Cessation of Menstruation Improves the Correlation of FPG to Hemoglobin A1c in Caucasian Women

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BACKGROUND: Anemia is known to cause spurious hemoglobin A1c (HbA1c) results. The effect of menstruation on HbA1c was tested by correlating it to FPG in non-anemic premenopausal and in menopausal women.

METHOD: Non-diabetic, non-obese middle-aged Caucasian women were classified as premenopausal or menopausal. Hemogram, FPG, and A1c results were obtained.

RESULTS: Hemoglobin concentrations were lower in the premenopausal group. FPG showed a poor correlation to A1c value overall (r = 0.251, p = 0.001) which was improved by multiplying the A1c % by the total hemoglobin concentration to create an absolute A1c value (r = 0.362, p = 0.000). When the data was sorted by menopause status, the correlation of FPG to Absolute A1c improved (r = 0.463, p = 0.000) in the menopausal women, but remained low (r = 0.283, p = 0.005) in the premenopausal women.

CONCLUSIONS: Menstruation may be a significant factor affecting the accuracy of A1c concentrations.

ABBREVIATIONS: A1c = hemoglobin A1c; BMI = body mass index; CI = confidence interval, FPG = fasting plasma glucose; HbA1c = hemoglobin A1c; MBG = mean blood glucose; MCV: mean corpuscular volume; NGSP = National Glycohemoglobin Standardization Program; RDW = red blood cell distribution width.

INDEX TERMS: Absolute A1c; A1c; glucose; menopause; menstruation.

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In the past 24 years, the prevalence of diagnosed diabetes has increased 59% for American women. In 2004, 7.8% of white females between the ages of 45 and 64 had diagnosed diabetes, with an average age at diagnosis of 46.4 years.[1] Chronic complications of diabetes include retinopathy, kidney disease, neuropathy, and cardiovascular disease, and can be delayed and diminished by tight glycemic control for both type 1 and type 2 diabetics.[2-5] Fasting plasma glucose (FPG) is the preferred screening tool for diabetes mellitus, and a FPG ≥ 126 mg/dL (7.0 mmol/L) that is reproduced on a separate occasion is diagnostic for diabetes.[6] Hemoglobin A1c has long been recognized as the best test of long term glycemic status for both type 1 and type 2 diabetics, and elevations in A1c are linked to higher incidence of chronic complications.[2-4,7] Glycation is a continuous process, and the long life of red blood cells gives the A1c test a look back capability over the lifetime of those cells.[8]

Technical problems with A1c measurement that cause a spurious result have been discussed in depth, and encompass the inclusion of labile A1c in the total A1c, the inability to distinguish glycated hemoglobin from carbamylated hemoglobin, and the positive and negative effects of variant hemoglobins on A1c concentration.[8,9] The National Glycohemoglobin Standardization Program was established to certify methods as traceable to the Diabetes Control and Complications Trial Reference Method.[10,11]

Nathan and colleagues[7] determined a linear relationship between mean blood glucose (MBG) and A1c in 21 diabetics, showing MBG (mg/dL) = 33.3*HbA1c – 86, r = 0.958.

Recently Rohlfing and colleagues[12] examined 1439 patients and refined the relationship to mean plasma glucose MPG (mg/dL) = 35.6*A1c – 77.3, r = 0.82. When they examined fasting glucose alone, the correlation to A1c was lower, r = 0.69. Pathologic conditions can cause discrepant A1c values, invalidating the correlation to plasma glucose. A1c values are lower in hemolytic disease and hemoglobinopathies with decreased red blood cell survival.[13] Iron deficiency anemia causes increased A1c values in diabetics[14,15] and non-diabetics.[16] Spuriously low values of A1c were found in diabetic subjects with anemia, and correction of the anemia raised the A1c.[17] Subtler differences remain to be discovered. Kilpatrick and coworkers reported an intra-individual variance of six percent for glycated hemoglobin, but an 85% inter-individual variance.[18] Rates of glycation differ among individuals, and A1c concentration is also affected by the duration of the diabetic status.[19]

Menstruation has not been investigated as a significant cause of A1c inaccuracy, even though it is a leading cause of iron deficiency anemia. The average age of menopause in American women is 51, close to the average age of diagnosis for diabetes. Many women experience significant blood loss during perimenopause, a several year span of time prior to menopause. This work looks at middle-aged, non-obese, non-diabetic Caucasian women, distinguishing them as premenopausal or menopausal, and compares the hemoglobin values between the groups. It correlates A1c to FPG, and Hemoglobin to FPG in both groups. It introduces a parameter called "Absolute A1c", the product of total hemoglobin* A1c and tests its correlation to FPG in both groups.

MATERIALS AND METHODS

Population

Two hundred women between the ages of 40 and 54 were recruited by newspaper, television, and radio announcement in the Pensacola, Florida area between November 2004 and May 2005. Participants were interviewed to determine their health status, race, age, and menopause status. Some women had a previous hysterectomy and were not aware of their menopause status; these women were grouped with the menopausal women. Participants were weighed and height measured, and BMI was calculated as weight in kg/height in m^2. Participants were excluded if they were outside the specified age range, obese (BMI $\geq$ 30), were known diabetics or had a fasting plasma glucose (FPG) $\geq$ 126 mg/dL (7.0 mmol/L), or if they took medication to lower their cholesterol. Since race other than Caucasian is a risk for diabetes, and only 7.8% of the enrollees were not Caucasian, these women were excluded from this analysis. 169 subjects remained. For the correlation with FPG and A1c, women with hemoglobin < 11.0 g/dL or MCV < 80 fL were disqualified (n = 5). All of the subjects were in good health. This study was approved by the Institutional Review Board at the University of West Florida; all subjects provided informed consent.

Laboratory analysis

Subjects were instructed to fast between ten and 12 hours, and blood was collected in the morning. Blood for glucose analysis was collected in serum separator tubes, and centrifuged within 60 minutes of collection. Glucose was performed on a Dade RXL using the Hexokinase G-6-PD method. Hemoglobin A1c was collected in EDTA and was performed on a Tosoh A1c 2.2+. This instrument is an ion exchange HPLC method approved by the NGSP, it is not affected by carbamylation or HbAS, it separates labile A1c from stable A1c, and printouts alert the operator when the instrument is unable to identify unusual elution peaks. Hemoglobin concentrations were performed by the Cyanmethemoglobin method and MCV and RDW by electrical impedance on a Beckman Coulter HMX. All assays were performed at West Florida Hospital, Pensacola, Florida within one day of collection by a qualified technical staff. The between run imprecision (CV) for glucose, A1c, hemoglobin, MCV and RDW were 1.7%, 2.9%, 0.9%, 0.7%, and 1.2%, respectively. Absolute A1c value was calculated as the A1c %* total hemoglobin in g/L.

Data analysis

Descriptive statistical analysis was performed using Microsoft Excel. Student's t-test of unequal variances was performed for age, FPG, hemoglobin. Student's t-test of equal variance was performed for A1c, Absolute A1c, and MCV. Confidence intervals are 95%. Correlations and box plots were generated with SPSS v.12. Pearson correlation coefficients (r) represent two tailed significance.

RESULTS

All of the women were between 40 and 54 years old, Caucasian, non-diabetic, and normal to overweight. The women were grouped as premenopausal (n = 100) or postmenopausal (n = 69). None of the women reported anemia in their health history. One woman had a hemoglobin concentration of 5.9 g/dL; as an extreme outlier she was excluded. The premenopausal women had a mean hemoglobin of 13.6 g/dL (95% Confidence interval 13.5 – 13.8). The menopausal women had a mean hemoglobin of 13.9 g/L (95% Confidence interval 13.7 – 14.1), p = 0.003. Median hemoglobins were 13.6 g/dL for the premenopausal women and 13.8 g/dL for the

menopausal women. Boxplot analysis showed that the premenopausal women had lower hemoglobin concentrations in general, ranging from 9.8 g/dL to 15.4 g/dL compared to the menopausal women with 12.6 g/dL to 15.9 g/dL (Figure 1). Since iron deficiency anemia is known to prolong red cell survival and increase A1c results, four additional premenopausal women were excluded from the following analysis based on low hemoglobin or MCV.

The two sets of women were similar with respect to BMI, FPG, A1c, MCV, and RDW; they differed in age ($p < 0.001$) and Hemoglobin concentration ($p = 0.009$). Comparison of the two groups is shown in Table 1. FPG showed a weak correlation to A1c value overall (r = 0.251, $p = 0.001$). FPG showed a better correlation to A1c % in the menopausal group (r = 0.218, $p = 0.034$ for the premenopausal group, r = 0.296, $p = 0.013$ for the menopausal group), shown in Figure 2, A and B.

Total hemoglobin correlated with FPG in the menopausal women (r = 0.385, $p = 0.001$), but not with the premenopausal women (r = 0.130, $p = 0.209$). Absolute A1c values were generated in g/L (A1c %* total hemoglobin) shown in Table 1; the two groups of women were significantly different ($p = 0.008$). Absolute A1c correlated significantly to FPG (r = 0.362, $p = 0.000$). The correlation between Absolute A1c was stronger in the postmenopausal women (r = 0.463, $p = 0.000$) than the premenopausal women (r = 0.283, $p = 0.005$), and is shown in Figure 2, C and D.

DISCUSSION

Previous studies have found a less than perfect correlation between A1c and FPG even in non-diabetics. As manufacturers correct the technical factors affecting accuracy of those assays, pre-analytical factors come more into focus. Hemoglobin is glycated in a glucose-dose dependent fashion to form A1c. Total hemoglobin concentration has not been considered in this equation except in the instances where turnover of red cell mass is noticeably altered. Menstrual blood loss is an innocuous factor here, in that most of the premenopausal women would not be classified as anemic. Comparing the hemoglobin ranges of premenopausal to menopausal women shows the effect of that menstrual loss (Figure 1).

This study showed a positive correlation between total hemoglobin and FPG in the menopausal women (r = 0.385, $p = 0.001$). This is not unexpected. Serum hematocrit and serum ferritin levels have been shown to be positively associated with plasma glucose levels in both diabetics and in subjects with insulin resistance syndrome.[20-25] In a study of nondiabetic non-obese Caucasian women in a Pennsylvania population,[26] hemoglobin correlated significantly with FPG in women aged 54 to 70 years (r = 0.344, $p = 0.000$, n = 119, unpublished results). Incorporating the total hemoglobin and A1c into Absolute A1c in the present study improved its correlation to FPG from 0.296 to 0.463. This suggests that both the glucose and the hemoglobin concentrations are relevant in the glycation process.

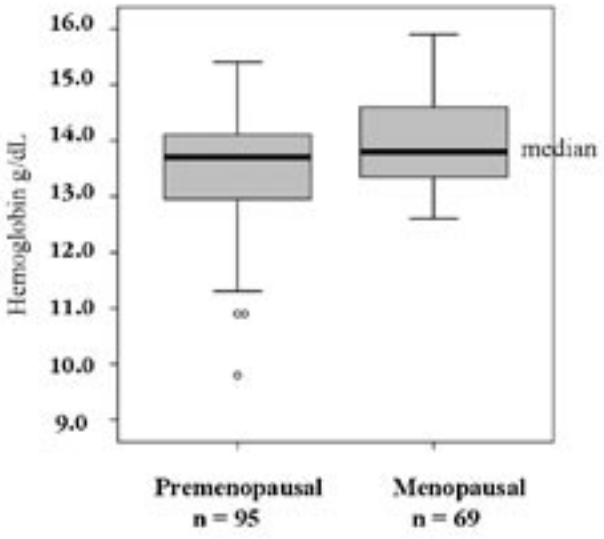
Table 1. Descriptive statistics of non-anemic subjects

	Premenopausal Mean (95% CI)		Menopausal Mean (95% CI)		*p* value
Age	46.1	(45.3 – 47.0)	50.0	(49.3 – 50.7)	<0.001**
BMI	23.2	(22.6 – 23.8)	23.6	(22.8 – 24.3)	0.190
FPG mg/dL	88.0	(88 – 90)	88.0	(86 – 90)	0.500
A1c %	5.4	(5.3 – 5.4)	5.4	(5.3 – 5.5)	0.081
Hemoglobin g/dL	13.6	(13.5 – 13.8)	13.9	(13.7 – 14.1)	0.009**
MCV fL	90.5	(89.8 – 91.2)	90.1	(89.2 – 91.0)	0.311
RDW	12.3	(12.2 – 12.5)	12.2	(12.1 – 12.4)	0.189
Absolute A1c g/L	7.29	(7.18 – 7.41)	7.55	(7.39 – 7.71)	0.008**
n		95		69	

*indicates significance at <0.05; **indicates significance at <0.01

Total hemoglobin did not correlate to glucose in the premenopausal women (r = 0.130, p = 0.209). This data is also consistent with findings from the Pennsylvania study: hemoglobin did not correlate to FPG in women aged 40 to 50 years (r = 0.208, p = 0.103, n = 63, unpublished data). Incorporating the total hemoglobin and A1c into Absolute A1c improved its correlation to FPG, but to a lesser extent than in the menopausal women (from 0.218 to 0.283). This finding suggests that menstrual blood loss has a pre-analytical effect on A1c. Since menstruation is not a pathologic process it has been overlooked as a source of variation in A1c accuracy. Women with heavy periods can become iron deficient; in fact 20% of women will become iron deficient sometime during their reproductive years.[27] The paradox is this: While the iron deficiency should make the A1c concentration higher, the blood loss and replacement with younger cells should make the A1c concentration lower. In this study, five percent of the premenopausal women were suspected to have iron deficiency and therefore inaccurate A1c results. Even after they were excluded from the analysis the premenopausal group still had a poor correlation to FPG when compared to the non-menstruating group, consistent with a variable pattern of red blood cell turnover.

Figure 1. Hemoglobin ranges for premenopausal and menopausal women



The hemoglobin range is wider and the concentrations are lower for premenopausal compared to menopausal women. Ranges are depicted in quartiles. Error bars represent the extremes. The box represents quartile two and quartile three. The line represents the median. Open circles represent weak outliers.

This study suggests that menstruation during middle age can have a significant effect on the accuracy on A1c, and that effect goes away at menopause. This is a significant finding, because half of the women who develop type 2 diabetes do so in perimenopause.[28] The average age for diagnosis of diabetes in white American women is about 46 years,[1] the average age for her menopause is 51, with perimenopause occurring as early as 35 or as late as 55.[28] Women in perimenopause suffer from irregular menstruation. In a study of 500 perimenopausal women 18% reported more frequent and/or heavier periods with irregular bleeding between periods.[29] Clearly the accuracy of the A1c result is dependent upon consistent hemoglobin concentration. This study showed a significant difference in hemoglobin concentration between premenopausal and menopausal women with similar MCV and RDW.

This work introduces the parameter "Absolute A1c", derived as the product of total hemoglobin and %A1c. It is intriguing that this parameter showed a superior correlation to FPG. It is logical that A1c formation is dependent upon the concentration of both glucose and hemoglobin. This parameter is very appealing, given the association of insulin resistance with increased iron stores,[23-25] and after thorough study may prove to be a valuable diagnostic tool. It is important to interpret these results within the specifications of this study. The subjects were not diabetic, so the range of glucose and A1c was limited. Furthermore, only 169 subjects were tested. Mean blood glucose would be a stronger tester than FPG, but that data was not collected. Subjects with hemoglobinopathies may have different results. This study uses Absolute A1c as a tool to examine the differences between premenopausal and menopausal Caucasian women, and it is clear that these groups are different from each other. It will be important to test the validity of Absolute A1c and the effect of menstruation on A1c in non-Caucasian women, as these women represent a significant at-risk population. The prevalence of diabetes in African Americans is 1.8 times that of whites, the prevalence in Hispanics is 1.7 times that of whites, and American Indians are 2.2 times as likely to have diabetes as whites of a similar age.[30]

It will be interesting to test Absolute A1c in other groups, including those with iron deficiency, men, and older women. Coban and colleagues[16] have already speculated that iron deficient individuals may have increased A1c in part due to a higher glucose to hemoglobin ratio. One study restricted to men showed a low variation in Glycohemoglobin,[31] which is consistent with the expectation of stable hemoglobin levels. Older women of all races have an increased prevalence of diabetes[30] and will benefit from improvements in the indicators of glycemic control.

Strengths of this study lie in its limitation of variables: the subjects are non-diabetics so overall glucose variation was limited, none of the subjects were obese so that blood volume was comparable in the subjects, and they all were the same sex and similar in age. It is unlikely that any participants had a hemoglobinopathy based on race and the ability of the HPLC analyzer to flag unexpected eluate peaks. One weakness of this study is that each set of data – FPG and A1c – was obtained only once from each participant. Women were dichotomized as premenopausal or menopausal based on patient reporting. Since this study revolved around blood loss from menstruation, women who had hysterectomies were included with the menopausal women. There was no method to determine which non-menopausal women were in peri-menopause, therefore all of the menstruating women were analyzed as one group. Hormonal changes may have some effect on glucose and A1c; this was not explored.

A1c results are valuable in long term maintenance and assessment of diabetes, and both the American Diabetes Association[6] and the American Heart Association recommend a target goal A1c < 7% to reduce the risk for micro and macrovascular disease in women.[32] The Standards of Medical Care in Diabetes[6] do not include an assessment of total hemoglobin in diabetes management, and men and women have the same target A1c goal. Clinicians and researchers should be aware that perimenopausal patterns of bleeding may cause inconsistent A1c values, and incorporate patient history of menstrual and other bleeding, self monitoring blood glucose results, and hemogram results (including hemoglobin, MCV and RDW) with A1c for a thorough assessment of glycemic control.

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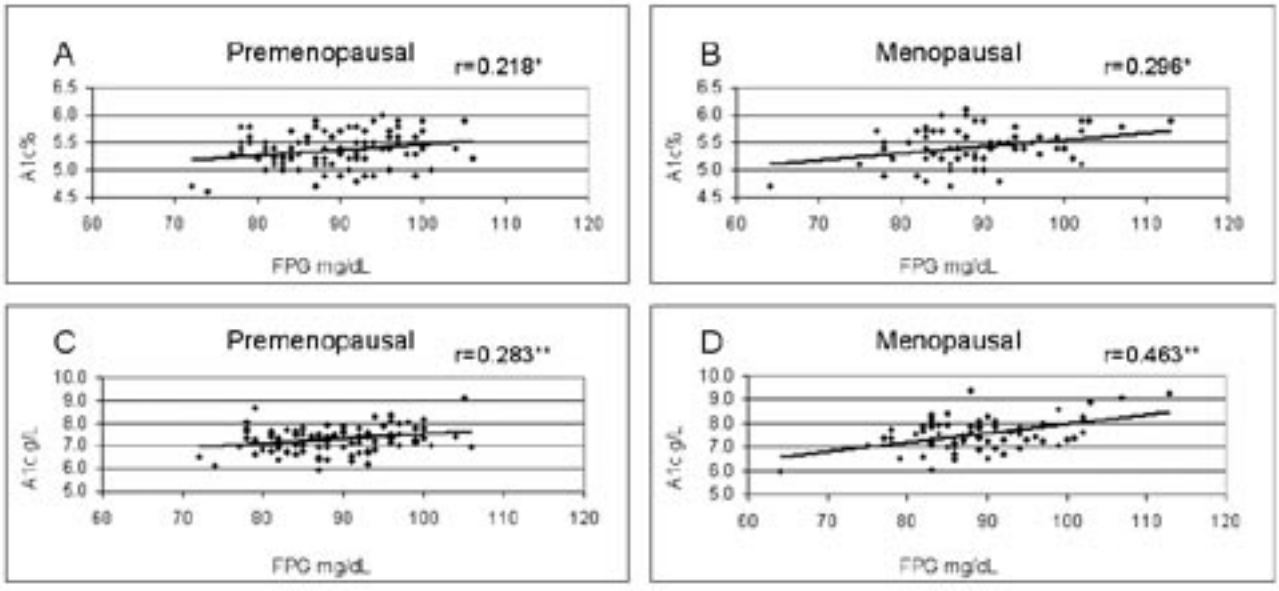
Figure 2. Correlation of FPG with A1c in premenopausal and menopausal women



Correlation of A1c % is stronger in menopausal women than premenopausal women (compare B to A). Absolute A1c correlates better than A1c % to FPG in premenopausal women (compare C to A) and menopausal women (compare D to B).

*indicates significance at <0.05; **indicates significance at <0.01

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