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The diagnosis of gestational diabetes mellitus using a 75g oral glucose tolerance test: a prospective observational study.

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Abstract:	Background Screening for Gestational Diabetes Mellitus (GDM) is controversial. This prospective study compared different sets of diagnostic cut-off points on plasma glucose measurements following a 75g Oral Glucose Tolerance Test (OGTT). Methods Women who had maternal risk factors for GDM were recruited at their convenience at their first prenatal visit and consented to a one-step OGTT at 26-28 weeks gestation. All women fulfilling the World Health Organization (WHO) 2013 diagnostic criteria received standard care for GDM. Findings Of the 202 women, 139 (69%) had one risk factor for GDM and 63 (31%) had >1. Using the WHO criteria, 53% (n=108) had GDM compared with 35% (n=71) using Canadian criteria and 18% (=36) using National Institute for Health Care Excellence criteria (NICE) criteria (both p<0·001). Of the 108 women, 50% (n=54) required pharmacological treatment to control hyperglycaemia. If the Canadian criteria were applied, 11/54 (20.4%) women would not have received hypoglycaemics. If the NICE criteria were applied, 36/54 (66.7%) women would not have received hypoglycaemics. Maternal insulin, HOMA-IR and C-peptide measured at the time of the OGTT showed evidence of increased insulin resistance in women who had GDM based on the WHO criteria but who had a normal OGTT based on the Canadian or NICE criteria. Interpretation Under stringent research conditions, the prevalence of GDM was higher using the WHO rather than the Canadian or NICE diagnostic criteria. Our findings also suggest that the Canadian and NICE criteria are not identifying women who may benefit from improved glycaemic control.

Title: The diagnosis of gestational diabetes mellitus using a 75g oral glucose tolerance test: a prospective observational study.

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Abstract

Background
Screening for Gestational Diabetes Mellitus (GDM) is controversial. This prospective study compared different sets of diagnostic cut-off points on plasma glucose measurements following a 75g Oral Glucose Tolerance Test (OGTT).

Methods
Women who had maternal risk factors for GDM were recruited at their convenience at their first prenatal visit and consented to a one-step OGTT at 26-28 weeks gestation. All women fulfilling the World Health Organization (WHO) 2013 diagnostic criteria received standard care for GDM.

Findings
Of the 202 women, 139 (69%) had one risk factor for GDM and 63 (31%) had >1. Using the WHO criteria, 53% (n=108) had GDM compared with 35% (n=71) using Canadian criteria and 18% (=36) using National Institute for Health Care Excellence criteria (NICE) criteria (both p<0·001). Of the 108 women, 50% (n=54) required pharmacological treatment to control hyperglycaemia. If the Canadian criteria were applied, 11/54 (20.4%) women would not have received hypoglycaemics. If the NICE criteria were applied, 36/54 (66.7%) women would not have received hypoglycaemics. Maternal insulin, HOMA-IR and C-peptide measured at the time of the OGTT showed evidence of increased insulin resistance in women who had GDM based on the WHO criteria but who had a normal OGTT based on the Canadian or NICE criteria.

Interpretation
Under stringent research conditions, the prevalence of GDM was higher using the WHO rather than the Canadian or NICE diagnostic criteria. Our findings also suggest that the Canadian and NICE criteria are not identifying women who may benefit from improved glycaemic control.

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Research in context

Evidence before this study

We searched the Pubmed database for studies published from the year that the Hyperglycaemia and Adverse Pregnancy Outcomes (HAPO) study was first outlined (from 1st of Jan 2002 - 31st of Jan 2020) with the search terms "gestational diabetes mellitus (GDM) diagnostic criteria" and "75-gram oral glucose tolerance test (OGTT)". The search was restricted to English language publications. In addition to this, the relevant national guidelines on the diagnosis of GDM and their associated literature were searched.

Previous studies conducted which have compared GDM diagnostic criteria have failed to consider the implication of glucose sample handling measures on the rate of GDM. Furthermore, the associations between GDM with the measured clinical outcomes are derived where the GDM rate is questionable due to a lack of adherence to the highest international sample handling practices.

Added value of this study

With the application of stringent glucose sample handling procedures (which have been updated since the HAPO study), our study compared three previous recommended sets of diagnostic criteria that utilise the 2-hour 75 gram OGTT (the World Health Organisation 2013 criteria (endorsed by two thirds of European guidelines and one of the two sets of criteria endorsed by the American Diabetes Association), the Canadian Diabetes Association criteria and the National Institute for Health and Care Excellence (NICE) adopted in England and Wales) in terms of GDM prevalence, the need for hypoglycaemic treatment and biochemical evidence of increased insulin resistance. Our findings suggest that many cases of maternal hyperglycaemia requiring pharmacological interventions may be missed under the current Canadian and in particular, NICE criteria.

Implications of all the available evidence

The current NICE guideline for the diagnosis and management of diabetes in pregnancy is due for review in 2020 and our study provides timely evidence that the GDM diagnostic criteria in particular need to be reviewed. This is necessary to facilitate future epidemiological studies on GDM. The optimal balance between diagnostic sensitivity and specificity needs to be achieved which will require consideration of studies conducted with adherence to the glucose sample handling methodology that ensures the accuracy of GDM diagnosis.

Introduction

Until 2010, the diagnosis of Gestational Diabetes Mellitus (GDM) worldwide was usually based on a three-hour 100g Oral Glucose Tolerance Test (OGTT).[1] The diagnosis required at least two out of four abnormal values and the diagnostic cut-off measurements of maternal glucose were predicted on the risk of the woman developing Type 2 diabetes mellitus later in life. In the United States of America (USA) screening was usually universal and involved a two-step process with a 50g Oral Glucose Challenge Test (GCT) followed by the 100g OGTT if the GCT was abnormal.[2] In Europe and elsewhere, screening was usually one-step and selective based on risk factors.[3]

In 2008, the Hyperglycemia and Adverse Pregnancy Outcomes (HAPO) Study Research Group published the findings from an international 15 centre observational study which enrolled 25,505 women and examined the relationship between mild hyperglycaemia and pregnancy outcomes.[4] In the absence of treatment, they reported a continuous relationship between maternal hyperglycaemia and large for gestational age (LGA) neonates, primary caesarean section, neonatal hypoglycaemia and increased cord blood C-peptide (as a surrogate for fetal hyperinsulinaemia).

Based on the HAPO findings, the International Association for Diabetes in Pregnancy Study Group (IADPSG) developed new diagnostic criteria based on a 2-hour 75g OGTT.[5] The cut-off thresholds for GDM were made more sensitive and only one abnormal value out of three maternal plasma samples was needed for the diagnosis. Based on the recommended criteria, 16·1% of the participants in HAPO would retrospectively have been diagnosed with GDM in addition to the 1·8% with more severe hyperglycaemia who had been excluded from the study. The overall retrospective 17·9% rate compares with an estimated rate of GDM complicating 6% of pregnancies in the USA in 2009.[6]

These new criteria were endorsed subsequently by the World Health Organization (WHO).[7,8] However, the IADPSG criteria have proved highly contentious.[9,10] Implementation of the new criteria has resulted in a major increase in the prevalence of GDM and an associated increase in investigations, obstetric interventions, multidisciplinary care and financial costs.[11,12]

The American College of Obstetricians and Gynecologists, however, continued with the 100g OGGT. The American Diabetes Association initially supported the 75g OGTT but now accepts that either the 75 g or the 100g test is acceptable.[6] In a survey of 28 European countries with national guidelines, 68% recommended the 2013 WHO criteria but 32% did not.[3] Others have accepted the 75g OGTT but have recommended different diagnostic cut-off points.[13,14]

This prospective study compared the WHO 2013 diagnostic cut-off points for the 2-hour 75g OGTT with sets of criteria recommended by the Canadian Diabetes Association and by the National Institute for Health and Care Excellence (NICE) in England and Wales (see Table 1).[13,14]

Methods

Women were recruited at their convenience after sonographic confirmation of an ongoing singleton pregnancy during their first antenatal visit. Clinical and sociodemographic details were recorded and computerised by a trained midwife as part of the woman's medical records. Body Mass Index (BMI) was calculated after measurement of weight and height. If the woman had a risk factor for GDM according to the 2010 national guidelines, informed written consent was obtained and she was given an appointment for a one-step 75g OGTT at 26-28 weeks gestation.[15]

There was strict adherence to preanalytical and analytical laboratory standards for the measurement of glucose.[16,17] The blood samples for the fasting, 1-hour and 2-hour samples were collected in a sodium fluoride additive tube (Sarstedt Fluoride EDTA S-Monovette 2.7mL). These samples were placed immediately on an ice-water slurry and transported to the laboratory within 30 minutes by a single researcher (EO'M) for centrifugation to prevent glycolysis. Glucose was measured via the hexokinase method on the Beckman Coulter AU640 analyser in the hospital laboratory which is nationally accredited to the International Organization for Standardization (ISO) 15189 by the Irish National Accreditation Board (coefficient of variation (CV)% of the analyser was 2·0% at 5·7mmol/L and 2·0% at 13·0 mmol/L).

At the time of the fasting plasma glucose sample, an additional venous sample was collected in an EDTA tube (Sarstedt EDTA Monovette 7·5mL). Samples were stored in the refrigerator at 4°C prior to centrifugation at 1500 rotations per minute for 15 minutes. The individual plasma and red cell aliquots were pipetted into 1ml cryotubes and stored at -80°C for the duration of the study. The plasma samples were then thawed and transferred to 96 well plates and transported on liquid nitrogen to the test laboratory on liquid. The Bio-plex Pro Human Diabetes Assay (Bio-Rad Laboratories, Cat #171A7001M, Lot #64213365) was used for analysis by a commercial company with Good Manufacturing Practice and International Standard for Organization 13485 and 9001 compliance. A total of ten analytes were measured, including c-peptide and insulin (which allowed the calculation of HOMA-IR). All measurements were completed successfully and the calibration curve for each analyte was satisfactory with an R^2>0·9. Analyte measurements were reported in pg/mL. The intra-assay % coefficient of variation (CV) and inter-assay % CV specified by the manufacturer are 3-6% CV and 2-6% CV respectively.[18]

If GDM was diagnosed based on the 2013 WHO criteria the woman was given an appointment for a group session on dietary advice and capillary glucose monitoring. Women with diet controlled GDM attended for routine antenatal care. If glycaemic control was inadequate with diet control only, they were referred to the multidisciplinary GDM service for treatment with metformin or insulin depending on the degree of hyperglycaemia.

Women who took part in the study were given an appointment to attend for a growth scan at 37 weeks. At this time the GDM treatment modality was recorded by the principal researcher. Pregnancy and delivery details were computerised immediately after delivery before postnatal hospital discharge.

Statistical analysis was performed using SPSS version 24 and the online statistical program Vassarstats.[19] The distribution of continuous data was assessed for normality using descriptive statistics for skewness and kurtosis and visual inspection of the distribution histograms. Data that were normally distributed are presented as mean (standard deviation) and non-normally distributed data are presented as median (interquartile range). Descriptive statistics were used to characterise the study population. Non-parametric tests were used to compare median values of non-normally distributed continuous variables. Vassarstats was used to assess for the significance of the difference between two independent proportions for categorial outcome variables. Box and whisker plots were generated to show the biomarker levels according to the GDM diagnostic criteria.

The study was approved by the Hospital Research Ethics Committee (Study 14-2017).

Results

The characteristics of the study population analysed by the results of the OGTT are shown in Table 2. Of the 202 women studied, 139 (69%) had one risk factor for GDM and 63 (31%) had >1.

Using the 2013 WHO criteria, 53·5% (n=108) of women were diagnosed with GDM compared with 35·1% (n=71) using Canadian criteria and 18.8% (n=38) using NICE criteria (p <0·001). As the Canadian cut-off is higher for all three maternal samples, all the women who had GDM based on the WHO criteria had GDM based on the Canadian criteria. Three women had GDM based on the NICE criteria but not based on the WHO criteria with the lower 2-hr threshold.

The Venn diagrams in Figures 2-4 show the distribution of the positive tests for each criteria when international standards to inhibit glycolysis were strictly implemented. With the WHO criteria, 88% (n=95) of those diagnosed with GDM had an abnormal FPG (including 60·2% (n=65) with an abnormal FPG only) and only 0·9% (n=1) required the 2-hour test only to make the diagnosis. Using the Canadian criteria, 84·5% (n=60) of those diagnosed with GDM could be identified with an abnormal FPG (including 56·3% (n=40) with an abnormal FPG only) and no cases required the 2-hour test only to make the diagnosis. In contrast, 10 out of the 38 (26·3%) women with GDM based on the NICE criteria had an abnormal 2-hour test.

Of the women with GDM based on the WHO criteria who required insulin to control their hyperglycaemia, 16/18 would have received insulin if the Canadian criteria were applied and 11/18 if the NICE criteria were applied. Of the 36 women with GDM based on the WHO criteria treated with metformin, 27/36 also met the Canadian criteria while 7/36 met the NICE criteria (Table 3).

The clinical outcomes of primary Caesarean section (CS) rate and birthweight (BW) $\geq 90^{th}$ percentile for the cohort are also presented in Table 3, stratified according to the different diagnostic criteria. The WHO negative cohort were used as the comparison group. Women treated for GDM based on the WHO criteria did not have a higher rate of primary CS or a Large for Gestational Age (LGA) infant compared with the WHO negative cohort. However, the study was not powered to demonstrate differences in these clinical outcomes.

Table 3 also shows the median levels of insulin, HOMA-IR and C-peptide levels measured at the time of the OGTT prior to any treatment. Irrespective of the diagnostic criteria used, those women diagnosed with GDM had higher median levels of insulin, c-peptide and HOMA-IR compared to the women who had a normal WHO OGTT (p<0·05). Women who had a positive WHO OGTT and either a negative Canadian or NICE OGTT also had higher median biomarkers consistent with increased insulin resistance.

Table 4 compares the insulin, HOMA-IR and C-peptide measurements of women who needed treatment for a positive WHO OGTT with those of women with a normal OGTT. If is notable that women who need pharmacological treatment after their OGTT are likely to show evidence of increased insulin resistance.

Figure 5-7 show box and whisker plots for insulin, C-peptide and HOMA-IR levels according to GDM diagnostic criteria with the WHO negative cohort as the reference group.

Discussion

We found that 53·5% of 202 women screened selectively with a one-step 75g OGTT had GDM using the 2013 WHO criteria. This compared with prevalences of 35·1% using Canadian criteria (p<0.001) and 18·8% using NICE criteria (p<0.001). The differences were not surprising given the different diagnostic thresholds (Table 1). The rates of GDM with all three sets of criteria were higher than previous reports, but this may be explained by the stricter implementation of laboratory standards in our study.

We also found that many women with a positive WHO OGTT who received metformin or insulin to control maternal hyperglycaemia as pregnancy advanced would not have received pharmacological treatment if the Canadian or NICE criteria were applied. Women who had GDM, irrespective of the diagnostic criteria, were more likely to have increased insulin, C-peptide and HOMA-IR measurements at the time of the OGTT which is consistent with increased insulin resistance.[20,21]

This study has strengths. The study population was well characterised and all women had sonographic dating of their pregnancy. BMI was calculated accurately at the first antenatal visit and, unlike HAPO, not at the time of the OGTT when women were often well advanced in pregnancy. GDM was diagnosed following a one-step 75g OGTT and had not been screened previously with either a fasting plasma glucose (FPG) or 50g oral GCT. Our preanalytical laboratory standards followed the latest guidelines, particularly for the inhibition of glycolysis, and were more stringent than the HAPO study and subsequent studies on OGTT measurements.

A potential limitation is that the numbers of participants were small and in a single centre. However, the study design meant that a single researcher (EO'M) could ensure strict adherence to laboratory methodology, and the women were managed clinically in a standardised way by the same multidisciplinary team if GDM was diagnosed. The study was not powered to detect statistical significance in clinical outcomes. However, the differences in maternal biomarkers depending on the GDM diagnostic criteria were statistically significant.

A recently updated Cochrane review on different strategies for diagnosing GDM identified a total of seven small trials with 1420 (range 30-386) women.[22] The quality of the evidence was assessed as very low and no conclusion could be reached. In particular, none of the trials compared the 2013 WHO criteria with other criteria. Laboratory standards were not considered.

In the HAPO study, after excluding 1·8% of women with severe hyperglycaemia, 16·1% of the 25,505 women had an abnormal OGTT post-hoc based on the IADPSG recommendations adopted by WHO in 2013. This gave an average rate of 17·9% of GDM with universal screening. However, there was wide variation retrospectively in GDM rates from 9·3% in Beersheba, Israel to 25·5% in Bellflower, California.[23] There were also wide variations across the 15 centres in the number of cases that could be diagnosed based on the FPG alone (with a diagnostic FPG in 24% of those with GDM in Bangkok, Thailand compared to 70% in Bridgetown, Barbados).

The reason for the wide variation in post hoc GDM rates between the HAPO centres was unexplained. One possibility was population differences. Another possibility was variation in the preanalytical laboratory handling of samples. The HAPO study protocol had incorporated the 2002 American laboratory standards for the inhibition of glycolysis.[24] It recommended that the venous sample should be centrifuged within 60 minutes of phlebotomy and that the sample should be placed on ice in a tube containing the glycolysis inhibitor sodium fluoride. There were problems identified with the preanalytical handling of neonatal cord glucose samples in the HAPO study and following an intervention the processing of samples within 60 minutes increased from 48·4% to 70·8%.[20] To our knowledge the adherence to laboratory protocols across the 15 centres has not been published.

In 2011, revised international laboratory standards for glucose samples recommended centrifugation within 30 minutes and that samples in fluoride additive tubes should be placed immediately in an ice-water slurry.[16] If that cannot be achieved, a tube containing a rapidly effective glycolysis inhibitor, such as citrate buffer, should be used.

The application of the 2011 standards in our study meant that glycolysis was inhibited more effectively than in the HAPO study. This minimised variations in the handling of samples. Our results show that if the 2013 WHO criteria are applied and the laboratory standards strictly implemented, the number of women diagnosed with GDM will increase further globally. Our results also suggest that if the 2011 laboratory standards are implemented, then the 2-hour sample may not be necessary for diagnosis. This would also decrease preanalytical glycolysis in the FPG and 1-hour samples by shortening the phlebotomy-centrifugation interval, reduce financial costs and make the 75g OGTT less time consuming for women.

A recent editorial on the subject of inhibiting glycolysis when measuring maternal plasma glucose supported the use of stricter preanalytical laboratory standards as used in our study.[17] It also highlighted the importance of improved diagnostic sensitivity in epidemiology studies post HAPO.

The customary practice with the 75g OGTT is to send all three samples in batches to the laboratory after the 2-hour sample is taken.[25] Glycolysis takes place at a rate of 5-7% per hour and therefore, glucose is most likely to be underestimated in the FPG sample.[26] As the diagnosis of GDM is made on one, not two, samples with the 75g OGTT, underestimating maternal glucose may lead to the diagnosis of GDM being missed.

This is also more likely to occur if the 1-hour sample rather than the 2-hour sample is omitted. In a previous study conducted by our research group of 155 women screened for GDM with a 75g OGTT where the WHO 2013 criteria were applied with strict preanalytical sample handling, all cases of GDM were diagnosed on fasting or 1-hour samples. The 2-hour test was not needed to diagnose any additional cases of GDM.[27]

Variations in the phlebotomy-centrifugation interval between hospitals may explain the variations in GDM rates between centres reported in the HAPO study and in other reports.[4]

A major source of contention is the statistical methods used to decide the post HAPO IADPSG thresholds for the three plasma samples. It was based on an arbitrary 1·75 odds ratio (OR) of an increased risk of certain adverse pregnancy outcomes compared with mean plasma glucose concentrations. The pregnancy outcomes used were increased BW >90th centile (OR 1·39 95% CI 1·32-1·44), primary CS (OR 1·11 95% CI 1·06-1·15), neonatal hypoglycaemia (OR 1·08 95% CI 0·98-1·19), and cord blood C-peptide measurements >90th centile (OR 1·55 95% CI 1·47-1·64).

The clinical risks of maternal hyperglycaemia associated with the IADPSG 1·75 OR in the HAPO study were low. The risk of neonatal hypoglycaemia was not statistically significant and testing was not universal. The risk of primary CS was not analysed by parity or BMI at the first antenatal visit. LGA was not analysed for confounders such as obesity in early pregnancy. The clinical implications of a cord C-peptide level > 90th centile are uncertain. The Canadian criteria are also arbitrary but are based on a less sensitive 2·0 OR for an increased risk of the adverse outcomes.[13]

The cut-off threshold recommended by the guideline development group (GDG) in NICE for the diagnosis of GDM was based on consideration of a de novo, highly complex, theoretical modelling of 14 different maternal plasma glucose thresholds which were part of both the 1999 and the 2013 WHO criteria.[28] The focus was on cost effectiveness in predicting specified maternal and neonatal clinical outcomes. NICE used data from four of the 15 HAPO centres (two in the UK and two in Australia). It is notable that the only 1-hr threshold considered in the modelling was that used in the 2013 WHO recommendations and no consideration was given to laboratory standards.

The NICE criteria recommended by the GDG were based on economic considerations rather than clinical factors. The 2015 report recognised the limitations of the theoretical modelling and suggested updating the recommendations within 3-5 years. A surveillance proposal consultation document released by NICE in 2018 proposed not updating the guideline as planned.[29] The decision was taken because the limited amount of recent evidence was broadly consistent with the 2013 recommendations and because of concerns about capacity within the maternity services to cope with an increase in GDM cases. It anticipated that the evidence base for diabetes in pregnancy, including the diagnostic criteria for GDM, would be further developed in the near future and therefore the next scheduled review should take place in 2020.

It is concerning that the current NICE criteria are based on economic and capacity considerations, yet our observations suggest that many cases of maternal hyperglycaemia requiring pharmacological interventions may be missed under the current NICE diagnostic cut-off points. Our findings also suggest that the absence of a 1-hour threshold is a serious omission with the NICE OGTT. We believe that the planned NICE review should proceed in 2020 as originally advised.

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Author contribution: MT and EOM designed the study. EOM conducted the study and collected the data. EOM, CR and MT conducted the data analysis. All authors (EOM, CM, ROK, LM SS and MT) interpreted the data, drafted and revised the manuscript and approved the final version of the manuscript for publication. All authors agree to be accountable for all aspects of the work.

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Table 1. Outline of the three GDM diagnostic criteria using a one-step 75 g OGTT

	Fasting (mmol/L)	1-hour (mmol/L)	2-hour (mmol/L)
WHO 2013	≥5·1	≥10·0	≥8·5
Canadian Diabetes Association 2013	≥5·3	≥10·6	≥9·0
NICE 2015	≥5·6	-	≥7·8

Table 2. Comparison of maternal characteristics and delivery outcomes according to whether they fulfilled different diagnostic criteria for gestational diabetes mellitus (GDM).

	WHO negative (n=94)	**WHO positive (n=108)**	**Canadian positive (n=71)**	**NICE positive (n=38)****
Age (years, mean, SD)	31·3 (5·3)	31·6 (5·3)	32·6 (5·1)	34·6 (3·7)
Obese (%,n)	42·6% (40)	68·5% (74)[1c]	67·6% (48)[1b]	63·9% (23)
Irish nativity (%,n)	85·1% (80)	75·9% (82)	71·8% (51)	72·2% (26)
Nulliparas (%,n)	37·2% (35)	36·1% (39)	26·8% (19)	25·0% (9)
1 RF for GDM (%,n)	77·7% (73)	61·1% (66)[1a]	54·9% (39)[1b]	55·6% (20)[1a]
>/=2 RF for GDM (%,n)	22·3% (21)	38·9% (42)[1a]	45·1% (32)[1b]	44·4% (16)[1a]

Abbreviations - RF - risk factor, GDM - gestational diabetes mellitus, WHO - World Health Organization , NICE - National Institute for Health and Care Excellence (United Kingdom).
**Note for the NICE criteria, 3 women would test positive based on the 2 hour value (>/= 7·8), who did not meet the criteria for WHO (2 HOUR >/= 8·5), thus they did not receive treatment for GDM.
Significance is shown as follows:
Reference group: [1] WHO negative
P value: [a] <0·050, [b] <0·005, [c] <0·001

Table 3. Clinical outcomes and biomarker levels amongst the cohort stratified according to the diagnostic criteria used.

	WHO negative[#] (n=90)	WHO positive (n=108)	Canadian positive (n=71)	NICE positive (n=38)	WHO positive but Canadian negative (n=47)	WHO positive but NICE negative (n=75)
GDM Pharmacological treatment -Metformin (%,n) -Insulin (%,n)	 0.0% 0.0%	 33.3% (36) 16.7% (18)	 38.0% (27) 22.6% (16)	 21.2% (7) 33.3% (11)	 25.5% (12) 10.6% (5)	 38.3% (28) 10.6% (8)
Primary CS^ (%,n)	22.4% (17)	25.0% (20)	26.5% (13)	29.1% (7)	25.6% (10)	22.0% (13)
BW ≥ 90%ile (%,n)~	7.5% (7)	3.7% (4)	5.6% (4)	2.6% (1)	10.6% (5)	14.7% (11)
Insulin (pg/ml) (median, IQR)**	205.0 (133.3)	317.9 (178.8)[a]	329.5 (205.6)[a]	328.9 (186.3)[b]	289.5 (145.1)[c]	301.0 (184.6)[b]
HOMA-IR (median, IQR)**	1.2 (0.9)	2.2 (1.4)[a]	2.4 (1.6)[a]	2.5 (1.7)[a]	1.8 (0.9)[a]	2.0 (1.2)[a]
C-peptide (pg/ml) (median, IQR)**	1346.0 (632.9)	1882.6 (592.1)[a]	1914.4 (555.9)[a]	1924.9 (510.2)[a]	1730.2 (625.1)[b]	1869.5 (641.3)[a]

Abbreviations: WHO - World Health Organization, NICE- National Institute for Health and Care Excellence (United Kingdom), GDM - gestational diabetes mellitus, CS-Caesarean Section, BW - Birth weight

[#]Three women who were WHO negative but NICE positive based on a 2 hour value 7.8-8.4 mmol/L were excluded from this group.

^Primary CS - all CS performed in women with no prior CS (denominators for the groups: WHO negative -76, WHO positive - 80, Canadian positive - 49, NICE positive - 24, WHO positive, Canadian negative 39, WHO positive NICE negative 59

~ The numbers are too small to assess statistical significance

** Cohort numbers for the biomarker data, WHO negative - 89, WHO positive - 105, Canadian positive - 70, NICE positive - 37, WHO positive, Canadian negative - 45, WHO positive, NICE negative - 70.

Reference group - WHO negative

P value significance level; a <0.001, b <0.005, c <0.050

Table 4 Insulin, C-peptide and HOMA-IR levels according to GDM treatment cohort (based on the WHO 2013 criteria).

	Insulin (pg/ml, median, IQR)	C-peptide (pg/ml, median, IQR)	HOMA-IR (median, IQR)
Non-GDM (n=88)	206.1 (131.1)	1344.1 (641.7)	1.2 (0.8)
GDM - diet controlled (n=56)	304.0 (175.8)[1b]	1784.4 (707.8)[1a]	2.1 (1.3)[1a]
GDM - metformin (n=34)	329.5 (229.3)	1881.7 (615.7)[1a]	2.2 (0.6)[1b]
GDM - insulin (n=18)	345.9 (166.7)	1945.2 (431.8)[1a]	2.7 (1.6)[1b]

Reference group: 1: Non-GDM
Significance level: a <0.001, b <0.005

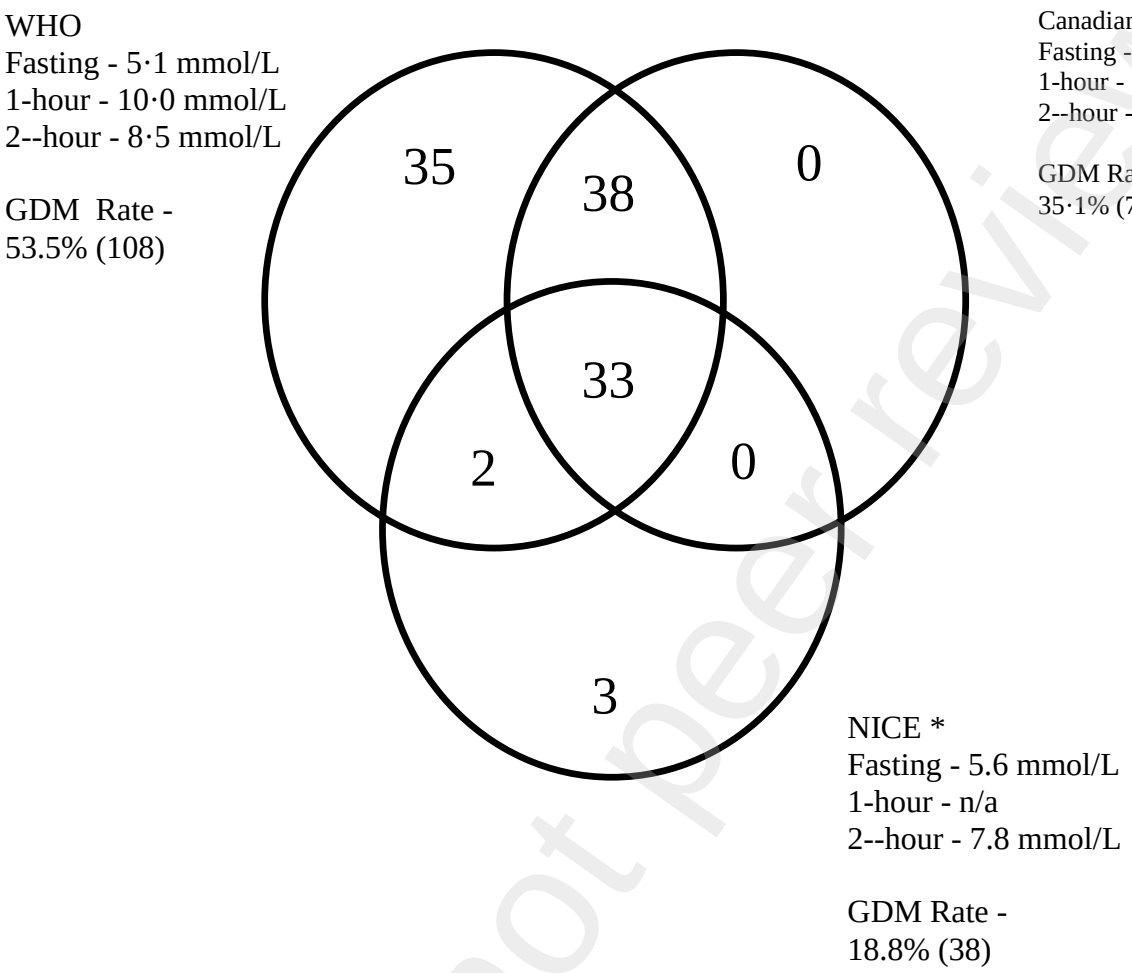

Figure 1. Distribution of the positive results according to the WHO, Canadian and NICE criteria for diagnosis of gestational diabetes mellitus

*Of the 38 women that would be diagnosed according to the NICE criteria, 3 were based on a 2-hour value ≥7.8mmol/L which did not meet the WHO criteria for diagnosis (≥8.5 mmol/L), thus they did not receive treatment for GDM

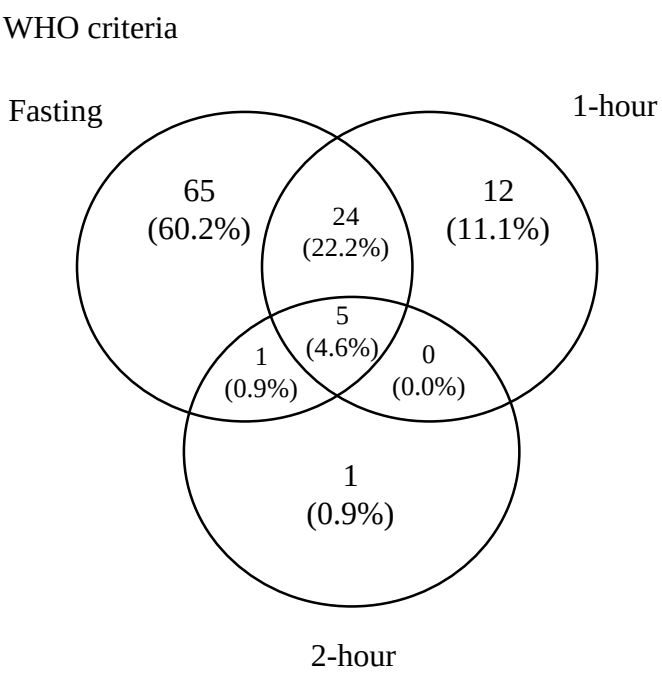

Figure 2. Distribution of positive oral glucose tolerance test results with the WHO criteria in the setting of strict preanalytical sample handling.

Canadian criteria

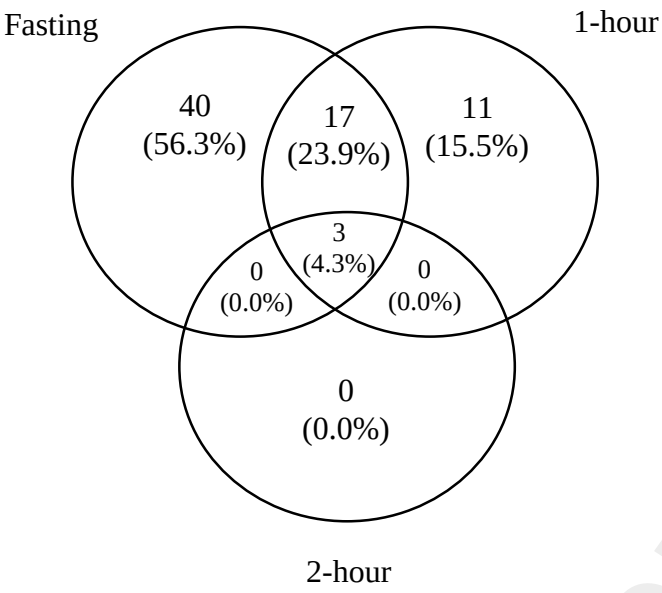

Figure 3. Distribution of positive oral glucose tolerance test results with the Canadian criteria in the setting of strict preanalytical sample handling.

NICE criteria

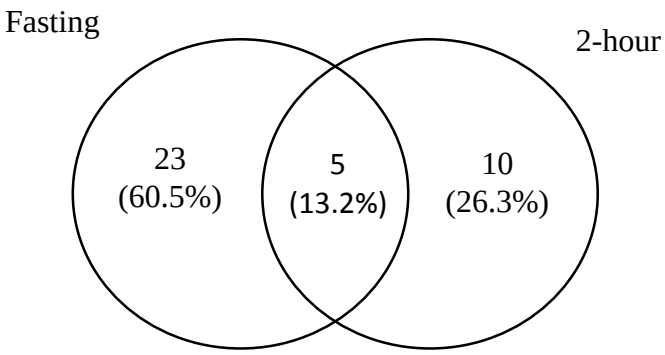

Figure 4. Distribution of positive oral glucose tolerance test results with the NICE criteria in the setting of strict preanalytical sample handling.

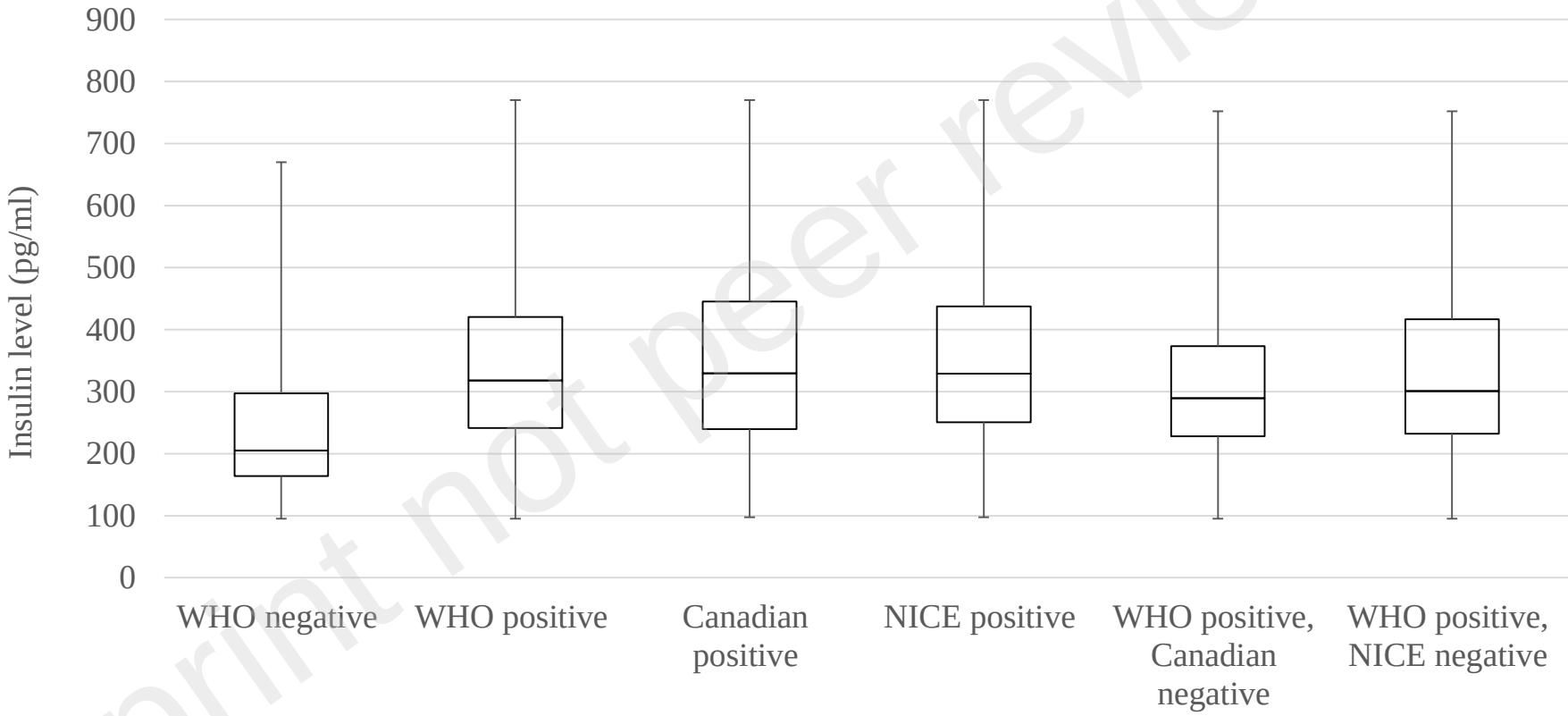

Figure 5. Box and whisker plot of the insulin levels according to the GDM diagnostic criteria

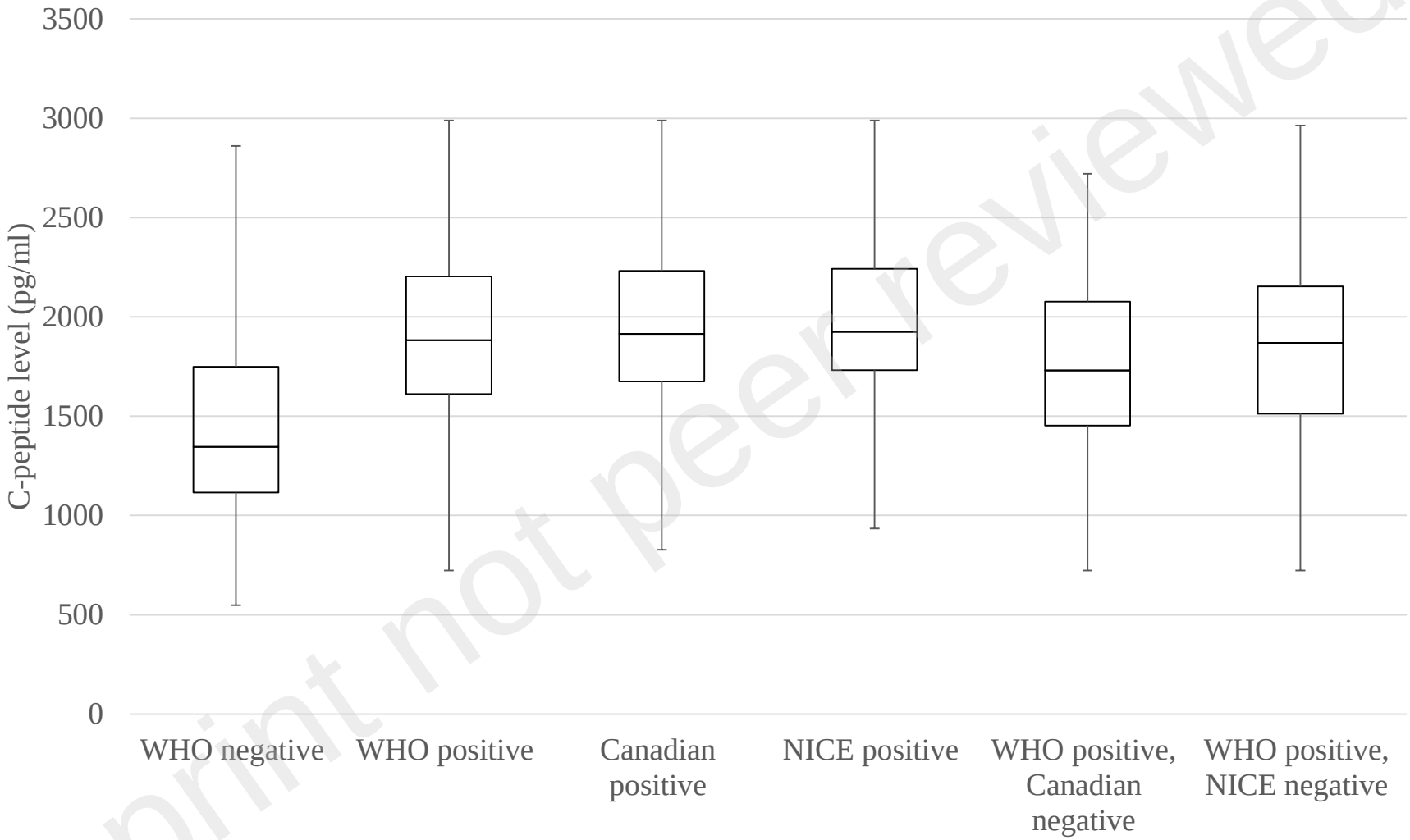

Figure 6. Box and whisker plot of the c-peptide levels according to the GDM diagnostic criteria

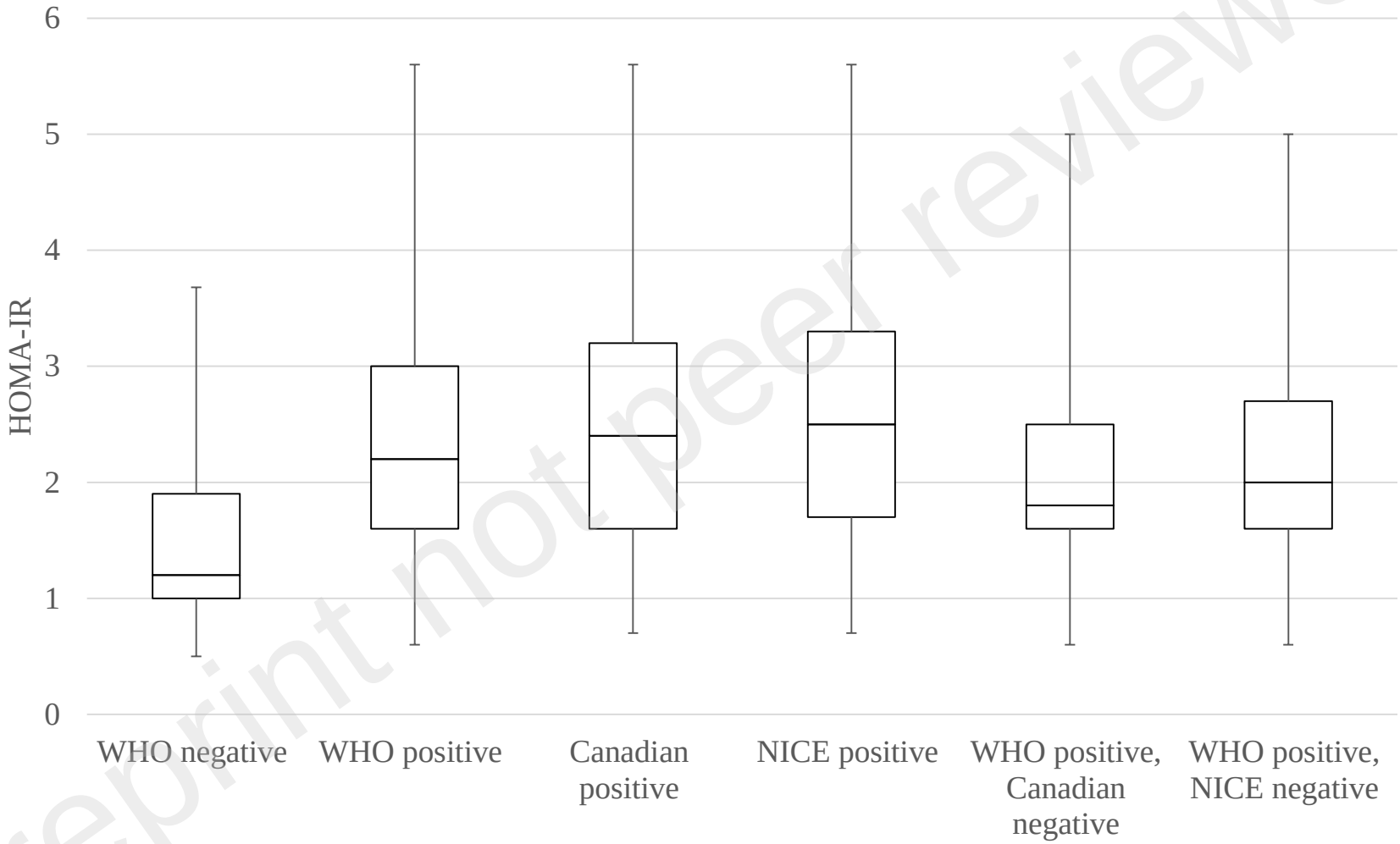

Figure 7. Box and whisker plot of the HOMA-IR levels according to the GDM diagnostic criteria