thresholds of maternal hyperglycaemia from the WHO 2013 criteria for gestational diabetes identify women with a higher genetic risk for type 2 diabetes [version 1; peer review: 1 not approved]

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Abstract

Background: Using genetic scores for fasting plasma glucose (FPG GS) and type 2 diabetes (T2D GS), we investigated whether the 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.

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=173; 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 different diagnostic categories that are part of 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.

Keywords
Gestational diabetes, genetic scores, fasting plasma glucose, type 2 diabetes

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Introduction

Gestational diabetes mellitus (GDM) has been variably defined since criteria were first developed over 50 years ago. 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 ≥7.8 mmol/L post 75 g oral glucose load as part of an oral glucose tolerance test (OGTT), measured between 24 and 28 weeks gestation. However, lesser degrees of maternal fasting hyperglycaemia have long been associated with a higher risk for adverse perinatal outcomes, so a FPG ≥6.1 mmol/L (indicative of impaired fasting glycaemia in the non-pregnant population) was also integrated into the WHO criteria.

The Hyperglycaemia and Adverse Pregnancy Outcome (HAPO) Study 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.

WHO recommended the adoption of IADPSG in 2013, 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 vs 9.4% prevalence for WHO 1999 criteria). 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. A proportion of this risk can be attributed to genetic predisposition, since genome wide association study (GWAS) data from large, non-pregnant population-based studies have identified multiple loci associated with FPG and type 2 diabetes and some of these are shared with GDM.

Methods

Study population

Women of European ancestry with singleton pregnancies and without known pre-existing diabetes from the Hyperglycaemia and Adverse Pregnancy Outcome (HAPO) Study (n=2,628) and Atlantic Diabetes in Pregnancy (DIP) study (n=1,084) were included. The HAPO study was an observational, multi-centre study to which women were recruited during pregnancy if they were over 18 years of age. 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.

Sample collection and clinical characteristics

The study methods used in HAPO and DIP have been described in detail previously. 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 (where available) 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 hyperglycaemia 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.

Genotyping

Genotyping of individual SNPs in DNA samples from both the DIP and HAPO studies was carried out at LGC Genomics (Hoddesdon, UK), using the PCR-based KASP™ 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 seven 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 Table 1 and Table 2.

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 nine 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 one SNP that showed deviation from Hardy-Weinberg Equilibrium in the HAPO study (Bonferroni-corrected $P$ value <0.05; see Table 1 and Table 2). 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)), 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 analysed (see Figure 2 for formula). GS were generated for participants with complete data for all included SNPs 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.
Table 1. Fifteen SNPs associated with fasting plasma glucose (FPG) and used to construct the FPG genetic score.

| Chr:Pos (hg19) | SNP (proxy)* | Locus | Alleles (Effect/Other) | Effect Allele Frequencyb | Beta (mmol/L)c |
|----------------|--------------|-------|------------------------|-------------------------|---------------|
| 1:214159256    | rs340874     | PROX1 | C/T                    | 0.57                    | 0.013         |
| 2:27741237     | rs780094     | GCKR  | C/T                    | 0.62                    | 0.029         |
| 7:15064309     | rs2191349    | G6PC2 | C/T                    | 0.70                    | 0.075         |
| 7:44235668     | rs4607517    | GCK   | A/G                    | 0.18                    | 0.062         |
| 8:118184783    | rs13266634   | SLC30A8| C/T                   | 0.69                    | 0.027         |
| 9:4289050      | rs7034200    | GLIS3 | A/C                    | 0.48                    | 0.018         |
| 10:22773292    | rs780094     | GCKR  | C/T                    | 0.62                    | 0.029         |
| 11:47336320    | rs7944564    | MADD  | A/T                    | 0.72                    | 0.021         |
| 11:61571478    | rs174550     | FADS1 | T/C                    | 0.65                    | 0.017         |
| 11:92708710    | rs10830963   | MTNR1B| Q/C                    | 0.28                    | 0.067         |
| 15:62433962    | rs11071657   | C2CD4B| A/G                    | 0.62                    | 0.008         |

SNP, single nucleotide polymorphism.

*Proxy SNPs were genotyped and analysed in the Hyperglycemia and Adverse Pregnancy Outcome Study (both r² > 0.85 in 340,000 British white unrelated samples from UK Biobank).

bEffect allele frequency was calculated in 340,000 British white unrelated samples from the UK Biobank.

cBeta values were aligned to the trait-raising allele on the + strand (Human Genome Assembly Reference hg19). Source of SNPs and beta values: Dupuis et al., 2010.

We excluded rs10885122 (ADRA2A locus) due to deviation from Hardy-Weinberg Equilibrium in the Atlantic Diabetes In Pregnancy Study (Bonferroni corrected P < 0.05).

Table 2. Thirty-eight SNPs associated with type 2 diabetes (T2D) risk and used to construct the T2D genetic score.

| Chr:Pos (hg19) | SNP (proxy)* | Locus | Alleles (Effect/Other) | Effect Allele Frequencyb | Beta² | Source in which SNP was originally identified |
|----------------|--------------|-------|------------------------|-------------------------|-------|--------------------------------------------|
| 1:120526982    | rs1493694    | NOTCH2| T/C                    | 0.11                    | 0.110 | Zeggini et al., 2008²⁵                   |
| 1:214163675    | rs340835     | PROX1 | A/G                    | 0.49                    | 0.062 | Dupuis et al., 2010⁰                      |
| 2:27741237     | rs780094     | GCKR  | C/T                    | 0.62                    | 0.011 | Dupuis et al., 2010⁰                      |
| 2:43732828     | rs7578597    | THADA | T/C                    | 0.89                    | 0.141 | Zeggini et al., 2008²⁵                   |
| 2:60584819     | rs243021     | BCL11A| A/G                    | 0.46                    | 0.090 | Voight et al., 2010²⁶                    |
| 2:227093745    | rs2943641    | IRS1  | C/T                    | 0.65                    | 0.083 | Rung et al., 2009²⁷                      |
| 3:12393125     | rs1801282    | PPARG | C/G                    | 0.88                    | 0.138 | Altschuler et al., 2000²⁶                |
| 3:23336450     | rs7612463    | UBE2E2| C/A                    | 0.89                    | 0.102 | Yamauchi et al., 2010²⁹                  |
| 3:64711904     | rs4607103    | ADAMTS9| C/T                   | 0.76                    | 0.092 | Zeggini et al., 2008²⁵                   |
| 3:123065778    | rs11708067   | ADCY5 | A/G                    | 0.75                    | 0.097 | Dupuis et al., 2010⁰                     |
| Chr:Pos (hg19) | SNP (proxy)* | Locus | Alleles | Effect Allele Frequency | Beta | Source in which SNP was originally identified |
|----------------|-------------|-------|---------|-------------------------|------|---------------------------------------------|
| 4:6292915      | rs10010131  | WFS1  | G/A     | 0.60                    | 0.104| Sandhu et al., 2007^25                   |
| 5:76424949     | rs4457053   | ZBED3 | G/A     | 0.32                    | 0.150| Voight et al., 2010^26                   |
| 6:20661250     | rs7754840   | CDKAL1| C/G     | 0.31                    | 0.170| Zeggini et al., 2007^27                   |
| 7:28189411     | rs1635852   | JAZF1 | T/C     | 0.49                    | 0.120| Zeggini et al., 2008^28                   |
| 7:44235668     | rs4607517   | GCK   | A/G     | 0.18                    | 0.029| Dupuis et al., 2010^6                     |
| 7:130466854    | rs972283    | KLF14 | G/A     | 0.51                    | 0.099| Kong et al., 2009^32                     |
| 8:95937502     | rs7845219   | TP53/IP1| T/C     | 0.50                    | 0.093| Voight et al., 2010^4                     |
| 8:118184783    | rs13266634  | SLC30A8| C/T     | 0.69                    | 0.139| Sladek et al., 2007^23                   |
| 9:22133284     | rs10965250  | CDKN2A/B| G/A     | 0.83                    | 0.181| Zeggini et al., 2007^26                   |
| 9:81952128     | rs13292136  | KLF14 | G/A     | 0.52                    | 0.104| Kong et al., 2009^32                     |
| 10:12328010    | rs12779790  | CDC123/ | C/T     | 0.18                    | 0.088| Zeggini et al., 2008^26                   |
| 10:114758394   | rs7903146   | TCF7L2| T/C     | 0.29                    | 0.335| Grant et al., 2006^4                     |
| 11:1696849     | rs2334999   | HCCA2/DUSP8| T/C     | 0.42                    | 0.080| Kong et al., 2009^32                     |
| 12:2847069     | rs163184    | KCNQ1 | G/T     | 0.48                    | 0.083| Yasuda et al., 2008^35, Unoki et al., 2008^36 |
| 12:17408360    | rs5215      | KCNJ11| C/T     | 0.36                    | 0.089| Gloyn et al., 2003^37                     |
| 12:72430398    | rs1552224   | CENTD2| A/C     | 0.84                    | 0.123| Voight et al., 2010^4                     |
| 12:92673828    | rs1387153   | MTNR1B| T/C     | 0.29                    | 0.115| Prokopenko et al., 2009^38, Dupuis et al., 2010^19 |
| 12:66170163    | rs2612067   | HMGA2 | G/T     | 0.10                    | 0.180| Voight et al., 2010^26                   |
| 12:71613276    | rs1353362   | TSPAN8/LGR5| C/T     | 0.28                    | 0.103| Zeggini et al., 2008^26                   |
| 12:121402932   | rs7305618   | HNF1A | C/T     | 0.77                    | 0.112| Voight et al., 2010^38                   |
| 13:80717156    | rs1359790   | SPRY2 | G/A     | 0.71                    | 0.096| Shu et al., 2010^39                      |
| 15:62396389    | rs712432    | C2CD4A/B| A/G     | 0.57                    | 0.068| Yamauchi et al., 2010^30                  |
| 15:77747190    | rs7178572   | HMGA2 | G/A     | 0.71                    | 0.068| Koone et al., 2011^40                    |
| 15:80432222    | rs11634397  | ZFAND6| G/A     | 0.66                    | 0.102| Voight et al., 2010^38                   |
| 17:30698040    | rs4430796   | HNF1B | G/A     | 0.48                    | 0.130| Gudmundsson et al., 2007^77              |
| X:152908152    | rs2301142   | DUSP9 | A/G     | 0.85                    | 0.086| Voight et al., 2010^38                   |

^SNP, single nucleotide polymorphism.

^*Proxy SNPs were genotyped and analysed in the Hyperglycemia and Adverse Pregnancy Outcome Study (\(r^2 > 0.7\) in 340,000 British white unrelated samples from UK Biobank^24, except for at ADAMTS9 where \(r^2 = 0.45^24\)).

^Effect allele frequency was calculated in 340,000 British white unrelated samples from the UK Biobank^24.

^Beta values were aligned to the T2D-risk allele on the + strand (Human Genome Assembly Reference hg19). Beta value = log odds ratio for T2D from genome-wide association study meta-analysis of up to 8130 cases and 38987 controls, published in Voight et al. 2010^26.

We excluded rs8042680 (PRC1 locus, Atlantic Diabetes in Pregnancy Study) and rs1470579 (IGF2BP2 locus, Hyperglycemia and Adverse Pregnancy Outcome Study) from the T2D GS due to deviation from Hardy-Weinberg Equilibrium (Bonferroni-corrected \(P < 0.05\)). We additionally excluded rs11642841 (FTO locus) due to its primary effect on BMI^23.
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 analysed 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.

Ethics approval
Ethics approval was obtained from the Northwestern University Office for the Protection of Research Participants for HAPO (Protocol # 0353-001). 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 (Ref: 54/05) 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 summarised in Table 3 for HAPO and DIP, respectively. Women with a FPG \( \geq 5.1 \) 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 <0.05 after Bonferroni correction). In HAPO we observed a higher SBP for women diagnosed with GDM by FPG GS with FPG, 1-hour or 2-hour glucose in women with and without GDM (cases and controls) were analysed 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.

Weighted GS = \( \left( \frac{\text{sum (number of alleles for each SNP x Beta)}}{\text{sum of Beta values}} \right) \times \text{total number of SNPs} \)

Table 4. 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 hyperglycaemia only and by both fasting and either 1-hour or 2-hour criteria, compared with controls (Figure 3A, 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 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.

Discussion and conclusions
In this study of 3,712 pregnant women of European ancestry, we have confirmed that women diagnosed with GDM according to the WHO 2013 criteria have a raised genetic risk for type 2 diabetes and shown for the first time that this risk was raised across all of the different measures of glucose tolerance. 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 confirmed that FPG in pregnant women both with and without GDM was positively associated with a FPG GS generated using SNPs identified in a non-pregnant population. 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 \( \geq 8.5 \) mmol/L alone was expected. Maternal FPG was also associated with the T2D GS, which would be expected, as there are loci within the T2D GS which also raise fasting glucose (e.g. \( GCK, MTNR1B \)). The \( ADCY5 \) locus has also been found to be associated with 2-hour glucose values\(^4\). Thus, the
Table 3. Clinical characteristics for participants diagnosed with gestational diabetes (GDM) by the different criteria in the Hyperglycemia and Adverse Pregnancy Outcome Study (A) and the Atlantic Diabetes in Pregnancy Study (B).

(A) HAPO

| Variables                          | Controls with normal glucose | FPG ≥5.1 mmol/L only | 1-hr glucose≥10 mmol/L only | 2-hr glucose≥8.5 mmol/L only | Both (FPG ≥5.1 mmol/L and either 1-hr glucose≥10 mmol/L or 2-hr glucose≥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                                                                  |

(B) DIP

| Variables                          | Controls with normal glucose | FPG ≥5.1 mmol/L only | 1-hr glucose≥10 mmol/L only | 2-hr glucose≥8.5 mmol/L only | Both (FPG ≥5.1 mmol/L and either 1-hr glucose≥10 mmol/L or 2-hr glucose≥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 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 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 Study; FPG, fasting plasma glucose; HAPO, Hyperglycemia and Adverse Pregnancy Outcome Study; IQR, interquartile range; SBP, systolic blood pressure.

*The 1-hour and 2-hour glucose measures 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.

*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 4. Associations for fasting plasma glucose (FPG) and type 2 diabetes (T2D) genetic scores (GS) with different measures of glucose tolerance in women with and without diabetes in the Hyperglycemia and Adverse Pregnancy Outcome Study (HAPO).^a

| Glucose measure | 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.

^aThese analyses were performed in HAPO as it was a representative sample of pregnant women of European ancestry.

^bThe 1-hour and 2-hour glucose measures 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.

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

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

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Figure 3. Plots showing mean fasting plasma glucose (FPG) (A) or type 2 diabetes (T2D) (B) genetic score (GS) in each gestational diabetes (GDM) glucose diagnostic category in the Hyperglycemia and Adverse Pregnancy Outcome Study (HAPO) and Atlantic Diabetes in Pregnancy Study (DIP). The 1-hour and 2-hour 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-hour 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-hour glucose <10 mmol/L and 2-hour glucose <8.5 mmol/L. The 1-hour only group includes women with 1-hour glucose ≥10 mmol/L, FPG <5.1 mmol/L and 2-hour glucose <8.5 mmol/L. The 2-hour only group includes women with a 2-hour glucose ≥8.5 mmol/L, FPG <5.1 mmol/L and 1-hour glucose <10 mmol/L. The remaining group includes women with both a FPG ≥5.1 mmol/L and either a 1-hour glucose ≥10 mmol/L or 2-hour 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.
observation of a higher T2D GS in women meeting the fasting or 2-hour WHO 2013 criteria for GDM is not surprising. A GWAS for 1-hour glucose values was not available at the time of writing, but since we found the T2D GS to be associated with 1-hour glucose values in HAPO, it is likely that this explains the higher T2D GS seen in the women meeting this criterion for diagnosis of GDM, and will contribute to the higher T2D GS seen in women with both a fasting and either 1-hour or 2-hour hyperglycaemia. 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 a 1-hour glucose ≥10 mmol/L or 2-hour glucose ≥8.5 mmol/L. On the whole, the relationship between GDM and a higher T2D GS was clearest for women with a raised 2-hour glucose or a combination of raised fasting and 1-hour or 2-hour glucose, but studies with greater statistical power will be needed to confirm whether genetic risk of T2D is heterogeneous across the different thresholds of glucose tolerance that are part of the WHO 2013 criteria for GDM.

This work specifically examining the genetic risk of type 2 diabetes in women diagnosed with GDM according to different measures of glucose tolerance supports the results from the recent HAPO Follow-Up Study\(^6\), 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 hyperglycaemia 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\(^8\). 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)\(^6\) 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\(^6\). 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 slightly 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 therefore 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, regardless of how the diagnosis is met, 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\(^4\). This study has added to the literature confirming genetic predisposition to type 2 diabetes in women with GDM and supports the possibility that genetic testing could be a novel tool to help identify women at high risk for GDM at an early stage of pregnancy, helping to target screening and early intervention.

**Data availability**

**Underlying data**

Data is not freely available due to it consisting of potentially identifiable information, and as such is held securely to protect the interests of research participants in line with the guidance from the relevant ethics committees. However, the ethics committees will allow data analysed and generated in this study to be available to researchers through open collaboration. For access to the data used in this study please contact Dr Rachel Freathy (r.freathy@exeter.ac.uk) and Professor William Lowe Jr (wlowe@northwestern.edu) in relation to HAPO and Dr Rachel Freathy and Professor Fidelma Dunne (fidelma.dunne@nuigalway.ie) in relation to Atlantic DIP. Requests will be reviewed as soon as possible on receipt and will be facilitated with an agreement to ensure that data is transferred and held securely and results of new analyses shared with the relevant study investigators.
The websites describing the studies and other data available are [https://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study_id=phs000096.v4.p1](https://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study_id=phs000096.v4.p1) for HAPO and [http://atlanticdipireland.com/](http://atlanticdipireland.com/) for Atlantic DIP.

**Acknowledgements**

We acknowledge the work of the HAPO and DIP original investigators, whose names can be viewed in their original publications. We acknowledge the role of all professionals and families who contributed to HAPO and DIP. We acknowledge the use of the University of Exeter High-Performance Computing (HPC) facility in carrying out this work. Part of this study has been conducted using the UK Biobank Resource under Application 7036.

**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, 22 June 2020 (doi: [https://doi.org/10.1101/671057](https://doi.org/10.1101/671057)).

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The manuscript by Hughes A.E. and collaborators titled: All thresholds of maternal hyperglycaemia for the WHO 2013 criteria for gestational diabetes identify women with a higher genetic risk for type 2 diabetes” test the hypothesis that each diagnosed criteria have different genetic risk using a FPGGS and a T2D GS.

I have the following comments:

1. Please clarify what is the objective of the study and the clinical utility.

2. Previous studies have shown the limited utility GRS for T2 (and likely FPG) in the detection of women at risk for GDM, suggesting that either other genetic factors are the main driver of GDM or environmental exposures. The authors did not provide a convincing explanation in their discussion of the relevance of their findings.

3. Having an increase genetic risk for T2D or fasting hyperglycemia is irrelevant if the authors cannot show what are the clinical implications of this genetic susceptibility. It is known that women with GDM are at higher risk for developing T2D in the future, but the real question is whether a high genetic susceptibility or other clinical factors are the main drivers of this risk in women who already experienced GDM.

4. A major drawback of using GS is that they cannot provide mechanistic insights, so it is unclear how the use of GS for T2D and FPG for GDM can provide any relevant information in GDM.

5. Please provide a more comprehensive explanation for the GS calculation.

6. What is the definition of European ancestry: self-reported white or was based on population stratification analysis.
7. Pre-pregnancy BMI and maternal age are well known risk factors for GDM. I suggest supporting the statement that the association seen between the GS and the different diagnostic criteria are not driven by BMI with data. We cannot assume that variants have the same impact in BMI variability in non-pregnant and pregnant populations.

8. Table 4, please confirm that the outcomes follow normal distribution and provide results for DIP

Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
I cannot comment. A qualified statistician is required.

Are all the source data underlying the results available to ensure full reproducibility?
No

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: risk assessment

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Author Response 13 Oct 2020

Alice Hughes, University of Exeter, Exeter, UK

We thank the reviewer for their constructive comments on this work. With the help of these comments we have taken the opportunity to improve the manuscript. We have addressed their comments in turn below and updated the manuscript accordingly (Version 2).

1. Please clarify what is the objective of the study and the clinical utility.

Thank you for prompting us to clarify the message of the paper by stating the objective of the study. The objective of the study was to investigate whether there was a difference in genetic risk for fasting hyperglycaemia and type 2 diabetes according to the different
diagnostic thresholds of glucose tolerance as part of the WHO 2013 criteria for gestational diabetes (GDM). We originally stated this in the form of a hypothesis to test in the last paragraph of the introduction, but we have updated the introduction to refocus it as an objective of the study instead (paragraph 4 of the “Introduction” section).

In relation to the clinical utility of the study there are several points to consider. The thresholds for fasting, 1-hour and 2-hour glucose in the WHO 2013 criteria were chosen based on their Obstetric risks (i.e. primary caesarean section, large for gestational age baby, HAPO study reference: https://doi.org/10.1056/NEJMoa0707943). Although it is well known that women with a history of GDM have a higher risk of type 2 diabetes in later-life, it is not known if this differs according to how the diagnosis of GDM is met, and whether this may partly reflect underlying predisposing genetic factors. As expected, we observed a higher genetic risk for fasting hyperglycaemia in women who had a met the fasting plasma glucose criteria (>5.1 mmol/L). We observed a higher genetic risk for type 2 diabetes across all diagnostic categories. Therefore, these criteria identify an important group of women with a genetic risk for type 2 diabetes. This is important for the clinical community to be aware of, since there are many different criteria for GDM used across the world which utilise different criteria (for example, in the United Kingdom the fasting glucose cut-off is 5.6 mmol/L and in Denmark a fasting glucose is not included). Our findings support the findings of the HAPO Follow-Up Study, which showed women diagnosed with GDM according to the WHO 2013 criteria had higher rates of disorders of glucose metabolism 10 years later (Reference: https://doi.org/10.1001/jama.2018.11628). The genetic risk is of particular interest for women who meet the criteria for GDM due to a high 2-hour glucose, as we did not observe a significantly higher BMI in this group of women. Therefore, if they had not been identified as having GDM, these women may not necessarily be considered a high-risk group who would require monitoring and follow-up (e.g. with an annual HbA1c). We have added to the discussion to emphasise the potential implications for long-term follow-up (paragraph 6, “Discussion and conclusion” section).

Of course, it is not known whether preventative lifestyle interventions could modify this genetic risk; we touch on this in the discussion in relation women with a history of GDM in the Diabetes Prevention Program (DPP) trial (Reference: https://dx.doi.org/10.2337%2Fdc13-0700). A recent study suggested that a genetic score for type 2 diabetes was more strongly associated with developing type 2 diabetes in women with a history of GDM and a poor diet (Reference: https://dx.doi.org/10.1136%2Fbmjdrd-2019-000850). But, they also point out that the relationship between the T2D genetic risk score and incident type 2 diabetes was modest. We have added to this area of the discussion to provide a more balanced discussion on the potential implications of a high genetic risk for type 2 diabetes, emphasising the uncertainty of whether the genetic risk could be of benefit in targeting public health interventions in relation to post-GDM care (paragraphs 6 and 7).

2. Previous studies have shown the limited utility GRS for T2 (and likely FPG) in the detection of women at risk for GDM, suggesting that either other genetic factors are the main driver of GDM or environmental exposures. The authors did not provide a convincing explanation in their discussion of the relevance of their findings.

We agree that the genetic risk for fasting hyperglycaemia and type 2 diabetes will only explain a portion of risk for GDM. The genetic risk will not explain risks associated with BMI, maternal age, parity and socioeconomic deprivation. In addition, these genetic scores will
not explain the higher risks for GDM seen in women of African and South Asian heritage, for example. Whilst this study did not aim to assess the power of the genetic scores to diagnose or predict GDM, we have added to the discussion taking into account these considerations and referencing studies that have focussed on this as a new paragraph in the discussion (paragraph 5 of “Discussion and conclusion” section).

3. **Having an increase genetic risk for T2D or fasting hyperglycemia is irrelevant if the authors cannot show what are the clinical implications of this genetic susceptibility.** It is known that women with GDM are at higher risk for developing T2D in the future, but the real question is whether a high genetic susceptibility or other clinical factors are the main drivers of this risk in women who already experienced GDM.

We have partially responded to the clinical implications in response to comment 1 and point to the updated version of the discussion. Similar to comment number 2, we agree that genetic risk is likely to explain only a portion of risk for type 2 diabetes and we have added to the discussion to emphasise the importance of other factors (please see paragraph 6 of the “Discussion and conclusion” section and the response to comment 1 above). However, we also believe that genetic risk for type 2 diabetes and fasting hyperglycaemia are likely to explain part of the relationship between GDM and type 2 diabetes in later-life. We discuss this in more detail in paragraphs 4 and 6 of the “Discussion and conclusion” section.

4. **A major drawback of using GS is that they cannot provide mechanistic insights, so it is unclear how the use of GS for T2D and FPG for GDM can provide any relevant information in GDM.**

We believe that genetic scores provide important insights into the biology of GDM. For example, the risk loci included in the scores have been implicated in beta cell function, proinsulin secretion and impaired insulin action secondary to unfavourable metabolic patterns of adiposity and liver lipid metabolism (References: https://doi.org/10.1371/journal.pmed.1002654 and https://doi.org/10.1038/s41588-018-0084-1). The relevance of this to GDM was underlined in a recent paper by Powe et al. (Reference: https://doi.org/10.2337/db18-0203), which showed associations between genetic risk scores for fasting glucose, fasting insulin, and insulin secretion and sensitivity with GDM. In particular, they also observed strong associations between a fasting glucose GS and fasting glucose in pregnancy, similar to the associations seen outside of pregnancy, emphasising an important shared genetic predisposition. We did not seek to repeat the biological relevance of these associations demonstrated in this paper in our work, but rather show that there is a higher genetic risk for type 2 diabetes associated with the different measures of glucose tolerance in the WHO 2013 criteria for GDM. However, we agree that a discussion considering the likely underlying biology of genetic scores in relation to type 2 diabetes and GDM will help add to the context of the paper and have included a new paragraph in relation to this in the discussion (paragraph 4).

5. **Please provide a more comprehensive explanation for the GS calculation.**

We have provided a description of the genetic score (GS) calculation in the Methods section under the heading titled “Generating a genetic score for FPG and type 2 diabetes”. We have
also included the formula used in Figure 2. We have added a sentence referring to Tables 1 and 2 which show where the beta (effect size) was obtained for each SNP used in the score to clarify this further.

6. What is the definition of European ancestry: self-reported white or was based on population stratification analysis.

European ancestry was based on self-reported white ethnicity in both HAPO and DIP. We have added this to the Methods section under the heading titled “Study population”.

7. Pre-pregnancy BMI and maternal age are well known risk factors for GDM. I suggest supporting the statement that the association seen between the GS and the different diagnostic criteria are not driven by BMI with data. We cannot assume that variants have the same impact in BMI variability in non-pregnant and pregnant populations.

The SNPs at the risk loci included in the genetic scores influence glycaemic traits independent of BMI. We did not include SNPs which had their main effect on BMI (e.g. FTO). As BMI is not on the causal pathway between the genetic score and the outcome (GDM diagnostic criterion) it is not necessary to adjust for it in analyses, hence why it is not included in the data presented in this paper. Adjusting for BMI also has the potential to bias estimates due to collider-stratification bias (Reference: https://doi.org/10.1093/hmg/ddw433). For example, we observe a paradoxical negative correlation between the T2D GS and pre-pregnancy BMI in HAPO (Spearman's Rho -0.05, P value 0.01). This is not a true association and comes about because individuals with a higher genetic risk score are more likely to develop type 2 diabetes at a lower BMI than individuals with a lower genetic risk score. However, we agree that there are key differences in GDM risk according to BMI, which we have shown in the comparison of BMI between the different diagnostic criteria in Table 3 summarising the clinical characteristics of the participants in the studies. We have expanded on the importance of the role of BMI contributing to risk of type 2 diabetes and GDM in the discussion (see responses to Comments 1 and 3 and paragraphs 5 ad 6 of the “Discussion and conclusions” section).

8. Table 4, please confirm that the outcomes follow normal distribution and provide results for DIP.

Fasting glucose, 1-hour and 2-hour glucose have a positive skew in both HAPO and DIP (hence their presentation of median with IQR in Table 3). A normal distribution of outcomes is not a pre-requisite for linear regression models (Reference: https://doi.org/10.1017/CBO9780511790942), so we do not think this is of concern for this analysis, but we are happy to supply additional information if the reviewer would like this. We have now included a second part to the table with the associations in DIP (which had a similar pattern of associations to HAPO), but with a cautionary note (footnote b to Table 4) that the beta coefficients seen in a case-control study design will not be applicable to a general pregnant population due to the relative over-representation of cases of GDM. Now we have included these analyses P values for association have been corrected for 24 comparisons and we have updated the Methods section under the heading “Statistical analyses”.
**Competing Interests:** We have no competing interests to declare.