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ABSTRACT

Aims/Introduction: We examined the association between hemoglobin A1c (HbA1c) and anemia, which was categorized into three groups according to mean corpuscular volume (MCV), as well as the association between hemoglobin in the non-anemic range and HbA1c.

Materials and Methods: We used the 2016 health checkup data from 36,422 workers without diabetes. Anemic people were divided into three groups based on MCV: <80, 80–90 and >90 fL. Non-anemic people were divided into four groups based on their hemoglobin levels. We carried out multiple linear regression models to estimate the means and 95% confidence intervals (CIs) of HbA1c.

Results: For men, 0.2% had anemia with MCV <80 fL, 0.5% had anemia with MCV 80–90 fL, 0.9% had anemia with MCV >90 fL and 98.4% had no anemia. For women, the corresponding values were 6.1, 6.4, 2.8 and 84.7%, respectively. The adjusted mean HbA1c (%) values for men with anemia with MCV <80, 80–90 and >90 fL were 5.67 (95% CI 5.60–5.74), 5.58 (95% CI 5.54–5.62) and 5.41 (95% CI 5.37–5.44), respectively. Among men without anemia, HbA1c (%) increased from 5.36 (95% CI 5.34–5.39) in those with hemoglobin ≥17.5 mg/dL to 5.45 (95% CI 5.45–5.46) in those with hemoglobin 13.0 to <14.5 mg/dL (*P* for trend <0.001). The HbA1c values were higher in men with anemia with MCV <80 fL or MCV 80–90 fL, but lower in men with MCV >90 fL, compared with non-anemic men with hemoglobin 13.0 to <14.5 mg/dL (All *P* < 0.001). Similar findings were observed in women.

Conclusions: We observed elevated HbA1c among anemic people with MCV <80 fL or MCV 80–90 fL, and decreased HbA1c among anemic people with MCV >90 fL, suggesting that different types of anemia might influence HbA1c differently. In addition, non-anemic people with lower hemoglobin levels had higher HbA1c levels, suggesting that hemoglobin levels are in need of consideration when interpreting HbA1c values among non-anemic people.

INTRODUCTION

Hemoglobin A1c (HbA1c) is a glycated hemoglobin formed by the non-enzymatic attachment of glucose to the N-terminal valine of the β-chain of hemoglobin[1]. HbA1c reflects the average blood glucose levels in the previous 2–3 months[2], and is a more convenient measurement than fasting plasma glucose, as it does not require fasting. Thus, HbA1c has been increasingly used for diagnosing diabetes and monitoring blood glucose levels in patients with diabetes[2–4].

Anemia can lead to falsely increased or decreased HbA1c levels, depending on the type of anemia[5]. A systematic review of 12 studies among people without known diabetes reported that iron deficiency anemia (IDA) was associated with a spurious increase in HbA1c[6]. Some types of non-iron deficiency anemia (non-IDA; e.g., hemolytic anemia) have been reported to falsely lower HbA1c levels[5]. However, just three studies assessed HbA1c in association with non-IDA[7–9]; thus, the impact of non-IDA on HbA1c remains elusive[6]. In addition, most studies included in the review had a small sample size (10 studies had a sample size of 50–423) and did not adjust for potential confounders (seven studies). In addition to research on anemia[6], several studies have examined the association between hemoglobin in the non-anemic range and HbA1c among non-diabetic people[7,10–13]. Although some studies have reported that HbA1c values increased with decreasing hemoglobin levels[10–12], others reported a positive association[7] or no association[13] between hemoglobin and HbA1c. The conflicting findings make it impossible to draw any conclusion about the influence of hemoglobin in the non-anemic range on HbA1c.

To fill the evidence gap, we carried out a cross-sectional analysis of data from a large working population in Japan. We examined the association of HbA1c with anemia, which was divided into three groups according to MCV. In addition, we tested whether hemoglobin in the non-anemic range is negatively associated with HbA1c.

METHODS

Study design

The Japan Epidemiology Collaboration on Occupational Health (J-ECOH) Study is an ongoing multicenter, health checkup-based cohort study among workers from several companies in Japan and has been described elsewhere[14,15]. Briefly, participants in the J-ECOH Study underwent routine physical and laboratory examinations each year. Additionally, a questionnaire that covered medical history, health-related lifestyle factors and work environment was completed. Annual health checkup data between January 2008 and December 2016 or between April 2008 and March 2017 were collected from >100,000 employees of 11 participating companies. The study protocol, including the consent procedure, was approved by the ethics committee of the National Center for Global Health and Medicine, Japan.

In the present study, the 2016 health checkup data (between January 2016 and December 2016 or between April 2016 and March 2017) were used to assess the association of anemia and hemoglobin in the non-anemic range with HbA1c.

Study participants

Of the 73,198 people aged ≥20 years who attended the 2016 health checkup, we excluded people with diabetes (n = 5,352, fasting glucose ≥126 mg/dL, HbA1c ≥6.5% or under treatment for diabetes). We further excluded people who had missing data on HbA1c (n = 2,257), blood glucose (n = 5) or medical treatment of diabetes (n = 146), and who had blood drawn while they were in a non-fasted state (n = 7,900). People with missing data on hemoglobin (n = 8,093) or MCV (n = 17,561) were also excluded. Some people met two or more of the exclusion criteria. Participants with a self-reported history of kidney disease (n = 798) or cancer (n = 438) were further excluded to eliminate the influence of these diseases on hemoglobin. We excluded those with missing data on potential confounders: body mass index (BMI; n = 6) and smoking status (n = 220). Finally, 36,422 participants (31,003 men and 5,419 women) remained for analysis.

Health checkup

Body height and weight were measured using a scale while the participants wore light clothes and no shoes. BMI was calculated as weight in kilograms divided by the square of height in meters. Smoking status was identified by a self-administered questionnaire. Plasma glucose was measured with either the enzymatic or glucose oxidase peroxidative electrode method. HbA1c was measured using a latex agglutination immunoassay, high-performance liquid chromatography or the enzymatic method. All of the laboratories involved in the health checkups of the participating companies received satisfactory scores (rank A or a score >95/100) from external quality control agencies.

Anemia

Anemia was defined as a hemoglobin level <13 g/dL for men and <12 g/dL for women according to the World Health Organization criteria[16]. We divided people with anemia into three groups based on MCV: <80, 80–90 and >90 fL, according to previous studies[17–20]. People without anemia were divided into four groups based on their hemoglobin levels (13.0 to <14.5, 14.5 to <16.0, 16.0 to <17.5 and ≥17.5 g/dL in men; 12.0 to <13.0, 13.0 to <14.0, 14.0 to <15.0 and ≥15.0 g/dL in women).

Statistical analysis

Characteristics of the study participants are described as the means and standard deviations for continuous variables, and percentages for categorical variables. We carried out multiple linear regression models to estimate the means and 95% confidence intervals (CIs) of HbA1c according to three anemia groups (MCV <80, 80–90 and >90 fL) and hemoglobin levels (in the non-anemic range). We first adjusted for age (years) and worksite, and then further adjusted for BMI (kg/m^2) and current smoking status (yes or no)[21,22]. In addition, we adjusted

for fasting plasma glucose (FPG; mg/dL), which has been established as a determining factor of HbA1c[23]. For people without anemia, trend associations were assessed by treating the categories of hemoglobin as ordinal numbers and modeling this variable as a continuous variable.

Alcohol drinking can be a potentially important confounder, as higher alcohol consumption has been associated with lower HbA1c levels[24], and heavy drinking can lead to macrocytic anemias[25]. In the J-ECOH Study, questions on alcohol consumption were markedly different across the participating companies. Thus, we carried out a sensitivity analysis with an additional adjustment for alcohol drinking (non-drinker, light drinkers [<46 g ethanol/day], heavy drinkers [≥46 g ethanol/day]) using data of one major company [$n = 17,770$])[26]. To exclude the impact of heavy drinking on the association between hemoglobin and HbA1c, we further repeated the analysis after excluding heavy drinkers (≥46 g ethanol/day). All

statistical analyses were carried out using SAS version 9.3 (SAS Institute, Cary, NC, USA). A two-sided $P < 0.05$ was considered statistically significant.

RESULTS

For men, 0.2% had anemia with MCV <80 fL, 0.5% had anemia with MCV 80–90 fL, 0.9% had anemia with MCV >90 fL and 98.4% had no anemia. For women, the corresponding values were 6.1, 6.4, 2.8 and 84.7%, respectively. Table 1 shows the characteristics of the study participants. Among people without anemia, those with higher hemoglobin levels were more likely to be current smokers, and had higher mean values of BMI and FPG.

People with anemia versus those without anemia
Figure 1 and Table S1 show that both men and women with anemia with MCV <80 fL or MCV 80–90 fL had higher mean

Table 1 | Characteristics of study participants according to hemoglobin category

	<13.0			Hemoglobin (g/dL)			
	MCV (fL)			13.0 to <14.5	14.5 to <16.0	16.0 to <17.5	≥17.5
	<80	80–90	>90				
Men							
n	51	152	272	6,537	17,440	6,139	412
Age (years)	48.5 ± 11.7	51.5 ± 10.6	53.1 ± 8.8	48.1 ± 10.3	45.5 ± 10.4	44.5 ± 10.5	46.8 ± 9.8
BMI (kg/m²)	22.6 ± 3.4	22.4 ± 3.0	22.2 ± 3.0	22.8 ± 2.9	23.6 ± 3.2	24.8 ± 3.7	26.0 ± 4.2
FPG (mg/dL)	99.3 ± 8.5	98.2 ± 9.4	97.1 ± 10.0	97.2 ± 9.2	97.4 ± 9.5	98.3 ± 10.2	98.6 ± 11.2
HbA1c (%)	5.71 ± 0.33	5.63 ± 0.34	5.44 ± 0.38	5.47 ± 0.30	5.43 ± 0.31	5.42 ± 0.33	5.44 ± 0.36
Current smoker (%)	27.5	13.2	34.6	29.4	34.2	40.3	54.1
Alcohol drinker (%)†							
Non-drinker	41.9	40.4	26.0	28.2	29.0	30.9	22.5
Drinker (<46 g ethanol/day)	51.6	54.6	63.8	62.6	62.4	59.8	62.6
Drinker (≥46 g ethanol/day)	6.5	5.1	10.2	9.2	8.6	9.3	14.8
	<12.0			12.0–<13.0	13.0–<14.0	14.0–<15.0	≥15.0
	MCV (fL)						
	<80	80–90	>90				
Women							
n	330	348	153	1,437	2,019	963	169
Age (years)	43.4 ± 6.6	43.4 ± 6.6	46.3 ± 11.3	45.3 ± 11.1	46.2 ± 11.1	47.4 ± 10.5	48.9 ± 9.5
BMI (kg/m²)	22.2 ± 3.8	21.5 ± 3.4	21.0 ± 3.0	21.6 ± 3.4	21.9 ± 3.6	22.4 ± 3.9	23.6 ± 4.6
FPG (mg/dL)	92.5 ± 7.4	91.2 ± 7.7	90.0 ± 7.4	91.4 ± 8.1	92.1 ± 8.8	93.9 ± 9.6	95.7 ± 10.5
HbA1c (%)	5.57 ± 0.29	5.51 ± 0.31	5.31 ± 0.34	5.42 ± 0.32	5.41 ± 0.33	5.40 ± 0.33	5.41 ± 0.33
Current smoker (%)	13.0	8.9	12.4	9.6	10.0	13.5	22.5
Alcohol drinker (%)†							
Non-drinker	65.2	72.0	67.6	66.5	62.2	66.4	54.2
Drinker (<46 g ethanol/day)	33.8	28.0	29.4	31.9	36.4	32.1	45.8
Drinker (≥46 g ethanol/day)	1.0	0	2.9	1.6	1.4	1.4	0

†Data were available for people in one major company (15,039 men and 2,731 women). BMI, body mass index; FPG, fasting plasma glucose; HbA1c, hemoglobin A1c; MCV, mean corpuscular volume.
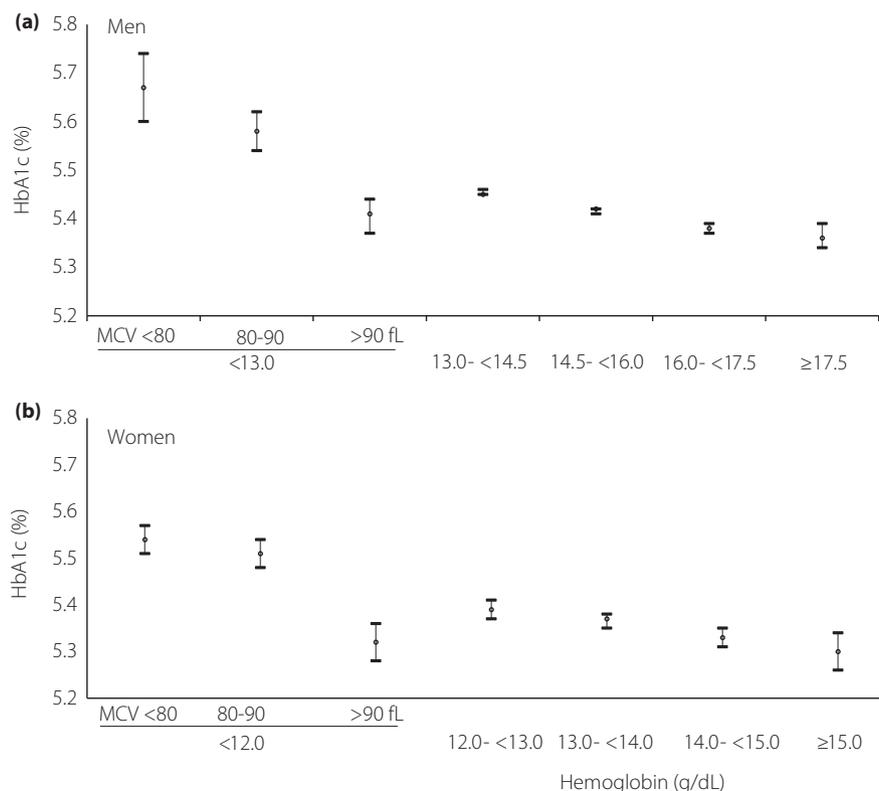
Figure 1 | Adjusted mean (95% confidence interval) of hemoglobin A1c (HbA1c; %) according to hemoglobin category for (a) men and (b) women. Adjusted for age (years), worksite, body mass index (kg/m^2), current smoker (yes or no) and fasting plasma glucose (mg/dL). MCV, mean corpuscular volume.

values of HbA1c than people without anemia after adjusting for age, worksite, BMI, smoking and fasting plasma glucose. The findings did not change after further adjustment for alcohol consumption (Table S2). Overall, men and women with anemia with MCV >90 fL had lower adjusted HbA1c values than people without anemia. The absolute difference in HbA1c (%) between men with anemia with MCV >90 fL and those with pre-anemia (hemoglobin 13.0 to <14.5 g/dL) was −0.05 (95% CI −0.08, −0.02). For women, the corresponding difference was −0.07 (95% CI −0.02, −0.11). People with anemia with MCV >90 fL had lower HbA1c values than people with anemia with MCV <80 fL or MCV 80–90 fL. The absolute difference in HbA1c (%) between men with anemia with MCV >90 fL and those with anemia with MCV <80 fL or MCV 80–90 fL was −0.27 (95% CI −0.34, −0.19) and −0.18 (95% CI −0.23, −0.12), respectively. For women, the corresponding values were −0.22 (95% CI −0.27, −0.17) and −0.19 (95% CI −0.23, −0.14), respectively.

Hemoglobin and HbA1c among people without anemia
Among people without anemia, a higher HbA1c level was associated with lower hemoglobin levels (Figure 1). After adjusting for age, worksite, BMI, smoking status and FPG (Table S1), the values of HbA1c (%) increased from 5.36 (5.34–5.39) in men with hemoglobin ≥17.5 mg/dL to 5.45 (95% CI 5.45–5.46) in

those with hemoglobin 13.0 to <14.5 mg/dL (*P* for trend <0.001). For women, the adjusted values of HbA1c (%) increased from 5.30 (95% CI 5.26–5.34) in those with hemoglobin ≥15.0 mg/dL to 5.39 (95% CI 5.37–5.41) in those with hemoglobin 12.0 to <13.0 mg/dL (*P* for trend <0.001).

Sensitivity analysis
There is no material difference in the prevalence of anemia among male non-drinkers, light drinkers and heavy drinkers, with the prevalence rate being 1.9, 1.6 and 1.5%, respectively. For women, the corresponding values are 17.5, 15.2 and 10.8%, respectively. The associations of HbA1c with anemia and hemoglobin in the non-anemic range in the sensitivity analysis are similar with that of the main analysis (Table S2).

DISCUSSION
In the present study, among a large working population in Japan, we found that both men and women with anemia with MCV <80 fL or MCV 80–90 fL had higher HbA1c levels than people without anemia, especially for those with MCV <80 fL. However, both men and women with anemia with MCV >90 fL had lower HbA1c than people without anemia. We observed an inverse association between HbA1c and hemoglobin in men and women without anemia. To our knowledge,

this is the first study to simultaneously investigate the association of HbA1c with anemia and hemoglobin in the non-anemic range in Japan.

In the J-ECOH Study, serum ferritin was not available, because it is not a mandatary item in the annual health checkup. However, as part of the J-ECOH Study, a nutritional epidemiological survey, named the Furukawa Nutrition and Health Study[27], was carried out in one of the companies participating in the J-ECOH Study, and additional blood tests, such as serum ferritin, were carried out. We extracted the 2012–2013 Furukawa Nutrition and Health Study survey data, in which serum ferritin was measured. Among 1,977 workers who had data on serum ferritin, 36 people had anemia. It was found that all eight anemic people with MCV <80 fL had serum ferritin <15 µg/L (IDA)[28], most anemic people with MCV 80–90 fL had IDA (13/16) and most anemic people with MCV > 90 fL had non-IDA (8/12). IDA and the thalassemia trait are the commonest causes of microcytic anemia (MCV <80 fL)[29]. However, it was reported that the thalassemia trait is rare (<1/1,000) in the Japanese general population[30]. Thus, in the present study, anemic people with MCV <80 fL might consist mainly of people with IDA. Anemia with MCV 80–90 fL represents mostly IDA, and anemia with MCV >90 fL represents mostly non-IDA.

The present finding of a higher HbA1c associated with anemia with MCV <80 fL (mainly IDA) or MCV 80–90 fL (mostly IDA) was generally in line with the findings of the previous systematic review, which showed that IDA spuriously increased HbA1c[6]. After the publication of the systematic review, one large study of 11,472 Korean adults also showed that people with IDA had higher HbA1c values than people without anemia (5.7 vs 5.6%)[31]. The present study is based on data from a large working population ($n = 36, 422$), controlled for potential confounders, such as smoking, BMI and alcohol consumption, and further adjusted for FPG, which is a glycemic determinant of HbA1c. Findings from the present study and previous studies[6,31] provide evidence that people with IDA had higher HbA1c values.

The exact mechanism through which IDA falsely increases HbA1c levels remains unclear. One proposed explanation is that iron deficiency might alter the quaternary structure of the hemoglobin molecule, which might result in more rapid glycation of the β-globin chains and thus lead to higher HbA1c values[32]. Another suggested mechanism is that the rise in HbA1c might be a result of reduced red blood cell production in IDA, leading to a higher than normal mean age of red blood cells (exposing people with IDA to a higher glycation time than people without anemia)[32]. However, questions have been raised regarding this mechanism, as some studies have shown that people with IDA have normal or even shortened red blood cell lifespans[33,34]. Because limited work has been carried out on the mechanism of increased HbA1c in IDA[32], further studies to confirm and elucidate the effect of IDA on HbA1c are required.

We found that anemic people with MCV >90 fL (mostly non-IDA) had lower HbA1c levels than people without anemia.

Similarly, two studies from Korea and the USA observed lower HbA1c values in people with non-IDA than in non-anemic people (5.44 vs 5.59%, 5.16 vs 5.31%, respectively)[7,31]. It was hypothesized that some subtypes of non-IDA, such as hemolytic anemia, might decrease HbA1c as a result of a shortened red blood cell lifespan (more rapid erythrocyte turnover)[35]. In contrast, non-IDA as a result of vitamin B_{12} or folic acid deficiencies have been associated with higher HbA1c levels[32,36]. Further identification of non-IDA subtypes might help to confirm the findings of the present study and previous studies[7,31]. Given that limited data exist on the effect of non-IDA on HbA1c, more research on this topic is required.

The present finding that hemoglobin in the non-anemic range was inversely associated with HbA1c is consistent with several previous studies[10–12]. Using data from non-anemic people who attended health checkups in the hospital setting, two cross-sectional studies in Japan[10] ($n = 32,605$) and Korea[11] ($n = 87,284$) found that HbA1c (%) increased by 0.03 and 0.04 per 1-g/dL decrease in hemoglobin, respectively. One recent study using data from the National Health and Nutrition Examination Survey (NHANES) 1999–2014 ($n = 22,974$) showed that HbA1c (%) increased by 0.05 when hemoglobin decreased by 1 g/dL[12]. In contrast, an earlier study using the NHANES 1999–2002 data ($n = 8,296$) reported that HbA1c (%) increased by 0.04 when hemoglobin increased by 1 g/dL[7], which is the only study that reported a positive association between HbA1c and hemoglobin. The present study extended the findings of an inverse association between hemoglobin and HbA1c to an apparently healthy working population (HbA1c [%] increased by approximately 0.03 for each 1 g/dL decrease in hemoglobin; Table S1). Taken together, the findings from the present study and previous studies[10–12] suggest that hemoglobin levels are in need of consideration when interpreting HbA1c values among non-anemic people.

The present study used contemporary health checkup data from a large working population, separated people with anemia into three groups (MCV <80 [mainly IDA], 80–90 [mostly IDA] and >90 fL [mostly non-IDA]), and adjusted for potential confounders and FPG. However, several limitations need to be considered. First, because of the lack of data on serum ferritin and other causes of microcytic anemia, such as lead toxicity and hematological disorders, we cannot further confirm the diagnosis of IDA. Second, the relatively small number of people with anemia precluded us from examining the association between the severity of anemia and HbA1c. Third, we did not have data on the duration of anemia, which might affect hemoglobin. Fourth, hemoglobin variants can interfere with HbA1c measurement[37]. However, we do not have data on hemoglobin variants, which makes us unable to exclude its impact on the association of HbA1c with anemia and hemoglobin in the non-anemic range. Finally, because the present study participants were workers in Japan, caution should be exercised in generalizing the present findings to the general population or other racial/ethnic groups.

In conclusion, we observed elevated HbA1c levels among anemic people with MCV <80 fL (mainly IDA) or MCV 80–90 fL (mostly IDA) and decreased HbA1c levels among anemic people with MCV >90 fL (mostly non-IDA), suggesting that IDA and non-IDA might influence HbA1c differently. Caution should be exercised when assessing people with anemia when their HbA1c levels are near 5.7 or 6.5%, which are cut-off values for prediabetes and diabetes, respectively. In addition, nonanemic workers with lower hemoglobin levels had higher levels of HbA1c. For a more accurate interpretation of HbA1c among nonanemic people, hemoglobin levels should be taken into consideration in the future.

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DISCLOSURE

The authors declare no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1 | Adjusted mean (95% confidence interval) of hemoglobin A1c (%) according to hemoglobin category.

Table S2 | Adjusted mean (95% confidence interval) of hemoglobin A1c (%) according to hemoglobin category (sensitivity analysis).