# Preconception hemoglobin A1c concentration in healthy women is not associated with fecundability or pregnancy loss

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**Objective:** To examine the relationship of preconception hemoglobin A1c, a marker of cumulative exposure to glucose over the preceding 2–3 months, with time to pregnancy, pregnancy loss, and live birth among fecund women without diagnosed diabetes or other medical diseases.
**Design:** A secondary analysis of a prospective cohort of women participating in the Effects of Aspirin in Gestation and Reproduction (EAGeR) trial.
**Setting:** Four US academic medical centers.
**Patient(s):** A total of 1,194 healthy women aged 18–40 years with a history of one or two pregnancy losses attempting spontaneous conception were observed for up to six cycles while attempting pregnancy and throughout pregnancy if they conceived.
**Intervention(s):** Not applicable.
**Main Outcome Measure(s):** Time to pregnancy, human chorionic gonadotropin pregnancy, clinical pregnancy, pregnancy loss, and live birth.
**Result(s):** Although increasing preconception A1c level was associated with reduced fecundability (fecundability odds ratio [FOR] per unit increase in A1c 0.74; 95% confidence interval [CI] 0.57, 0.96) in unadjusted models and models adjusted for age, race, smoking and treatment arm (FOR 0.79; 95% CI 0.60, 1.04), results were attenuated after further adjustment for body mass index (FOR 0.91; 95% CI 0.68, 1.21). Preconception A1c levels among women without diagnosed diabetes were not associated with live birth or pregnancy loss.
**Conclusions(s):** Among healthy women without diagnosed diabetes, we observed no association of A1c with live birth or pregnancy loss. The association between A1c and fecundability was influenced by body mass index, a strong risk factor for both diabetes and infertility. These data support current recommendations that preconception A1c screening should be reserved for patients with risk factors for diabetes.
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Data will be made accessible in an electronic repository after completion of the studies' analytical phases. The data, along with a set of guidelines for researchers applying for the use of the data, will be posted to a data-sharing site, NICHD Data and Specimen Hub (DASH) (https://dash.nichd.nih.gov/).
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Hemoglobin A1c, a marker of cumulative exposure to glucose over the preceding 2–3 months, is a convenient screening tool used to diagnose diabetes and monitor diabetic treatment (1). Elevations of A1c concentrations above the criteria diagnostic for diabetes in pregnant women with poorly controlled diabetes have been linked to early pregnancy loss (2, 3), stillbirth (2), and congenital anomalies (4). The reasons are uncertain but may include maternal dysglycemia promoting epigenetic modifications of the fetal genome (5), oxidative stress resulting in impaired embryogenesis (6), and altered angiogenesis impacting placental development and physiology (7). These observations have led the American College of Obstetricians and Gynecologists (ACOG) and the American Society for Reproductive Medicine (ASRM) to establish strict criteria for glucose targets for women with diabetes planning a pregnancy (8), as well as the ASRM to adopt A1c screening in women with a history of recurrent pregnancy loss (8). Women who are overweight or obese with one or more risk factors (family history of diabetes, high-risk race/ethnicity, hypertension, polycystic ovary syndrome [PCOS], physical inactivity, hyperlipidemia, hypertension, and/or cardiovascular disease) or have a history of gestational diabetes are currently recommended to undergo screening every 1–3 years (1). Despite the reproductive complications observed in overt diabetes, the relationship between the continuum of glucose exposure and impaired fecundability and pregnancy loss remains uncertain (9, 10).

To date, most studies of glucose/insulin metabolism in women without pregestational diabetes have been performed after pregnancy is established. Thus, it is unknown if implantation and early pregnancy loss are associated with higher levels of A1c in apparently healthy patients trying to conceive. The rigorous methodology and preconception recruitment necessary to capture fecundability and pregnancy loss have typically only included infertility populations (11, 12) or self-reported pregnancy status (13). Studies that examined preconception measures of glucose/insulin metabolism have not evaluated preconception A1c. (11–13). A1c reflects glycemic exposure over a period of time, as opposed to tests that reflect short-term glucose metabolism. These tests are prone to intra- and inter-individual variation and are inconvenient as they must be drawn in the fasting state (14). Furthermore, there is limited information regarding the measurement of glycemia in the normal range, as most studies investigate outcomes in association with medically defined cut-points for disease. Thus, our objective was to examine the association of preconception A1c, along with other measures of glucose metabolism, with time to pregnancy, human chorionic gonadotropin (hCG) pregnancy, clinical pregnancy, early pregnancy loss, and live birth among women with proven fecundity trying to conceive naturally.

## MATERIALS AND METHODS

This was an analysis of a prospective cohort from the Effects of Aspirin in Gestation and Reproduction (EAGeR) trial. The EAGeR trial was a multicenter, double-blind, randomized placebo-controlled trial that examined the effect of preconception low-dose aspirin on live birth from 2006 to 2012 at four clinical sites in the United States. Institutional Review Board approval was obtained at the data coordinating center and at all clinical sites, and each participant provided written informed consent. Participant safety was monitored by a Data Safety and Monitoring Board, and the trial was registered with ClinicalTrials.gov (NCT00467363). The study design and methods have been previously described (15).

### Study Design and Population

Participants in the EAGeR trial included women, aged 18–40-years, with a history of regular menstrual cycles (21–42 days in length) who were attempting to conceive spontaneously and had a history of 2 or fewer prior live births and 1-2 confirmed pregnancy losses before randomization. The participants had no known diagnosis of infertility or major health problems (e.g., diabetes, hypertension, PCOS). The exclusion criteria were the use of long-acting reversible contraception earlier than 12 months and/or hormonal contraception in the past 3 months. A total of 1,228 participants enrolled in the trial. The participation ended if they did not conceive in 6 months, after experiencing 2 hCG-detected losses, or after experiencing 1 clinical loss during the study. Women used ovulation predictor kits (Clearblue Easy Fertility Monitor; Inverness Medical) to provide adequate timing of intercourse.

### Study Procedures

At the baseline appointment, occurring before randomization and conception, participants completed questionnaires regarding age, race, education level, household income, employment, time from last pregnancy loss to enrollment, number of previous live births, and alcohol consumption. Physical activity was categorized as low, moderate, or high using the short-form version of the International Physical Activity Questionnaire (16).

Height and weight were measured by trained staff at enrollment and were used to calculate the body mass index (BMI). Waist circumference measurement was obtained with a measuring tape applied halfway between the xiphoid process and umbilicus, and the participant was asked to bend sideways to identify the natural waist. For the hip

measurement, the tape was applied at the level of the pubis symphysis. The waist-to-hip ratio was then calculated. Each measurement was obtained twice to ensure accuracy, and the average of both measures was used.

## Outcome Measures

Time to pregnancy, hCG pregnancy, clinical pregnancy, pregnancy loss, and live birth were the outcomes of interest in this study. Time to pregnancy was defined as the number of cycles that the participant was attempting to conceive before the presence of hCG. An hCG pregnancy was detected by urine pregnancy tests (Quidel Quickvue, Quidel Corporation, sensitive to 25 mIU/mL hCG) performed at the study site or home. Additional urinary specimens were collected in the clinic at the end of each menstrual cycle. During the participant's first and second cycle of study participation, urine was also collected at home daily and stored in home freezers until transfer to the study sites each month. All urine samples were stored frozen at −80 °C until assay. The spot urine samples from study visits and the samples from the last 10 days of the first two menstrual cycles were analyzed for the presence of free beta hCG to allow for more sensitive detection of pregnancy and very early pregnancy loss (Catalog No. 4221-16, Diagnostic Automation Inc.; Catalog No. RIS0011R, Bio-Vendor). Clinical pregnancies were confirmed by the evidence of clinical pregnancy on the study ultrasound during gestational weeks 6–7 (e.g., presence of gestational sac, presence of fetal heart tones, or clinical confirmation of pregnancy at a later stage). Pregnancy losses included hCG-detected and clinical losses before 20 weeks of gestation (i.e., early pregnancy loss). Human chorionic gonadotropin-detected losses were defined as a positive hCG without further progression to evidence of pregnancy on sonogram or clinically. Clinical losses were defined as pregnancy loss after ultrasound confirmation.

## Biochemical Analysis

Blood samples were collected at baseline, and fasting status was recorded during the blood draw. Serum was separated and stored frozen at −80 °C pending analysis. Preconception A1c concentration was measured in the whole blood that was collected at the baseline visit before randomization among those with available whole blood samples for analysis (n = 1,194; 97%). Analysis of glucose and insulin was restricted to women who reported that they had been fasting at the time of the baseline blood draw (n = 173; 14.1%).

A1c was measured in whole blood using nonporous ion-exchange high-performance liquid chromatography using the Tosoh Automated Analyzer HLC-723G8 (Tosoh G8) (Tosoh Bioscience, Inc. San Francisco, CA, and Tokyo, Japan). The reference range was 4.3%–6.0%, and the measurement range was 3.1%–19.0%. The interassay coefficients of variation (CVs) were 1.16% at 5.34% and 0.55% at 10.11%. Serum concentrations of fasting and nonfasting glucose and insulin were measured using a Roche COBAS 6000 chemistry autoanalyzer (Roche Diagnostics, Indianapolis, IN). The CVs were 1.3% at 97.2 mg/dL and 1.8% at 223.3 mg/dL for glucose, and 3.1% at 16.89 μU/mL and 3.1% at 52.67 μU/mL for insulin. Homeostatic model assessment of insulin resistance (HOMA-IR) was calculated by the following formula: fasting insulin (μU/mL) × fasting glucose (nmol/L)/22.5 (17).

## Statistical Analysis

A total of 1,228 participants enrolled in the trial, of which 1,194 had preconception A1c measures available. Participants were divided into tertiles of A1c. The tertiles were not evenly divided because A1c is reported with only 1 decimal place and many women had the same value. The first tertile included an A1c range of 3.8%–4.9%, the second tertile A1c of 5.0%–5.1%, and the third tertile A1c of 5.2%–7.5%. Participants with fasting biospecimens available were also divided into tertiles based on fasting glucose (n = 173), fasting insulin (n = 172), and HOMA-IR (n = 92). Patients who met the criteria for prediabetes (n = 30) and diabetes (n = 2) based on the A1c criteria set by the American Diabetes Association (5.7%–6.4% for prediabetes and ≥6.5% for diabetes [18]) were retained in our study as we were interested in A1c and glucose/insulin dynamics in the general population of women trying to conceive, including women with undiagnosed prediabetes and diabetes.

Demographic and baseline clinical characteristics and anthropometrics were examined by tertiles of preconception A1c levels using ANOVA for continuous variables and $\chi^2$ test for categorical variables. Discrete Cox proportional hazards regression models were used to assess preconception A1c and fecundability odds ratios (FORs) as well as 95% confidence intervals (CIs), accounting for left truncation in participants who were attempting conception before enrollment, and right censoring because of early withdrawal or the discontinuation of follow-up after 6 months. Models evaluated markers of glucose/insulin dynamics (A1c, fasting glucose, fasting insulin, and HOMA-IR) by tertiles comparing the first and third tertile to the middle (reference) tertile. The middle group was chosen as the reference group as it was hypothesized that increased or decreased A1c concentrations may be associated with fecundability and pregnancy loss. Although no departures from linearity were observed, continuous models are also presented. To examine the association of A1c levels with hCG-detected and clinical pregnancy and live birth, log-binomial regression models were used. To examine the association of A1c concentration with pregnancy loss, log-binomial regression models with inverse probability weights were used to estimate RR and 95% CIs. Weighted models were used to control for potential selection bias introduced by only including women with hCG-detected pregnancies (n = 766). Weights were generated from models including factors that may predict pregnancy, such as maternal age, parity, marital status, number of previous losses, and treatment assignment. In all analyses, two models were developed to adjust for potential confounders, one model adjusted for age, race, treatment arm, and smoking, with a second model that additionally adjusted for BMI. These two models were constructed to understand the role of BMI on these outcomes, given the strong associations observed between A1c concentration and BMI in prior studies (19–21). All analyses were conducted in SAS version 9.4 (SAS Institute, Cary, NC).

# RESULTS

## Participant Characteristics

Women in the highest A1c tertile were slightly older (29.5 ± 5.0 years) and more likely to be of non-White race (9.7%) in comparison to the other tertiles (28.8 ± 4.8 and 3.5%; 28.0 ± 4.6 years and 2.9%, respectively; Table 1). They were also more likely to report smoking in the previous year, had a longer time since their last pregnancy loss, and had a higher number of previous losses than women in the other A1c tertiles. There were no differences in physical activity, household income, employment, and parity.

Markers of adiposity, including BMI and waist-to-hip ratio, exhibited a pattern of increasing adiposity with increased A1c tertile. Specifically, women in the highest tertile had a mean BMI of 28.2 ± 7.7 kg/m$^2$, compared with 26.2 ± 6.5 kg/m$^2$ in the lowest tertile. In addition, markers of glucose metabolism, fasting and nonfasting serum glucose, fasting insulin, and HOMA-IR also increased with increasing the A1c tertile (Supplemental Table 1, available online).

## Time to Pregnancy

Increasing preconception A1c was associated with reduced fecundability (FOR 0.74; 95% CI 0.57, 0.96, per unit increase in A1c) in continuous models before adjustment, although this was attenuated after adjusting for age, race, smoking, and treatment arm (FOR 0.79; 95% CI 0.60, 1.04, per unit increase in A1c; [Table 2)]). This was further attenuated when additionally controlling for BMI (FOR 0.91; 95% CI 0.68, 1.21, per unit increase in A1c). There were no associations between A1c and fecundability when A1c was categorized into tertiles.

In addition, tertiles were created for fasting glucose (n = 173), fasting insulin (n = 173), and HOMA-IR (n = 92) when data were available. No differences in fecundability in unadjusted or adjusted models were present for each fasting marker of glucose/insulin metabolism either in continuous or tertile models (Table 2).

**Overall hCG-detected pregnancy, clinical pregnancy, and live birth.** A total of 300 (74.8%) women with A1c in the first tertile had an hCG-detected pregnancy, while 235 (71.0%) participants in the second (reference) tertile, and 231 (70.6%) in the third tertile had an hCG-detected pregnancy (Table 3). There were no associations with hCG-detected pregnancy observed across tertiles of A1c. Continuous models suggested that increasing A1c was associated with lower odds of hCG-detected pregnancy in unadjusted models (RR 0.85; 95% CI 0.77, 0.95, per unit increase in A1c). This remained in models adjusted for age, race, smoking, and treatment arm (RR 0.87; 95% CI 0.77, 0.97, per unit increase in A1c), although this result was attenuated in models that controlled for BMI in addition to the above covariates (RR 0.92; 95% CI 0.81, 1.04, per unit increase in A1c). A similar pattern was observed with clinical pregnancy.

A total of 225 (56.1%) women in the first A1c tertile had a live birth, while 176 (53.2%) women with an A1c in the second tertile, and 183 (56.0%) in the third tertile had a live birth. There was no association between preconception A1c levels with a live birth in continuous models or tertile of A1c in either unadjusted or adjusted models.

## Pregnancy Loss

Among all women who attained an hCG pregnancy, a total of 73 (24.3%) women with A1c in the first tertile had an early pregnancy loss, while 59 (25.1%) in the reference (middle) group and 46 (19.9%) in the third tertile had a pregnancy loss ≤ 20 weeks gestation (Table 4). Among all women with an hCG pregnancy, clinical pregnancy loss occurred in 53 (17.7%) women in the first tertile, 42 (17.9%) women in the second tertile, and 30 (13%) women in the third tertile. There was no increase in early pregnancy loss or clinical pregnancy loss with increasing A1c levels. However, A1c concentration in the third tertile was associated with a small decreased risk of pregnancy loss than the middle tertile after adjustment (RR 0.65, 95% CI 0.42, 1.01).

# DISCUSSION

This study provides novel data indicating no relationship between A1c concentration across the normal range, fecundability, and pregnancy loss among healthy women without a history of infertility. Although A1c concentration is a useful tool for the diagnosis and management of diabetes and clinically impaired glucose metabolism and obesity are linked to fecundability and pregnancy loss, glucose homeostasis in otherwise healthy women does not appear to be associated with these outcomes after accounting for adiposity. Thus, guidelines for A1c testing before pregnancy should remain unchanged, supporting preconception screening reserved only for women with diabetes or at risk for developing diabetes.

## Fecundability

Few previous studies have reported preconception measures of glucose metabolism and fecundability, and those available indicated mixed results based on acute measures of glucose status. Prior studies did not use A1c, which is a marker of the average recent glycemic status. In contrast to our findings, a cohort of presumed healthy nulliparous women attempting natural conception reported that higher preconception fasting glucose levels were associated with reduced fecundability after accounting for adiposity (13). Although the cohort had similar distributions of normoglycemia (96%), impaired glycemia (3%), and undiagnosed diabetes (1%) as the women in our study, the normoglycemic group was 90% normal or underweight by BMI (mean 21 kg/m$^2$, standard deviation [SD] 4.5) representing a markedly different adiposity distribution of women than our cohort (mean BMI 26.2 kg/m$^2$, SD 6.5). The observation of reduced fecundability with increasing A1c levels in our study was mitigated after adjustment with BMI, which is likely related to the close association of BMI and A1c. A study of infertile women undergoing in vitro fertilization (IVF) treatment reported no difference in preconception fasting glucose and insulin, HOMA-IR, or oral glucose tolerance test (OGTT) between women who became pregnant and those that did not (11). In contrast, a study in patients

## TABLE 1

**Demographics and baseline characteristics by tertile of preconception A1c levels.**

| | | Preconception A1c, % | | | |
|---|---|---|---|---|---|
| | Total | Tertile 1 (3.8–4.9) | Tertile 2 (5–5.1) | Tertile 3 (5.2–7.5) | *P* value |
| Participants, n | 1194 | 442 | 372 | 380 | |
| Age, y | 28.7 ± 4.8 | 28 ± 4.6 | 28.8 ± 4.8 | 29.5 ± 5 | .0001 |
| Body mass index, kg/m$^2$ | 26.2 ± 6.5 | 24.9 ± 5.2 | 25.8 ± 5.9 | 28.2 ± 7.7 | <.0001 |
| Waist-to-hip ratio | 0.81 ± 0.07 | 0.8 ± 0.07 | 0.81 ± 0.07 | 0.82 ± 0.08 | <.0001 |
| Race, n (%) | | | | | |
| White | 1131 (94.7) | 429 (97.1) | 359 (96.5) | 343 (90.3) | <.0001 |
| Others | 63 (5.3) | 13 (2.9) | 13 (3.5) | 37 (9.7) | |
| Education, n (%) | | | | | |
| ≤High School | 162 (13.6) | 50 (11.3) | 54 (14.5) | 58 (15.3) | .21 |
| >High School | 1031 (86.4) | 391 (88.7) | 318 (85.5) | 322 (84.7) | |
| Household income (annual), n (%) | | | | | |
| ≥$100,000 | 473 (39.6) | 166 (37.6) | 153 (41.1) | 154 (40.5) | .74 |
| $75,000–$99,999 | 147 (12.3) | 57 (12.9) | 43 (11.6) | 47 (12.4) | |
| $40,000–$74,999 | 176 (14.8) | 64 (14.5) | 62 (16.7) | 50 (13.2) | |
| $20,000–$39,999 | 306 (25.6) | 120 (27.2) | 91 (24.5) | 95 (25) | |
| ≤$19,999 | 91 (7.6) | 34 (7.7) | 23 (6.2) | 34 (8.9) | |
| Employed, n (%) | | | | | |
| Yes | 874 (75.9) | 332 (77.2) | 275 (76.4) | 267 (73.8) | .51 |
| No | 278 (24.1) | 98 (22.8) | 85 (23.6) | 95 (26.2) | |
| Time from last loss to randomization (mo), n (%) | | | | | |
| ≤4 mo | 633 (53.9) | 284 (65.6) | 195 (53.3) | 154 (41) | <.0001 |
| 5–8 mo | 215 (18.3) | 66 (15.2) | 65 (17.8) | 84 (22.3) | |
| 9–12 mo | 97 (8.3) | 25 (5.8) | 37 (10.1) | 35 (9.3) | |
| > 12 mo | 230 (19.6) | 58 (13.4) | 69 (18.9) | 103 (27.4) | |
| Previous live births, n (%) | | | | | |
| 0 | 555 (46.5) | 212 (48) | 179 (48.1) | 164 (43.2) | .14 |
| 1 | 431 (36.1) | 165 (37.3) | 131 (35.2) | 135 (35.5) | |
| 2 | 208 (17.4) | 65 (14.7) | 62 (16.7) | 81 (21.3) | |
| Previous losses, n (%) | | | | | |
| 1 | 800 (67) | 319 (72.2) | 234 (62.9) | 247 (65) | .01 |
| 2 | 394 (33) | 123 (27.8) | 138 (37.1) | 133 (35) | |
| Smoking in past year, n (%) | | | | | |
| Never | 1038 (87.6) | 395 (90) | 327 (88.6) | 316 (83.8) | .009 |
| <6 times/week | 85 (7.2) | 31 (7.1) | 25 (6.8) | 29 (7.7) | |
| Daily | 62 (5.2) | 13 (3) | 17 (4.6) | 32 (8.5) | |
| Alcohol consumption in past year, n (%) | | | | | |
| Often | 26 (2.2) | 12 (2.7) | 7 (1.9) | 7 (1.9) | .08 |
| Sometimes | 367 (31.1) | 127 (29.1) | 103 (28) | 137 (36.5) | |
| Never | 787 (66.7) | 298 (68.2) | 258 (70.1) | 231 (61.6) | |
| Physical Activity | | | | | |
| Low | 310 (26) | 103 (23.3) | 95 (25.5) | 112 (29.5) | .31 |
| Moderate | 489 (41) | 184 (41.6) | 159 (42.7) | 146 (38.4) | |
| High | 395 (33.1) | 155 (35.1) | 118 (31.7) | 122 (32.1) | |
| Low-dose aspirin treatment group | 598 (50.1) | 223 (50.5) | 187 (50.3) | 188 (49.5) | .96 |

*Note:* Values are mean ± SD or n (%) as indicated.

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with PCOS undergoing IVF found that impaired glucose tolerance using an OGTT, but not isolated impaired fasting glucose, was associated with a lower rate of conception than in those with normoglycemia (12). Based on these limited data, coupled with our findings that increasing A1c in the normal range was not associated with reduced fecundability after BMI adjustment, the glycemic status may not be an important marker for fecundability independent of the well-known impact of obesity (22, 23).

## Pregnancy Loss

Increasing A1c concentration was associated with a nonsignificant reduction in early pregnancy loss, as our loss rates per A1c tertile were relatively similar with 24.5%, 25.1%, and 20.1% for tertiles 1, 2, and 3, respectively, and are consistent with previously published loss rates in healthy populations (9). Prior studies in couples undergoing IVF yielded conflicting results; one found no association between OGTT parameters and pregnancy loss (11), while another study in women with PCOS indicated that impaired glucose tolerance by OGTT was associated with a higher rate of pregnancy loss than impaired fasting glucose or normoglycemia (12). However, insulin resistance, which is strongly associated with PCOS, is known to increase the risk of miscarriage, even in the absence of hyperglycemia, which may explain the latter study's findings (24). Indeed, hyperinsulinemia may interfere with implantation by decreasing the expression of proteins

## TABLE 2

**Association between preconception A1c concentration, fasting glucose and insulin, and HOMA-IR in relation to fecundability.**

**Preconception A1c, %**

| | Tertile 1 (3.8–4.9) | Tertile 2 (5–5.1) | Tertile 3 (5.2–7.5) | Continuous |
|---|---|---|---|---|
| N | 442 | 372 | 380 | |
| Unadjusted | 1.08 (0.89, 1.32) | Ref. | 1.01 (0.81, 1.24) | 0.74 (0.57, 0.96)[a] |
| Adjusted model 1 | 1.06 (0.87, 1.30) | Ref. | 1.05 (0.85, 1.30) | 0.79 (0.60, 1.04) |
| Adjusted model 2 | 1.04 (0.85, 1.27) | Ref. | 1.11 (0.90, 1.38) | 0.91 (0.68, 1.21) |

**Fasting glucose (mg/dL)[b]**

| | Tertile 1 (28–77) | Tertile 2 (78–85) | Tertile 3 (86–120) | Continuous |
|---|---|---|---|---|
| N | 58 | 61 | 54 | |
| Unadjusted | 1.59 (0.92, 2.74) | Ref. | 1.00 (0.55, 1.81) | 0.99 (0.96, 1.01) |
| Adjusted model 1 | 1.65 (0.94, 2.89) | Ref. | 0.94 (0.51, 1.75) | 0.98 (0.96, 1.01) |
| Adjusted model 2 | 1.56 (0.88, 2.76) | Ref. | 0.96 (0.52, 1.79) | 0.99 (0.97, 1.01) |

**Fasting insulin (mIU/L)[b]**

| | Tertile 1 (2.55–6.14) | Tertile 2 (6.2–10.67) | Tertile 3 (11.02–86.22) | Continuous |
|---|---|---|---|---|
| N | 57 | 58 | 57 | |
| Unadjusted | 0.88 (0.51, 1.53) | Ref. | 0.92 (0.53, 1.62) | 0.98 (0.95, 1.01) |
| Adjusted model 1 | 0.92 (0.52, 1.64) | Ref. | 0.98 (0.55, 1.75) | 0.98 (0.95, 1.01) |
| Adjusted model 2 | 0.83 (0.46, 1.49) | Ref. | 1.26 (0.67, 2.37) | 0.98 (0.96, 1.01) |

**HOMA-IR[b]**

| | Tertile 1 (0.44–1.17) | Tertile 2 (1.17–2.09) | Tertile 3 (2.19–25.54) | Continuous |
|---|---|---|---|---|
| N | 32 | 33 | 27 | |
| Unadjusted | 0.94 (0.54, 1.63) | Ref. | 0.89 (0.50, 1.56) | 0.91 (0.81, 1.03) |
| Adjusted model 1 | 0.92 (0.52, 1.61) | Ref. | 0.96 (0.51, 1.61) | 0.91 (0.81, 1.02) |
| Adjusted model 2 | 0.81 (0.45, 1.44) | Ref. | 1.12 (0.60, 2.10) | 0.93 (0.83, 1.04) |

*Note:* Model 1 adjusts for age, race, smoking, treatment arm (low-dose aspirin vs. placebo). Model 2 adjusts for age, race, smoking, treatment arm (low-dose aspirin vs. placebo), and body mass index. HOMA-IR = homeostatic model assessment of insulin resistance.
[a] $P < .05$.
[b] Fasting metabolic biomarkers were restricted to a subset of women who reported they had been fasting at the time of the baseline blood draw; (fasting glucose, n = 173; fasting insulin, n = 172; HOMA-IR, n = 172).

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## TABLE 3

**Association between preconception A1c concentration in relation to hCG-detected pregnancy, clinical pregnancy, and live birth.**

**Preconception A1c, %**

| | Tertile 1 (3.8–4.9) | Tertile 2 (5–5.1) | Tertile 3 (5.2–7.5) | Continuous |
|---|---|---|---|---|
| n | 401 | 331 | 327 | |
| **hCG-detected pregnancy** | | | | |
| **n (%)** | 300 (74.8) | 235 (71.0) | 231 (70.6) | |
| Unadjusted | 1.05 (0.96, 1.15) | Ref. | 1.00 (0.90, 1.10) | 0.85 (0.77, 0.95)[a] |
| Adjusted model 1 | 1.03 (0.95, 1.13) | Ref. | 1.00 (0.91, 1.10) | 0.87 (0.77, 0.97)[b] |
| Adjusted model 2 | 1.02 (0.94, 1.11) | Ref. | 1.02 (0.93, 1.12) | 0.92 (0.81, 1.04) |
| **Clinical pregnancy** | | | | |
| **n (%)** | 278 (69.3) | 215 (65.0) | 214 (65.4) | |
| Unadjusted | 1.07 (0.96, 1.18) | Ref. | 1.01 (0.90, 1.13) | 0.84 (0.74, 0.96)[b] |
| Adjusted model 1 | 1.04 (0.94, 1.15) | Ref. | 1.02 (0.91, 1.14) | 0.87 (0.76, 1.00) |
| Adjusted model 2 | 1.03 (0.93, 1.14) | Ref. | 1.05 (0.94, 1.17) | 0.93 (0.8, 1.08) |
| **Live birth** | | | | |
| **n (%)** | 225 (56.1) | 176 (53.2) | 183 (56.0) | |
| Unadjusted | 1.06 (0.92, 1.21) | Ref. | 1.05 (0.92, 1.21) | 0.89 (0.74, 1.05) |
| Adjusted model 1 | 1.03 (0.90, 1.17) | Ref. | 1.08 (0.94, 1.23) | 0.94 (0.79, 1.12) |
| Adjusted model 2 | 1.01 (0.89, 1.15) | Ref. | 1.13 (0.99, 1.29) | 1.04 (0.86, 1.26) |

*Note*: Model 1 adjusts for age, race, smoking, treatment arm (low-dose aspirin vs placebo). Model 2 adjusts for age, race, smoking, treatment arm (low-dose aspirin vs placebo), and body mass index. hCG = human chorionic gonadotropin.
[a] $P < .01$.
[b] $P < .05$.

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## TABLE 4

**Association between preconception A1c and early pregnancy loss.**

| | Preconception A1c, % | | | |
|---|---|---|---|---|
| | Tertile 1 (3.8–4.9) | Tertile 2 (5–5.1) | Tertile 3 (5.2–7.5) | Continuous |
| N | 300 | 235 | 231 | |
| **Any early pregnancy loss** | | | | |
| **n (%)** | 73 (24.3) | 59 (25.1) | 46 (19.9) | |
| Unadjusted | 0.95 (0.70, 1.28) | Ref. | 0.81 (0.58, 1.14) | 0.83 (0.54, 1.27) |
| Adjusted model 1 | 0.94 (0.70, 1.26) | Ref. | 0.76 (0.54, 1.06) | 0.76 (0.50, 1.15) |
| Adjusted model 2 | 0.96 (0.71, 1.29) | Ref. | 0.72 (0.51, 1.01) | 0.68 (0.44, 1.05) |
| **Clinical loss** | | | | |
| **n (%)** | 53 (17.7) | 42 (17.9) | 30 (13) | |
| Unadjusted | 0.99 (0.68, 1.43) | Ref. | 0.74 (0.48, 1.14) | 0.77 (0.45, 1.32) |
| Adjusted model 1 | 0.97 (0.67, 1.40) | Ref. | 0.69 (0.44, 1.06) | 0.75 (0.44, 1.28) |
| Adjusted model 2 | 1.00 (0.69, 1.44) | Ref. | 0.65 (0.42, 1.01) | 0.67 (0.39, 1.16) |

*Note:* Model 1 adjusts for age, race, smoking, treatment arm (low-dose aspirin vs. placebo). Model 2 adjusts for age, race, smoking, treatment arm (low-dose aspirin vs. placebo), and body mass index. Models restricted to women who achieved an hCG pregnancy, with inverse probability weights to control for selection bias introduced by only including women who achieved pregnancy during the study. Weights were estimated to control for factors that may predict pregnancy, such as maternal age, parity, marital status, number of previous losses, and treatment assignment (low-dose aspirin vs. placebo). Weighted log-binomial regression was used to estimate RRs and 95% CIs. hCG = human chorionic gonadotropin; RR = risk ratio; CI = confidence interval.

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associated with endometrial receptivity (25) and induces oxidative stress and apoptosis in the developing blastocyst (26). However, despite glucose metabolism being vital in early reproduction by promoting decidualization in preparation for implantation (27), these collective findings do not support a link between normal glycemic status and miscarriage risk. Still, the detrimental impact of hyperglycemia on the risk of pregnancy loss in pregnant women with diabetes has been previously reported, especially in poorly controlled disease (2, 28). Therefore, predisposing mechanisms other than glycemia itself, such as insulin resistance and inflammation, may be more valuable for identifying risk factors for early pregnancy loss.

This study had several strengths. It is the first to assess preconception A1c levels and time to pregnancy and pregnancy losses in women without preexisting diabetes. A1c cut-points were originally established based on the risk of developing medical complications (i.e., diabetic retinopathy), which may not necessarily apply to reproductive outcomes, and this study provides important data on fecundability and pregnancy loss within what is generally considered the 'normal' range. Additional analysis of fasting glucose and insulin data further supplemented our A1c findings. While the sample size was limited for fasting measurements, outcomes were consistent. Data were collected prospectively, and participants were observed before conception until delivery, allowing outcomes to include information on fecundability, early pregnancy loss (including losses unknown to the women in real-time), and overall live birth rate. Another strength of the study was the systematic identification of early pregnancy and, therefore, accurate detection of hCG pregnancies and clinical pregnancy losses. This contrasts with previous studies that evaluated A1c levels in pregnant women without assessing outcomes in early pregnancy (29–36). Results are also generalizable to a larger population of healthy women, as enrollment inclusion criteria required women with proven fecundity instead of recruitment of an infertile population. However, the population was predominately White women of higher socioeconomic class without a diagnosis of PCOS, known diabetes, and/or glucose-related conditions, including hypertension, which somewhat limits generalizability. We were also not able to identify conditions that alter erythrocyte survival, which may falsely elevate or lower A1c concentrations (37). For example, iron-deficiency anemia disease, which is relatively common in reproductive-age women, can inappropriately increase A1c levels. Other less common conditions, such as hemoglobinopathies, may also interfere with test accuracy (38). In addition, racial differences in A1c levels have been noted, although variations are small and unlikely to be clinically significant (14).

## CONCLUSION

We found no relationship between preconception A1c in women without known metabolic disease and fecundability, live birth, and pregnancy loss. The association between A1c concentration and fecundability was influenced by BMI, a strong risk factor for both diabetes and infertility. Our data support guidelines set forth by the ACOG and the ASRM to reserve evaluation of A1c during preconception planning only for women who are diabetic or who meet criteria for diabetes screening based on clinical risk factors such as elevated BMI. Identifying informative targets to predict risk for impaired fecundity and early pregnancy loss remains a pressing need.

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