Reference Intervals for Hemoglobin A1c in Pregnant Women: Data from an Italian Multicenter Study

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Background: The reference intervals for hemoglobin A1c (Hb A1c) in pregnant women without diabetes are not well defined, and few examples of reference intervals established by networks of different laboratories are available.

Methods: Five Italian Diabetic Care Units were involved in the study. Data were collected from 445 pregnant women without diabetes, selected on the basis of glucose challenge test results, and from 384 nonpregnant control women. The Hb A1c measurements were performed with HPLC systems aligned to the Diabetes Control and Complications Trial. Plasma glucose measurements were also performed locally. Both Hb A1c and glucose measurements were harmonized by running appropriate external quality assessment schemes. The reference intervals were calculated in terms of nonparametric 2.5th to 97.5th percentiles with 0.90 confidence intervals.

Results: The Hb A1c measurements were reproducible (CV 2.0%) and accurate [mean (SE) difference from the target values, −0.10 (0.06)%]. Glucose measurements were also reproducible (mean CV = 3.2%) and accurate [difference from the target values, −0.01 (0.04) mmol/L]. To calculate common reference intervals, we merged the data collected in the different centers. The Hb A1c reference intervals were 4.0%–5.5% for pregnant nondiabetic women and 4.8%–6.2% for nonpregnant controls.

Conclusions: Healthy pregnant women have lower Hb A1c concentrations than nonpregnant women. The reference intervals for Hb A1c in pregnant women should therefore be lower than those currently in use.

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Diabetes in pregnant women is associated with increased occurrence of both fetal and maternal adverse events, including macrosomia, congenital malformations, spontaneous abortion, perinatal mortality, and preeclampsia (1, 2). The close relationship between the development of such complications and maternal hyperglycemia has been widely documented. Several studies have also shown that strict glycemic control before conception and throughout the gestational period can improve the outcome of pregnancies in women with diabetes, reducing the risk of complications to a rate similar to that found in uncomplicated pregnancies (3–5). As a consequence, the improvement of glycemic control is considered a major topic in the management of pregnancies complicated by diabetes.

In addition to self-measurement of capillary blood glucose, hemoglobin A1c (Hb A1c)11 measurements are an established tool in the assessment of glycemic control (6).

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Received December 5, 2005; accepted March 22, 2006.

Previously published online at DOI: 10.1373/clinchem.2005.064899

11 Nonstandard abbreviations: Hb A1c, hemoglobin A1c; DCCT, Diabetes Control and Complications Trial; GCT, glucose challenge test; EQAS, external quality assessment scheme; HK/G6PD, hexokinase/glucose-6-phosphate dehydrogenase; and GDM, gestational diabetes mellitus.
The American Diabetes Association recommendations state that Hb A1c concentrations <1% above the upper limit of the reference interval should be achieved before and during pregnancy to assure a good glycemic state (7). Although the Hb A1c reference intervals for the general population are well established, reference intervals for healthy pregnant women are not clearly defined. Available study data are scarce and often were obtained on a limited study population or by use of outdated analytical methods (8–10). Moreover, recent evidence has shown that despite effective preconception care and planned pregnancies providing good glycemic control in early pregnancy with optimal Hb A1c concentrations, the development of diabetes-associated complications cannot always be prevented (11, 12). These considerations highlight the need to carefully revise the target for glycemic control during an uncomplicated pregnancy.

In an Italian multicenter study, we used a Diabetes Control and Complications Trial (DCCT)-aligned method to evaluate Hb A1c reference intervals in a large number of healthy pregnant Caucasian women.

**Materials and Methods**

**STUDY PARTICIPANTS**

Five Italian Diabetic Care Units (center 1, located in Cagliari; center 2, located in Milano at H. Maggiore Ca’ Granda; center 3, located in Milano at S. Raffaele Hospital; center 4, located in Padova; and center 5, located in Pisa) participated in the study. All patients gave oral, informed consent to participate in the study. A total of 949 pregnant women attending the centers as outpatients for routine prenatal clinical care were screened with the 50 g, 1-h glucose challenge test (GCT) (13) and Hb A1c measurements. GCT was usually performed between the 24th and 27th weeks of pregnancy (range, 15–36 weeks), and Hb A1c was measured on the same day. All of the participants were Caucasian women 16 to 43 years of age. Healthy participants with negative GCT results and no previous history of gestational diabetes were selected for the group of nondiabetic pregnant women (n = 445). Women with a positive GCT result underwent a diagnostic oral glucose tolerance test (OGTT) with 100 g of glucose according to the criteria of Coustan and Carpenter (14). On the basis of OGTT results, a group of 145 women with gestational diabetes and 70 women with 1 abnormal glucose tolerance test value [isolated gestational hyperglycemia (IGH)] were selected. Patients with gestational diabetes were put on a controlled diet, and their fasting and postprandial capillary glucose concentrations were self-monitored. Insulin treatment was started if glucose concentrations were not satisfactory. Metabolic and obstetric monitoring were continued on all patients until delivery.

Women with positive GCT but negative OGTT results were not included in the study (n = 182) because these patients have been shown to have clinical outcomes different from those whose GCT results are within the reference interval (15).

Data collected from routine laboratory tests by one of the authors (A.M.) from a group of age-matched nonpregnant healthy women without diabetes or impaired fasting glucose (n = 384) were used as controls (16).

**ANALYTICAL METHODS**

Hb A1c was determined in EDTA-anticoagulated fresh blood samples, and the measurements were performed locally in the laboratories of the centers involved in the study. Two laboratories (centers 1 and 4) used the Menarini HA 8140 HPLC analyzer (A. Menarini Diagnostics), and the remaining 3 centers used the Menarini HA 8160 system. The alignment among the different instruments was evaluated by a proper external quality assessment scheme (EQAS) with control materials with confirmed commutability, with a DCCT-assigned Hb A1c content ranging from 5.3% to 9.6%. Such materials, prepared by the European Reference Laboratory for Glycohemoglobin with DCCT target values (Queen Beatrix Hospital, Winterswijk, The Netherlands), were aliquots of batches already used for a professional Italian EQAS, run essentially as described previously (17). Sets of 5 lyophilized controls were distributed to the participants, who were asked to analyze these materials in 2 different replicates during the whole study.

Plasma glucose measurements were performed on standard clinical chemistry analyzers, as reported here. One center (center 1) used the glucose oxidase-peroxidase method on a Roche Hitachi 704 analyzer, 2 centers (centers 2 and 3) used the hexokinase/glucose-6-phosphate dehydrogenase (HK/G6PD) method implemented on 2 Roche modular analyzers, 1 center (center 4) used the HK/G6PD method on a Mega Merck analyzer, and 1 center (center 5) used the HK/G6PD method on a Dade Dimension analyzer. The spectrophotometric reference method (18) calibrated with NIST Standard Reference Material 917b, was used in 1 center (center 3) to assign target glucose values, traceable to NIST, to a set of frozen serum pools to be used in the EQAS study, established and performed as for Hb A1c.

**INTERPRETATION OF GLUCOSE TOLERANCE TESTS**

For GCT interpretation, a GCT result was considered normal when the glucose concentration was <7.7 mmol/L (140 mg/dL) 1 h after the ingestion of 50 g of glucose (13). A diagnostic OGTT was performed in the fasting state by use of a 100-g oral glucose load and 3-h determinations. The Coustan–Carpenter criteria (14) were used in the interpretation of the OGTT: fasting, 5.2 mmol/L (95 mg/dL); 1 h, 9.9 mmol/L (180 mg/dL); 2 h, 8.5 mmol/L (155 mg/dL); 3 h, 7.7 mmol/L (140 mg/dL). Gestational diabetes mellitus (GDM) was diagnosed when at least 2 of the 4 plasma glucose results obtained in the test were at or exceeded the cutoff values, and IGH was
diagnosed when 1 of the 4 results was above the corresponding cutoff limit.

**STATISTICAL ANALYSIS**

The reference intervals were calculated according to the recommendations of the IFCC. We used RefVal, Ver. 4.01, a program designed ad hoc by H.E. Solberg (19, 20). Five tests to determine the gaussian distributions were performed by this program: the coefficients of skewness and kurtosis, the Kolmogorov–Smirnov test, the Cramér–von Mises test, and the Anderson–Darling test. The reference intervals bounded by the 2.5th and 97.5th percentiles were therefore calculated by means of nonparametric estimates, together with the 0.90 confidence intervals, when appropriate.

Comparisons among groups were performed with the Mann–Whitney rank-sum test, and correlations were estimated by the coefficient of determination ($r^2$). These analyses were done with the SigmaStat package (software release Ver. 3.0; SPSS). $P < 0.05$ was considered statistically significant.

**Results**

**ANALYTICAL PERFORMANCE**

The analytical quality of Hb A1c measurements performed locally in the different centers was evaluated by analysis of a set of control materials with an assigned Hb A1c value. The reproducibility, as judged on the basis of the replicates between different measurements on the same controls, was satisfactory, with a mean analytical CV of 2.0% (range, 0.6%–3.9%). With regard to accuracy, the mean (SE) differences between the measured glucose values and the reference method values were 0.06 (0.07) mmol/L for center 1, –0.14 (0.05) mmol/L for center 2, 0.08 (0.02) mmol/L for center 3, 0.02 (0.06) mmol/L for center 4, and –0.03 (0.06) mmol/L for center 5.

**Hb A1c reference intervals**

Within the framework of a study of gestational diabetes, we evaluated the Hb A1c concentrations in 4 different categories of participants. Among the pregnant women, we distinguished those with negative GCT results (GCT–), those with IGH, and those with GDM. Separately, a set of nonpregnant, nondiabetic women were studied as controls. The results for the different groups are presented in Table 1 and Fig. 1.

Each of the 3 categories of pregnant women had significantly lower mean Hb A1c values than did nonpregnant women (Fig. 1). The Hb A1c results for nondiabetic pregnant women were also analyzed separately at different gestational periods (Table 2). A small but significant increase in Hb A1c values was observed late in the pregnancies, at 28–36 weeks of gestation. To more closely evaluate a possible relationship between Hb A1c and gestational age, we plotted Hb A1c data from the nondiabetic women with respect to weeks of pregnancy (Fig. 2); the correlation between the 2 was very low ($r = 0.141; P = 0.0028$).

**Discussion**

Our results show that Hb A1c is significantly decreased in pregnancy and that different reference intervals should be established for healthy pregnant women and pregnant women with glucose intolerance or gestational diabetes.

Because establishing reference intervals is a laborious and often expensive procedure, alternative approaches have been proposed (21–24), and our data follow the strategy of merging the experiences of different diabetic care units to study glycemic control in healthy pregnant women to better monitor patients affected by diabetes in pregnancy.

<table>
<thead>
<tr>
<th>Table 1. Hb A1c reference intervals in pregnant and nonpregnant healthy women.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hb A1c, %</strong></td>
</tr>
<tr>
<td><strong>Groups</strong></td>
</tr>
<tr>
<td>---------------------</td>
</tr>
<tr>
<td>Pregnant</td>
</tr>
<tr>
<td>Nonpregnant</td>
</tr>
</tbody>
</table>

* CI, confidence interval.

b $P$ values of the medians between the 2 groups (Mann–Whitney rank-sum test).

c Values in parentheses indicate number of tests for goodness-of-fit with $P < 0.05$. 
In our approach, although the centers did not use exactly the same analytical techniques, a strict EQAS study allowed us to evaluate the analytical performance of the various centers; thus, the traceability to the reference method can be demonstrated. The data we collected show that the biases for Hb A1c and glucose were negligible with respect to the actual analytical goals for these analytes \(10\%\) and \(2.5\%\), respectively, according to Sacks et al. (16). The reproducibility of the methods was within the analytical goals \(3\%\) for Hb A1c and \(3.3\%\) for glucose (16), on average, for both analytes. For these reasons, we merged the data collected in the different centers to calculate common reference intervals.

Our evaluation of Hb A1c reference intervals in pregnancy was performed on a consistent number of women by use of a DCCT-aligned Hb A1c method, as addressed in a consensus statement (25). Our results mainly show that Hb A1c concentrations in healthy pregnant women are lower than those in nonpregnant, nondiabetic women of comparable age. Our findings are in good agreement with those obtained by Nielsen et al. (26), who demonstrated a decrease of the upper reference limit of Hb A1c from 6.3% before pregnancy to 5.7% in early pregnancy and 5.6% in the third trimester. A decrease in the upper reference limit for Hb A1c was also reported by O’Kane et al. (27), who determined an upper limit of 5.9% in nondiabetic pregnant women and 6.5% in the general population. Data from other authors, although not as comparable because they were obtained on a smaller number of individuals or by Hb A1c methods not aligned to the DCCT, also indicated decreased Hb A1c concentrations during uncomplicated pregnancies (8–10).

To our knowledge, this is the first study on Hb A1c reference intervals in Caucasian pregnant women, calculated with a statistical elaboration in agreement to IFCC recommendations (19).

We observed a small increase in Hb A1c values in the last 2 months of pregnancy. Controversial data are re-

### Table 2. Hb A1c reference intervals calculated at different gestational ages in uncomplicated pregnancies (GCT—women).

<table>
<thead>
<tr>
<th>Weeks of pregnancy</th>
<th>n</th>
<th>Median</th>
<th>2.5th</th>
<th>97.5th</th>
<th>Range</th>
<th>P</th>
<th>Type of distribution*</th>
</tr>
</thead>
<tbody>
<tr>
<td>15–24</td>
<td>126</td>
<td>4.8</td>
<td>3.8</td>
<td>5.5</td>
<td>3.5–5.7</td>
<td>NS</td>
<td>Nongaussian (4)</td>
</tr>
<tr>
<td>25–27</td>
<td>251</td>
<td>4.8</td>
<td>4.0</td>
<td>5.5</td>
<td>3.3–5.6</td>
<td>NS</td>
<td>Nongaussian (4)</td>
</tr>
<tr>
<td>28–36</td>
<td>68</td>
<td>5.0</td>
<td>4.4</td>
<td>5.5</td>
<td>4.3–5.6</td>
<td>0.003</td>
<td>Nongaussian (3)</td>
</tr>
<tr>
<td>Entire group (15–36 weeks)</td>
<td>445</td>
<td>4.8</td>
<td>4.0</td>
<td>5.5</td>
<td>3.3–5.7</td>
<td></td>
<td>Nongaussian (5)</td>
</tr>
</tbody>
</table>

* CI, confidence interval; NS, not significantly different.

b P values were obtained from the comparisons with the entire group.

c Values in parentheses indicate number of tests for goodness-of-fit with \(P<0.05\).

d Confidence intervals could not be determined.

e \(P<0.001\) vs the group 15–24 weeks pregnant; \(P=0.002\) vs the group 25–27 weeks pregnant.
ported in the literature about this point: some authors confirmed this finding (28), whereas others did not detect differences among trimesters (27) or reported an additional decrease in Hb A1c values in late pregnancy (26). These lower Hb A1c concentrations found in pregnancy might be related to the decrease in plasma glucose values and to the shortened erythrocyte life span that occur during pregnancy (29).

Unfortunately, Hb A1c is not useful in differentiating patients with GDM or IGH from nondiabetic pregnant women, perhaps because in IGH and GDM, only minor glucose intolerances develop for the first time during pregnancy. For essentially the same reason, we would not expect to find a strong correlation between Hb A1c and fasting plasma glucose during pregnancy. Moreover, as shown by the DCCT study, Hb A1c is more strongly correlated to the daily mean glucose concentration than to fasting plasma glucose (30).

The described decrease in Hb A1c concentrations in uncomplicated pregnancies has important clinical implications for the assessment of glycemic control in pregnant women with diabetes. Management of glycemic control in diabetic pregnancies is usually performed with reference to that established for the nonpregnant state. Such levels are now believed to rather inadequately reflect the real metabolic state during pregnancy because they appear to be insufficient for preventing the occurrence of typical diabetes-related complications and the pregnancy outcomes are not comparable to those of nondiabetic women (31). Recently, Parretti et al. (32), using a glucometer to evaluate fasting, postprandial, and nocturnal glucose values, showed that in healthy pregnant women, these values are lower than previously believed on the basis of studies performed on small numbers of hospitalized patients. These results have been more recently confirmed by Yogev et al. (33), who used a continuous glucose monitoring system in healthy pregnant women.

In conclusion, our results confirm the results of previous studies indicating that the targets for Hb A1c during pregnancy need to be revised and should be lower than those currently used.

We thank Louise Benazzi (Istituto Tecnologie Biomediche, Consiglio Nazionale delle Ricerche, Milano, Italy) for critically revising the manuscript and A. Menarini Diagnostics for supporting the study. This work was also partially supported by a Ministero dell’Istruzione, dell’Università e della Ricerca (MIUR) FIRST 2004 grant (to A.M.).

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