Hemoglobin A1c values are affected by hemoglobin level and gender in non-anemic Koreans

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ABSTRACT
Aims/Introduction: To evaluate whether hemoglobin A1c (HbA1c) levels are affected by hemoglobin level and gender.

Materials and Methods: A cross-sectional analysis was carried out in a sample of 87,284 non-diabetic Koreans without anemia who participated in comprehensive health check-ups between January and December 2009 at the Kangbuk Samsung Hospital Total Healthcare Center in Seoul, Korea. We categorized men and women separately according to fasting plasma glucose and hemoglobin level to carry out the analysis.

Results: HbA1c increased steadily with increasing fasting plasma glucose level. Both men and women with lower hemoglobin had significantly higher HbA1c at a given fasting glucose level, and this result was consistent across the fasting glucose quintiles within the non-diabetic range. Women had a lower mean hemoglobin value compared with men, and women had higher HbA1c levels at a given fasting glucose level consistently across the fasting glucose deciles. There was also a gender-specific association between age and HbA1c (P < 0.001 for interaction).

Conclusions: HbA1c values were affected by hemoglobin level and gender in non-anemic Koreans. Thus, hemoglobin level and gender should be considered in the diagnosis of diabetes using HbA1c.

INTRODUCTION
Hemoglobin A1 (HbA1c) is the gold-standard measure for the assessment of glycemic control, and has recently been recommended for use in the diagnosis of diabetes. Because of the long lifespan of erythrocytes, HbA1c levels reflect average glycemia over a long-term period of time (2–3 months). However, HbA1c measurements have several limitations, and interpretation of HbA1c levels can be problematic.

Plasma glucose molecules can attach to the hemoglobin in erythrocytes through non-enzymatic glycation, which results in a small hemoglobin variant of HbA1c. Any conditions that affect hemoglobin features, erythrocyte turnover and hemoglobin glycation could influence HbA1c values independent of glycemia. Thus, the relationship between mean glycemia and the HbA1c value might not be the same in all people. The HbA1c result is calculated as the ratio of glycated hemoglobin to total hemoglobin, suggesting that the hemoglobin level could affect HbA1c test results independently of glycemia. However, the association between hemoglobin levels and HbA1c results had not been investigated. Here we examined the relationship between HbA1c and hemoglobin levels in non-diabetic and non-anemic Koreans. In addition, we compared the HbA1c level between men and women because of the well-known difference in hemoglobin level between genders.
MATERIALS AND METHODS
Participants
More than 80,000 people undergo comprehensive health check-ups each year at the Kangbuk Samsung Hospital Total Healthcare Center, Seoul, Korea. Most seek medical check-ups either on their own initiative or because their employers cover the cost of medical check-ups for employees and their families. All anthropometric data, laboratory tests, results of radiological images and coded answers to self-report questionnaires were stored in electronic medical records.

Initial data were obtained from 97,972 participants aged over 20 years-of-age who participated in comprehensive health check-ups between January and December 2009. Among these participants, 10,688 were excluded for the following reasons: (i) anemia with hemoglobin level < 13.5 g/dL in men (n = 999) and < 11.5 g/dL in women (n = 3,370); (ii) above reference range of hemoglobin with > 17.5 g/dL in men (n = 683) and > 16 g/dL in women (n = 23); (iii) self-reported diabetes (n = 3,648), fasting plasma glucose concentration ≥ 126 mg/dL (n = 2,876), or HbA1c ≥ 6.5% (n = 3,339); (iv) chronic kidney disease with creatinine ≥ 1.5 mg/dL (n = 310); and (v) absence of HbA1c data (n = 34). After applying the above exclusion criteria, the total number of participants eligible for the study was 87,284 (52,360 men and 34,924 women).

Measurements
Blood samples were collected from the antecubital vein after an overnight fast. Fasting blood glucose was measured using Bayer Reagent Packs on an automated chemistry analyzer (Advia 1650™ Autoanalyzer; Bayer Diagnostics, Leverkusen, Germany). The blood glucose concentration was determined using the hexokinase method. HbA1c determination was based on the turbidimetric inhibition immunoassay using a Cobas Integra 800 automatic analyzer (Roche Diagnostics, Rotkreuz, Switzerland) with a reference value range of 4.4–6.4%. HbA1c measurements were standardized to the reference method aligned with the Diabetes Control and Complication Trial (DCCT) and the National Glycohemoglobin Standardization Program (NGSP) standards. The intra-assay coefficient of variation (CV) was 2.3% and interassay CV was 2.4%, both of which are within the NGSP acceptable range. Anthropometric and other biochemical variables were measured as described previously. All of the laboratory tests were carried out at the same laboratory. Hemoglobin was measured using a flow cytometry and semiconductor diode laser systems on a fully-automated hematology analyzer (Sysmex XE-2100; Sysmex Cooperation, Kobe, Japan).

The present study was exempted from the requirement of informed consent by the institutional review board, because researchers only accessed the database for analytical purposes, and personal information was not accessed. The study was approved by the institutional review board at Kangbuk Samsung Hospital.

RESULTS
Clinical characteristics of participants are shown in Table 1. The mean age and body mass index of participants was 52.36 ± 12.3 years and 22.8 ± 3.6 kg/m², respectively. A fasting glucose-stratified comparison of HbA1c between hemoglobin tertiles is shown in Figure 1. HbA1c increased steadily with increasing fasting plasma glucose level. Multiple linear regression analysis for HbA1c also showed a positive association between fasting plasma glucose and HbA1c (β = 0.012, P < 0.001 in both men and women; Table 2). Participants with lower hemoglobin had significantly higher HbA1c at a given fasting glucose level in both men and women, and this result was consistent across the fasting glucose quintiles (Figure 1). Furthermore, HbA1c decreased steadily with increasing hemoglobin level (Figure 2). Multiple linear regression analysis for HbA1c also showed a negative correlation between hemoglobin level and HbA1c value (β = −0.040, P < 0.001 in men; β = −0.053, P < 0.001 in women; Table 2).
Table 1 | Clinical characteristics of study participants by gender

|                  | Male     | Female    | P-value* | Total   |
|------------------|----------|-----------|----------|---------|
| n                | 52,360 (60.2%) | 34,924 (39.8%) |          | 87,284  |
| Age (years)      | 42.1 ± 8.1 | 42.2 ± 9.0 | >0.8     | 42.1 ± 8.5 |
| Fasting glucose (mg/dL) | 95.4 ± 8.9 | 92.1 ± 8.1 | <0.001   | 94.1 ± 8.7 |
| HbA1c (%)        | 5.55 ± 0.26 | 5.54 ± 0.27 | <0.001   | 5.55 ± 0.27 |
| Hb (g/dL)        | 15.5 ± 0.8 | 13.2 ± 0.8 | <0.001   | 14.5 ± 1.37 |
| Body mass index (kg/m²) | 24.4 ± 2.8 | 22.2 ± 3.0 | <0.001   | 23.4 ± 3.1 |
| Systolic BP (mmHg) | 116.1 ± 11.4 | 111.0 ± 12.6 | <0.001   | 114.0 ± 12.1 |
| LDL-C (mg/dL)    | 118.6 ± 27.4 | 106.8 ± 29.0 | <0.001   | 113.9 ± 29.7 |
| HDL-C (mg/dL)    | 51.8 ± 11.0 | 61.8 ± 13.5 | <0.001   | 55.8 ± 13.0 |
| Triglyceride (mg/dL) | 146.6 ± 91.3 | 94.2 ± 54.2 | <0.001   | 125.3 ± 82.2 |
| Total cholesterol (mg/dL) | 1993 ± 330 | 1915 ± 331 | <0.001   | 1961 ± 33.2 |

Data are n (%) or means ± standard deviation. *Estimated by unpaired t-test. BP, blood pressure; Hb, hemoglobin; HbA1c, hemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance; LDL, low density lipoprotein-cholesterol.

Table 2 | Multiple linear regression analyses for hemoglobin A1c

| Independent variable | Men            | Women           | All*          |
|----------------------|----------------|-----------------|---------------|
| Intercept            | 4.948          | 5.049           | 4.937         |
| Age                  | 0.003          | 0.005           | 0.004         |
| Fasting glucose (mg/dL) | 0.012         | 0.012           | 0.012         |
| Hemoglobin (g/dL)    | -0.04          | -0.053          | -0.044        |
| Sex (male/women)     | -              | -               | 0.068         |

*All p < 0.053. *Estimated by unpaired t-test. **Estimated by unpaired t-test. BP, blood pressure; Hb, hemoglobin; HbA1c, hemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance; LDL, low density lipoprotein-cholesterol.

Figure 1 | Glucose-stratified comparison of hemoglobin A1c (HbA1c) between hemoglobin tertiles in (a) men and (b) women. Adjusted for age. *P < 0.001 (Tukey’s post-hoc analysis of two way ANOVA) between all three different combinations of hemoglobin (Hb) groups in women and men, respectively. CI, confidence interval.

Women had a lower mean hemoglobin value compared with men (13.2 g/dL vs 15.5 g/dL; Table 1). When we compared HbA1c between genders for a given fasting glucose level, women had higher HbA1c levels consistently across the fasting glucose deciles (Figure 3a). However, after adjustment for hemoglobin level, the estimated HbA1c was lower in women than in men consistently across fasting glucose deciles (Figure 3b). HbA1c level was also analyzed by age and gender (Figure 3c). We found that estimated HbA1c increased with age in both men and women after adjustment for hemoglobin and fasting glucose. We also detected a gender-specific association between age and HbA1c (P < 0.001 for interaction), and the gap in HbA1c between genders narrowed in age groups older than 50–54 years.

DISCUSSION
We found that HbA1c values are affected by hemoglobin levels. The HbA1c value at a given fasting plasma glucose level differed according to hemoglobin level in both men and women. Participants with lower hemoglobin had higher HbA1c at the same fasting glucose level, and this finding was consistent across the non-diabetic range of fasting plasma glucose. The HbA1c level increased by approximately 0.1%, coinciding with
a decrease of 2 g/dL in the hemoglobin level at the same fasting glucose level in participants without anemia. The present results suggest that within the non-diabetic glycemic range, hemoglobin level is associated with HbA1c value independent of glycemia.

In the present study, women had higher values of HbA1c compared with men at similar fasting blood glucose levels. Mean red blood cell mass and hemoglobin levels are known to be lower in women than in men7, although the reason for this difference has never been clearly explained. Likewise, we found that the mean level of hemoglobin was 2.3 g/dL lower in women than in men. This inverse relationship between HbA1c and hemoglobin level is also found in other populations. In addition, ethnic differences in HbA1c have been reported for many years, showing that HbA1c levels in African–American samples are higher than in individuals of European descent at similar blood glucose levels5,9–11. Although most studies suggested that there could be population and ethnic differences in red blood cell turnover and intra- or extracellular environment, as well as genetic variation in hemoglobin glycation, the underlying reasons for ethnic differences in HbA1c are not fully

Figure 2 | Relationship between hemoglobin A1c (HbA1c) and hemoglobin deciles. (a) Adjusted for age, sex and fasting glucose. (b,c) Adjusted for age and fasting glucose. CI, confidence interval.

Figure 3 | Fasting glucose-stratified comparison of hemoglobin A1c (HbA1c) between genders. (a) Adjusted for age. (b) Adjusted for age and hemoglobin. CI, confidence interval.
Anemia is a well-known confounder that influences HbA1c values. Hemolytic anemia decreases HbA1c levels because of increased production of younger red blood cells, which contain hemoglobin with less exposure to ambient glycemia. Conversely, aplastic anemia, which increases the average age of circulating erythrocytes, leads to an increase in the concentration of HbA1c independent of glycemia. In the present study, participants with anemia were excluded and hemoglobin levels were within the normal range in all participants. Thus, the observed differences in hemoglobin were not as a result of anemia.

A limitation of the present study was that we had only fasting glucose data. Fasting plasma glucose does not reflect mean glucose. Not all individuals with the same or similar level of fasting plasma glucose are at the same glycemic status. Thus, further studies should include other measurements, such as a meal tolerance test or continuous glucose monitoring. Nevertheless, the large sample size and consistent results of the present study strengthen our findings.

Individuals with lower hemoglobin without anemia had higher HbA1c at the same fasting glucose level within the non-diabetic range. Women had higher values of HbA1c compared with men at similar fasting glucose levels, and this difference in HbA1c is thought to be attributed to lower hemoglobin levels in women. However, after adjustment for hemoglobin level, HbA1c remained consistently lower in women compared with men. This finding showed not only that hemoglobin level might affect HbA1c values independently of glycemia, but also that gender itself might affect HbA1c independently of hemoglobin levels.

In conclusion, HbA1c values are affected by both hemoglobin level and gender in non-anemic Koreans. We suggest that hemoglobin level and gender should be considered in the diagnosis of diabetes using HbA1c.

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