The 75-g Glucose Tolerance Test in Pregnancy

A reference range determined on a low-risk population and related to selected pregnancy outcomes

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OBJECTIVE — To determine a reference range for the 75-g glucose tolerance test (GTT) in pregnancy using a group of women at low risk for gestational diabetes mellitus (GDM) and to determine the validity of this reference range by examining selected pregnancy outcomes for glucose-tolerant women with a 2-h result on the GTT up to 1.0 mmol/l below the diagnostic level for GDM compared with treated women with GDM.

RESEARCH DESIGN AND METHODS — The reference range for the GTT was determined in 573 Caucasian women with an age <25 years and a BMI of <25 kg/m^2. Selected pregnancy outcomes were compared between 272 treated women with GDM (diagnosed on the basis of a 2-h glucose level ≥8.0 mmol/l) and 308 women with a 2-h glucose level of 7.0–7.9 mmol/l.

RESULTS — There was 95% confidence that at least 95% of all the fasting glucose levels are ≤5.1 mmol/l (92 mg/dl) and 95% confidence that at least 95% of all the 2-h glucose levels were ≤7.8 mmol/l (140 mg/dl). Treated women with GDM had a significantly reduced rate of large-for-gestational-age infants compared with glucose-tolerant women, without any increase in the rate of small-for-gestational-age infants or obstetric interventions.

CONCLUSIONS — The reference range for the GTT in pregnancy should be determined on a low-risk population rather than on a total population. Consideration should be given to lowering the fasting glucose level to 5.0 mmol/l (90 mg/dl) and the 2-h level to 7.8 mmol/l (140 mg/dl). Glucose-tolerant women below this relatively low reference range have an increased rate of large-for-gestational-age infants and may benefit from treatment.

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Gestational diabetes mellitus (GDM) is defined as any degree of glucose intolerance with onset or first recognition during pregnancy (1). A diagnosis of GDM has implications for the immediate outcome of the pregnancy as well as the long-term health of both the infant and the mother (2).

The definitive diagnosis of GDM is based on the results of a glucose tolerance test (GTT). However, there is no international agreement about the amount of glucose to be used or the diagnostic glucose levels (3). While ideally a diagnosis of GDM should be outcome-based and relevant to both the immediate and long-term consequences, this is unlikely to be achieved in the near future. A statistical approach has been frequently used.

The difficulty with using a statistical approach to define a reference range is the absence of a bimodal distribution of glucose results. It is not practical to determine a reference range after arbitrarily defining and excluding women with GDM, while a range determined on a total population will necessarily include many women with GDM.

A recent position statement of the American Diabetes Association has recommended that pregnant women with low-risk factors for GDM need not be tested (1). Determination of a reference range for the GTT on these low-risk women should assist in defining normal glucose tolerance in pregnancy.

The difficulty with using outcomes to define GDM is that the majority of women with GDM, however defined, are treated. Given this problem, one way of determining if the diagnostic category of GDM includes most women at risk would be to compare the outcomes of treated women with GDM with the outcomes of glucose-tolerant women.

There were two aims of this study. The first was to determine a reference range for the GTT in pregnancy in women deemed to be at low risk of this disorder. The second was to examine selected pregnancy outcomes in treated women with GDM (based on a 2-h glucose level ≥8.0 mmol/l after a 75-g GTT) compared with glucose-tolerant women with a 2-h glucose level between 7.0 and 7.9 mmol/l.

RESEARCH DESIGN AND METHODS

This study was carried out in the Illawarra area, New South Wales, Australia. The Illawarra area is based around the city of Wollongong and has a population of ~280,000 people. There are two public hospitals in the area offering prenatal clinic services and several obstetricians in private practice.

Over an 18-month period, January 1993 to June 1994, all women attending the prenatal clinics at Wollongong and Shellharbour hospitals were tested for GDM. All women were tested at the beginning of the 3rd trimester with a 75-g GTT in the morning after an overnight fast. No

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Abbreviations: GDM, gestational diabetes mellitus; GTT, glucose tolerance test; LGA, large-for-gestational-age; SGA, small-for-gestational-age; WHO, World Health Organization.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

preliminary challenge test was used. For a majority of women, venous samples for plasma glucose estimation were taken while fasting and 2 h after the 75-g glucose solution. For local logistical reasons, some women only had the 2-h sample taken after the GTT. We have called this simplified procedure the modified GTT (4).

In addition, for a variable but consecutive period over this 18-month period, all women were tested for GDM who were attended by three obstetricians in private practice and a general practitioner with an obstetric interest.

GDM was diagnosed, according to the criteria of the Australasian Diabetes in Pregnancy Society (ADIPS) (5), if the fasting glucose level was ≥5.5 mmol/l and/or the 2-h glucose level was ≥8.0 mmol/l.

Data collected on all women included their age at the time of glucose tolerance testing, gestational week of testing, pre-conception weight by recall, height, country of birth, and the results of the GTT. Women attending the prenatal clinic at the Wollongong Hospital had the presence or absence of a family history of diabetes in a first-degree relation recorded. The country of birth was used for the classification of ethnic origin. The countries of birth were grouped into geographical areas: Australia (non-aboriginal), Northern Europe, Southern Europe, India, Asia, Pacific Islands, Aboriginal, and others. The women from Australia and Northern and Southern Europe were grouped together and called Caucasian. Only the data on women who had a singleton pregnancy have been included.

Women were considered to be in a low-risk group for GDM if they were of Caucasian origin, were aged <25 years, and had a BMI <25 kg/m^2.

Large-for-gestational-age (LGA) or small-for-gestational-age (SGA) babies were defined as more than the 90th percentile or less than the 10th percentile, respectively, for a locally determined sex-specific birthweight for deliveries from 37 to 41 weeks (inclusive).

From January 1993 until September 1997, the results of all glucose tolerance testing at the Wollongong Hospital were retained. From this database it was possible to determine the number of women with a 2-h glucose level of 7.0–7.9 mmol/l (i.e., up to 1.0 mmol/l below the 2-h diagnostic level for GDM). The hospital medical record and the New South Wales Midwives Data Collection form were reviewed to determine selected pregnancy outcomes.

These outcomes were compared with the outcomes of women from the same hospital prenatal clinic who had been diagnosed with GDM and referred to one of us (R.M.) for medical management. All women were treated in a standard way as previously described (6). All women were seen by a diabetes educator and did home glucose monitoring. All women were seen by a dietitian and given individual dietary advice. The diet was designed to achieve normal weight gain during the pregnancy with 45–55% of energy being derived from carbohydrates, which were distributed as evenly as practical throughout the day. Twice-daily insulin was used if the capillary glucose 1 h after meals was ≥8.0 mmol/l.

Approval for this research was granted by the University of Wollongong Human Research Ethics Committee.

Unless otherwise specified, results have been expressed as the mean ± SD. Statistical tests used were as follows: two-sample *t* test for the difference between the means, Mann-Whitney *U* test for the difference between medians, and a test based on the normal distribution for the difference between two proportions. The Shapiro-Wilk test was used to examine whether a sample came from a normal distribution. Bartlett's test of homogeneity of variances was used to examine if the variance of the test scores of the Australian, Northern European, and Southern European women were equal. Results were considered significant if $P < 0.05$.

RESULTS

There were 2,907 women, comprising 1,953 from the prenatal clinics and 954 from private obstetric care providers, who were tested for GDM over the 18-month period of January 1993 to June 1994. Of these 2,907 women, 1,909 (65.7%) had both a fasting and 2-h glucose level after the GTT while 998 (34.3%) had only a 2-h glucose level. Details about the presence or absence of a family history of diabetes in a first-degree relation were available for the 1,542 women attending the Wollongong Hospital prenatal clinic (53.0% of the total), being positive for 225 (14.6%) and negative for 1,317 (85.4%).

There were 2,108 women (72.5%) from Australia, 294 (10.1%) from Northern Europe, 247 (8.5%) from Southern Europe, 5 (0.2%) from India, 20 (0.7%) Aboriginal, 21 (0.7%) from the Pacific Islands, 90 (3.1%) from Asia, and 122 (4.3%) were from other countries. There were 2,648 (91.1%) women of Caucasian origin and deemed to be in a lower risk group for GDM compared with women from the other ethnic backgrounds. Of these women, there were 573 (21.6%) who were both aged <25 years and who had a BMI <25 kg/m^2. Of these 573 women, all had the result of the 2-h glucose level, and 242 also had the result of the fasting glucose level.

On the basis of a 2-h glucose ≥8.0 mmol/l, GDM was found in 183 of 2,907 (6.3%) of the total population and in 16 of 573 (2.8%) of the low-risk women. Overall, GDM in the low-risk women accounted for 16 of the 183 (8.7%) cases.

Of the 1,542 women who attended Wollongong Hospital, 299 were in the low-risk category. Data about a family history of diabetes were available for 154 low-risk women who had had a fasting glucose level on the GTT. For the 18 women with a positive family history (11.6%), the glucose was 4.02 ± 0.53 mmol/l with a median of 3.90 mmol/l, and for women with a negative family history (n = 136), the glucose was 4.12 ± 0.42 mmol/l with a median of 4.05 mmol/l. These means were not significantly different.

Data about a family history of diabetes were available for 299 low-risk women who had a 2-h level on the GTT. For the 37 women with a positive family history (12.3%), the glucose was 5.94 ± 1.66 mmol/l with a median of 5.4 mmol/l, and for women with a negative family history (n = 262), the glucose was 5.62 ± 1.14 mmol/l with a median of 5.5 mmol/l. These means were not significantly different.

For the total population, mean fasting glucose was 4.27 ± 0.46 mmol/l and the 2-h level was 5.78 ± 1.35 mmol/l. The mean + 2 SD fasting and 2-h glucose levels were 5.2 and 8.5 mmol/l, respectively.

A fasting glucose level was available for 242 of the 573 low-risk women and was 4.20 ± 0.42 mmol/l with a median of 4.20 mmol/l. The mean + 2 SD fasting glucose was 5.04 mmol/l. The 2-h glucose level was 5.55 ± 1.15 mmol/l with a median of 5.40 mmol/l. The mean ± 2 SD 2-h glucose was 7.85 mmol/l. The 95th percentile was 7.53 mmol/l and the 97.5th percentile was 8.07 mmol/l.

The Shapiro-Wilk test was used to see if the results of either the fasting or 2-h samples could be regarded as having a normal distribution. Both tests were rejected: fasting; W = 0.91, $P < 0.0001$ and 2 h; W = 0.96, $P < 0.00001$.

In clinical practice, there is only concern about the upper range of the glucose

values in an otherwise healthy obstetric population. As the samples did not have a normal distribution, one-sided nonparametric tolerance intervals were calculated. There is 95% confidence that at least 95% of all the fasting glucose levels are ≤5.1 mmol/l and that at least 95% of all the 2-h glucose levels are ≤7.8 mmol/l

There were 3,384 women attending the Wollongong Hospital who had a GTT over the 57-month period of January 1993 to September 1997 and who had a singleton pregnancy delivered between 37 and 41 weeks (inclusive). Of these women, 272 (8.0%) had GDM and 308 (9.1%) had a 2-h glucose level between 7.0 and 7.9 mmol/l. The women with GDM were aged 29.1 ± 5.6 years and delivered at 39.3 ± 1.3 weeks. The glucose-tolerant women were aged 30.5 ± 5.6 years and delivered at 39.4 ± 1.1 weeks. The results of selected pregnancy outcomes for treated women with GDM and the glucose-tolerant women with a 2-h glucose level of 7.0–7.9 mmol/l are shown in Table 1.

Table 1—*Selected pregnancy outcomes for treated women with GDM and pregnant women with a 2-h glucose level of 7.0–7.9 mmol/l*

	Women with a 2-h glucose of 7.0–7.9 mmol/l	Treated women with GDM
n	308	272
Induced	68 (24.4)	67 (26.6)
Elective section	29 (9.4)	20 (7.4)
Obstetric intervention (emergency section, forceps, vacuum)	55 (19.4)	48 (19.0)
LGA	62 (20.1)	33 (12.1)*
SGA	24 (7.8)	34 (12.5)
>4,000 g	60 (19.5)	33 (12.1)†
<2,500 g	3 (1.0)	6 (2.2)

Data are *n* or *n* (%). Data for "induced" and "obstetric intervention" exclude women with an elective section. There was one perinatal death in the group of women with a 2-h glucose level of 7.0–7.9 mmol/l and two perinatal deaths for the treated women with GDM. There were 49 treated women with GDM (18.0%) who required treatment with insulin with a daily dose of 38.6 (13.4) U. *$P < 0.01$; †$P < 0.02$.

There were no statistically significant differences between the two groups with respect to obstetric interventions or the percentage of small babies defined as either SGA or <2,500 g. However, women with treated GDM had a significant reduction in the percentage of large babies defined as either LGA or >4,000 g.

CONCLUSIONS — Ideally, the parameters used to define a disorder should be determined in relation to the development of adverse outcomes specific to, or related to, that disorder. For example, this is the situation with respect to hypertension (7), lipid levels (8), and the recent readjustment of the diagnostic criteria for diabetes (9).

Outcome data, both immediate and long term, are not available for correlation with glucose levels in pregnancy, and for this reason a statistical approach to the definition of GDM is frequently used. However, a statistical reference range for glucose levels in pregnancy to define GDM has some limitations. If a total population is used, then this will, of necessity, include all of the women with GDM, however defined, and thus have relatively higher glucose levels. A reference range determined after exclusion of women with GDM will, of necessity, be arbitrary.

A locally derived reference range on a total obstetric population in an area where there is a high proportion of women at risk will have higher glucose values than that derived in a more representative population. A higher reference range may exclude some women from treatment who would have had this benefit if they lived elsewhere. The prevalence of GDM is increasing. In Australia, for example, at one hospital where the same testing procedure and diagnostic criteria have been used for more than 2 decades, the prevalence has more than doubled for all ethnic groups (10). Therefore, a contemporaneous population-based reference range may well have higher glucose values than one derived on the same population some years before.

In the U.S., Sacks et al. (11) found, after certain exclusions, that the mean + 2 SD 2-h glucose level after a 75-g GTT was 8.9 mmol/l. However, the population tested included a high proportion of women with risk factors; an Hispanic background was present in 61.5%, and a family history of diabetes was present in 41.6%. In Europe, Lind et al. (12) found the 95th percentile of the 2-h glucose was 9.0 mmol/l. This population represented a wide cross-section of the European community and had an age-range from 15 to 43 years and a BMI range from 18 to 47 kg/m^2. A family history of diabetes was present in only 5%. In Australia, Martin et al. (13), using an unselected population with a low proportion of women from a high-risk ethnic background, found the 95th percentile of the 2-h glucose was 7.8 mmol/l. In healthy pregnant women in Nigeria, Famuyiwa et al. (14) found the mean + 2 SD 2-h glucose at the beginning of the 3rd trimester was 6.2 mmol/l. In our total population, the mean + 2 SD 2-h glucose level was 8.5 mmol/l.

What the above-cited series have shown, and there is general support from the literature, is that a statistically derived reference range is invariably higher in populations where there is an increased number of women with acknowledged risk factors. It has been well argued that it does not make "biological sense" to derive population-specific criteria (3). There is no evidence that a fetus in a high-risk population is more resistant to the adverse effects of maternal hyperglycemia.

The development of GDM is associated with certain maternal risk factors. A possible way of overcoming some of the methodological problems mentioned above would be to determine a reference range in women with low-risk factors.

In the series herein reported, we have tried to derive a reference range for the 75-g GTT in pregnancy by examining only the results from low-risk women. For this purpose we have used data only from Caucasian women with a BMI <25 kg/m^2 and aged <25 years. The mean + 2 SD 2-h glucose level was 7.85 mmol/l. There was 95% confidence that at least 95% of all the 2-h glucose levels were <7.8 mmol/l. This figure is the same as the lower range of the World Health Organization (WHO) classification of impaired glucose tolerance (15). A "rounded up" figure of 8.0 mmol/l is commonly used in Australia for the diagnosis of GDM (5).

Lowering the 2-h diagnostic glucose level for GDM after the 75-g GTT to 7.8 mmol/l (140 mg/dl) will result in a higher number of women being diagnosed with this condition. This will be particularly pro-

nounced in areas where there are a considerable number of women with high-risk factors. Determining a reference range for the GTT on a population that includes a considerable proportion of women at risk is effectively a statistical rationalization of services that may disadvantage women below the diagnostic levels who, in another population, would be diagnosed and treated as having GDM.

With respect to the fasting glucose level, Sacks et al. (11) found the mean + 2 SD to be 5.6 mmol/l, Lind et al. (12) found the 95th percentile to be 5.2 mmol/l, and Martin et al. (13) found the 95th percentile to be 5.1 mmol/l. In Nigeria (14), the mean + 2 SD was 5.2 mmol/l. In our series, the mean + 2 SD fasting glucose level for the total population was 5.2 mmol/l and for low-risk women it was 5.0 mmol/l. Both of these fasting levels are considerably lower than the 5.5 mmol/l currently used for diagnosis of GDM in Australia.

However, very few women are diagnosed with GDM on the basis of an elevated fasting glucose level alone. When the fasting glucose is elevated and the 2-h glucose is normal there is a strong suspicion that the subject has not fasted (12). For this reason, the fasting glucose level is not favored by the WHO (16) and in clinical practice the 2-h level alone is preferred (17–19). A recent report of the American Diabetes Association (9) has recommended the lowering of the fasting glucose level for the nonpregnant population to make it more appropriate to the 2-h level as a predictor of diabetes-associated complications. Irrespective of any revision of the 2-h level, if the fasting glucose level is to retain any diagnostic relevance in pregnancy, it will need to be lowered to ~5.0 mmol/l (90 mg/dl).

In our series, a positive family history of diabetes in a first-degree relation was present in 12.3% of the low-risk population with no significant differences in either the fasting or the 2-h glucose level compared with women without a positive family history. Naylor et al. (20) also did not find a positive family history useful and others have commented that knowledge of this aspect may be unreliable (12). We feel that in a group of women from an ethnic background without a high risk of type 2 diabetes, the significance of a family history of diabetes is much reduced. For Anglo-Celtic women in Australia, the average age of onset of type 2 diabetes is 53.8 years (21). For many young women with GDM, their parents may not yet be of an age where they have developed diabetes.

There is a continuum of risk for adverse pregnancy outcomes as the maternal glucose level rises (11,22,23). Women with an abnormal challenge test and a subsequent normal GTT (24) and women with only one abnormal value on the GTT (25) have a more unfavorable pregnancy outcome than women with normal glucose values. Even glucose-tolerant women have an increased risk of an unfavorable pregnancy outcome with increasing maternal glucose levels (26). In particular, the proportion of LGA babies increases as the maternal glucose levels increase (26,27).

The diagnosis of GDM, based on a statistical reference range, should ideally be evaluated against pregnancy outcomes. However, this is difficult to do as most women with GDM are treated. However, if the diagnostic criteria are appropriate, then women categorized as having GDM, and being optimally treated, should have a pregnancy result equivalent to, but presumably no better than, glucose-tolerant women.

The medical management of women with GDM can reduce the rate of macrosomia to that of the overall obstetric population (6,27,28). Some recent reports, including an earlier one from our group using some of the patients used for comparative purposes in this study (29,30), have found that treated women with GDM have a more favorable outcome than subgroups of glucose-tolerant women.

In the series herein reported, women were diagnosed with GDM if the 2-h glucose level was ≥8.0 mmol/l. This is a relatively lower figure than many other diagnostic criteria (3) and should have included most women at risk of an unfavorable pregnancy outcome. However, women with treated GDM, compared with glucose-tolerant women with a 2-h glucose level between 7.0 and 7.9 mmol/l, had a significantly lower rate of LGA infants and of infants weighing >4,000 g. There was no increase in the rate of SGA infants or of obstetric interventions.

These data suggest that using a statistical approach in a low-risk population to define a relatively low reference range for the GTT in pregnancy will still miss a group of women with an increased risk of an adverse pregnancy outcome. The previous National Diabetes Data Group (NDDG) criteria (31) recognized that an increased risk could be found at a lower level, and it was suggested by some members of the working group that women with a 2-h glucose level of >6.6 mmol/l but <9.2 mmol/l should be classified as having impaired gestational glucose tolerance. Nasrat et al. (32) found that glucose-tolerant women with a 2-h glucose of >6.6 mmol/l had a glycemic, insulin, and C-peptide response similar to women with GDM and an increased rate of macrosomia. Examination of the figures available in the study of Sacks et al. (11) show an increase in the rate of macrosomia above 10%, which could be expected at around a 2-h glucose level of 6.4 mmol/l.

Determining a reference range for the GTT in pregnancy using a low-risk population, albeit also including some women with GDM, will result in lower glucose values than many criteria that are currently used. While this is a useful exercise, it does still not equate totally with the risk of an adverse pregnancy outcome. Women with a 2-h glucose level up to 1.0 mmol/l below the diagnostic category used in Australia have an increased rate of LGA infants compared with treated women with GDM. This group of women may also benefit from treatment similar to that given to women with GDM.

A higher diagnostic level or a requirement for more than one abnormal result for diagnosis will reduce the number of women with GDM but will also reduce the number of women receiving the advantages of treatment. In this study, we have used a cut-point for the diagnosis of GDM, which is relatively low by international standards. When it is considered that glucose-tolerant women with a 2-h result just less than the diagnostic value for GDM have a less favorable outcome, it is difficult to accept that a higher value should be justified. In addition, when the literature abounds with examples of women having one abnormal test result on either a preliminary challenge test or after the GTT having a less favorable pregnancy outcome, it is also difficult to justify a two-stage testing procedure or the need for more than one abnormal test result.

We feel that consideration should be given to reducing the fasting glucose level for the diagnosis of GDM to 5.0 mmol/l (90 mg/dl) and the 2-h glucose level to 7.8 mmol/l (140 mg/dl). More attention should be given, and research dedicated to, defining GDM on the basis of outcomes.

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