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Reference Intervals for Hemoglobin A_{1c} in Pregnant Women: Data from an Italian Multicenter Study

Andrea Mosca,[1*] Renata Paleari,[1] Maria G. Dalfrà,[2] Graziano Di Cianni,[3] Ilaria Cuccuru,[3] Giovanni Pellegrini,[4] Lucia Malloggi,[4] Matteo Bonomo,[5] Simona Granata,[6] Ferruccio Ceriotti,[7] Maria T. Castiglioni,[8] Marco Songini,[9] Giuliana Tocco,[9] Michela Masin,[2] Mario Plebani,[10] and Annunziata Lapolla[2]

Background: **The reference intervals for hemoglobin A_{1c} (Hb A_{1c}) 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 A_{1c} measurements were performed with HPLC systems aligned to the Diabetes Control and Complications Trial. Plasma glucose measurements were also performed locally. Both Hb A_{1c} 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 A_{1c} 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 A_{1c} 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 A_{1c} concentrations than nonpregnant women. The reference intervals for Hb A_{1c} in pregnant women should therefore be lower than those currently in use.**

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 A_{1c} (Hb A_{1c})[11] measurements are an established tool in the assessment of glycemic control *(6)*.

[1] Dipartimento di Scienze e Tecnologie Biomediche, Università degli Studi di Milano, Segrate (Milano), Italy.
[2] Dipartimento di Scienze Mediche e Chirurgiche, Università di Padova, Padova, Italy.
[3] Dipartimento di Endocrinologia e Metabolismo and [4] Laboratorio Analisi, Azienda Ospedaliera-Universitaria Pisa, Pisa, Italy.
[5] Centro Antidiabetico and [6] Laboratorio di Biochimica, H. Maggiore Ca' Granda, Milano, Italy.
[7] Diagnostica e Ricerca S. Raffaele spa and [8] Divisione di Ostetricia e Ginecologia, IRCCS H. San Raffaele, Milano, Italy.
[9] Centro Antidiabetico, Ospedale Brotzu, Cagliari, Italy.
[10] Servizio Medicina di Laboratorio, Azienda Ospedaliera-Università di Padova, Padova, Italy.
* Address correspondence to this author at: Dipartimento di Scienze e Tecnologie Biomediche, Università degli Studi di Milano, Via Fratelli Cervi 93, 20090 Segrate, Milano, Italy. Fax 39-02-5033-0414; e-mail andrea.mosca@unimi.it.
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[11] Nonstandard abbreviations: Hb A_{1c}, hemoglobin A_{1c}; DCCT, Diabetes Control and Complications Trial; GCT, glucose challenge test; OGTT, oral glucose tolerance test; IGH, isolated gestational hyperglycemia; 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 A_{1c} 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 A_{1c} 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 A_{1c} 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 A_{1c} 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 A_{1c} measurements. GCT was usually performed between the 24th and 27th weeks of pregnancy (range, 15–36 weeks), and Hb A_{1c} 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 A_{1c} 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 A_{1c} 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 A_{1c}.

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 A_{1c} measurements performed locally in the different centers was evaluated by analysis of a set of control materials with an assigned Hb A_{1c} 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 the accuracy, the mean (SE) differences between the measured Hb A_{1c} values and the target values, i.e., the deviations with respect to the DCCT values, were −0.1 (0.1)% for centers 1, 4, and 5; −0.3 (0.1)% for center 2; and, 0.1 (0.1)% for center 3. The agreement between the measured and the expected Hb A_{1c} values in the controls confirmed the accuracy of the measurements during the whole period of the investigation as well as the alignment of HPLC instruments used in the different laboratories. This finding was particularly relevant for pooling of results from different laboratories to establish the reference intervals for Hb A_{1c} in pregnancy.

Plasma glucose measurements showed good agreement among the centers according to the data collected from the EQAS study. The reproducibility, calculated from the replicates of the 6 pairs of control sera with NIST-traceable assigned values, was satisfactory, with a mean analytical CV of 3.2% (range, 1.0%–5.7%). 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 A_{1c} REFERENCE INTERVALS

Within the framework of a study of gestational diabetes, we evaluated the Hb A_{1c} 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 A_{1c} values than did nonpregnant women (Fig. 1). The Hb A_{1c} results for nondiabetic pregnant women were also analyzed separately at different gestational periods (Table 2). A small but significant increase in Hb A_{1c} values was observed late in the pregnancies, at 28–36 weeks of gestation. To more closely evaluate a possible relationship between Hb A_{1c} and gestational age, we plotted Hb A_{1c} 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 A_{1c} 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 1. Hb A_{1c} reference intervals in pregnant and nonpregnant healthy women.

		Hb A_{1c}, %					
			Percentiles (0.90 CI)[a]				
Groups	n	Median	2.5th	97.5th	Range (minimum–maximum)	P^b	Type of distribution[c]
Pregnant	445	4.8	4.0 (4.0–4.2)	5.5 (5.4–5.5)	3.3–5.7	<0.001	Nongaussian (5)
Nonpregnant	384	5.6	4.8 (4.6–4.9)	6.2 (6.1–6.2)	4.3–6.2		Nongaussian (4)

[a] 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 $P < 0.05$.

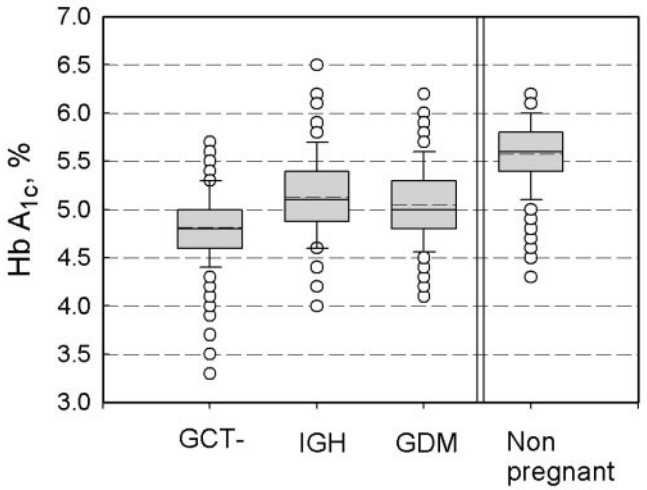

Fig. 1. Distribution of Hb A_{1c} values in nondiabetic pregnant women (GCT−; n = 445), in women with IGH (n = 70), in women with GDM (n = 145), and in the nonpregnant control group (n = 384).

$P < 0.001$ (comparison tests with Mann–Whitney rank-sum test) for all pregnant groups compared with the nonpregnant controls. *Error bars* indicate the 10th and 90th percentiles. *Top* and *bottom limits* of each *box* indicate the 75th and the 25th percentiles, respectively. The *solid line inside* each *box* represents the median, the *dashed line* the mean.

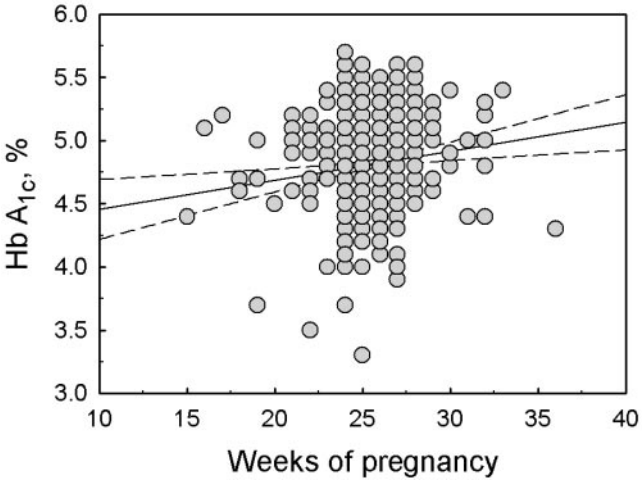

Fig. 2. Regression plot of Hb A_{1c} results obtained from nondiabetic pregnant women plotted against weeks of pregnancy.

Dashed lines represent confidence intervals of the regression. The correlation between Hb A_{1c} and the week of pregnancy was very weak ($r = 0.141$; $P = 0.0028$).

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 A_{1c} 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 A_{1c} 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 A_{1c} reference intervals in pregnancy was performed on a consistent number of women by use of a DCCT-aligned Hb A_{1c} method, as addressed in a consensus statement *(25)*. Our results mainly show that Hb A_{1c} 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 A_{1c} 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 A_{1c} 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 A_{1c} methods not aligned to the DCCT, also indicated decreased Hb A_{1c} concentrations during uncomplicated pregnancies *(8–10)*.

To our knowledge, this is the first study on Hb A_{1c} reference intervals in Caucasian pregnant women, calculated with a statistical elaboration in agreement to IFCC recommendations *(19)*.

We observed a small increase in Hb A_{1c} values in the last 2 months of pregnancy. Controversial data are re-

Table 2. Hb A_{1c} reference intervals calculated at different gestational ages in uncomplicated pregnancies (GCT− women).

		Hb A_{1c}, %					
			Percentiles (0.90 CI)[a]				
Weeks of pregnancy	n	Median	2.5th	97.5th	Range (minimum-maximum)	P^b	Type of distribution[c]
15–24	126	4.8	3.8 (3.5–4.1)	5.5 (5.3–5.7)	3.5–5.7	NS	Nongaussian (4)
25–27	251	4.8	4.0 (4.0–4.2)	5.5 (5.4–5.5)	3.3–5.6	NS	Nongaussian (4)
28–36	68	5.0	4.4[d]	5.5[d]	4.3–5.6	0.003[e]	Nongaussian (3)
Entire group (15–36 weeks)	445	4.8	4.0 (4.0–4.2)	5.5 (5.4–5.5)	3.3–5.7		Nongaussian (5)

[a] 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 A_{1c} values in late pregnancy *(26)*. These lower Hb A_{1c} 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 A_{1c} 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 A_{1c} and fasting plasma glucose during pregnancy, Moreover, as shown by the DCCT study, Hb A_{1c} is more strongly correlated to the daily mean glucose concentration than to fasting plasma glucose *(30)*.

The described decrease in Hb A_{1c} 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 of control 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 A_{1c} during pregnancy need to be revised and should be lower than those currently used.

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