RESEARCH ARTICLE

# Phenotypic and lifestyle determinants of HbA$_{1c}$ in the general population – The Hoorn Study

Willem Wisgerhof[1☯]*, Carolien Ruijgrok[1☯], Nicole R. den Braver[1], Karin J. Borgonjen—van den Berg[2], Amber A. W. A. van der Heijden[3], Petra J. M. Elders[3], Joline W. J. Beulens[1,4], Marjan Alssema[1,5]

1 Department of Epidemiology and Biostatistics, VU University Medical Center, Amsterdam Public Health Research Institute, Amsterdam, the Netherlands, 2 Department Agrotechnology and Food Sciences, Division of Human Nutrition, Wageningen University, Wageningen, the Netherlands, 3 Department of General Practice and Elderly Care Medicine, Amsterdam University Medical Centers, Amsterdam Public Health Research Institute, Amsterdam, the Netherlands, 4 Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht, the Netherlands, 5 Health Council of the Netherlands, The Hague, the Netherlands

☯ These authors contributed equally to this work.
* willemwisgerhof@gmail.com

## Abstract

### Aim

To investigate the relative contribution of phenotypic and lifestyle factors to HbA$_{1c}$, independent of fasting plasma glucose (FPG) and 2h post-load glucose (2hPG), in the general population.

### Methods

The study populations included 2309 participants without known diabetes from the first wave of the Hoorn Study (1989) and 2619 from the second wave (2006). Multivariate linear regression models were used to analyze the relationship between potential determinants and HbA$_{1c}$ in addition to FPG and 2hPG. The multivariate model was derived in the first wave of the Hoorn Study, and replicated in the second wave.

### Results

In both cohorts, independent of FPG and 2hPG, higher age, female sex, larger waist circumference, and smoking were associated with a higher HbA$_{1c}$ level. Larger hip circumference, higher BMI, higher alcohol consumption and vitamin C intake were associated with a lower HbA$_{1c}$ level. FPG and 2hPG together explained 41.0% (first wave) and 53.0% (second wave) of the total variance in HbA$_{1c}$. The combination of phenotypic and lifestyle determinants additionally explained 5.7% (first wave) and 3.9% (second wave).

## Conclusions

This study suggests that, independent of glucose, phenotypic and lifestyle factors are associated with HbA$_{1c}$, but the contribution is relatively small. These findings contribute to a better understanding of the low correlation between glucose levels and HbA$_{1c}$ in the general population.

## Introduction

Glycated haemoglobin (HbA$_{1c}$) is widely adopted as diagnostic marker for type 2 diabetes and reflects average plasma glucose levels over the prior two to three months [1, 2]. The use of HbA$_{1c}$ for diagnosis has several advantages over measurements of fasting plasma glucose (FPG) and 2h post-load plasma glucose (2hPG), which include lower intra-individual variation and more convenience in blood sampling conditions [3]. HbA$_{1c}$ has also been shown to be a more accurate marker than measures of glucose for future risk of diabetes complications, such as micro vascular complications and cardiovascular disease (CVD), in individuals with type 2 diabetes [4]. In addition, higher HbA$_{1c}$ is associated with higher risk for CVD and total mortality [5, 6]. This increased CVD and mortality risk with higher HbA$_{1c}$ is already seen at levels in the normal range [5, 6].

Nonetheless, controversy exists regarding the use of HbA$_{1c}$ as a diagnostic marker for type 2 diabetes in the general population, since several population-based studies have shown that correlations between glucose and HbA$_{1c}$ are relatively poor [7]. Furthermore, several studies have suggested that HbA$_{1c}$ levels are affected by other factors than glucose alone [8–10]. Multiple studies found that HbA$_{1c}$ might not be a reliable reflection of glycemic state in individuals with iron or vitamin B12 deficiency, renal or liver failure, short erythrocyte lifespan, rheumatoid arthritis, alcoholism, and in individuals who use aspirin, vitamin -C, -E supplements or antiretrovirals [11, 12]. To better understand the effects of such conditions on HbA$_{1c}$, a more comprehensive understanding of determinants of HbA$_{1c}$ is needed.

A small number of studies revealed that, independent of glucose, non-Hispanic black race, smoking, several genetic risk factors and higher age and BMI are associated with a higher HbA$_{1c}$ level, whereas higher alcohol consumption, haemoglobin mass and concentration are associated with a lower HbA$_{1c}$ level [13–16]. Another previous study showed that in individuals without diabetes, 2hPG explains only one quarter to one third of the variance in HbA$_{1c}$ [9]. Furthermore, researchers of the Lifeline Cohort Study recently found that a combination of clinical, lifestyle and genetic factors, in addition to FPG, explained 26.2% of the variance in HbA$_{1c}$, with FPG contributing 10.9% [13]. Exploring determinants in addition to both FPG and 2hPG provides better insight into the added value of other determinants, since the combination of FPG and 2hPG is a better reflection of glycemic state and diurnal glucose [17].

A better understanding of the variance in HbA$_{1c}$ is relevant for the interpretation of HbA$_{1c}$ levels in the general population. The present study therefore aims to investigate the relative contribution of previously identified determinants to HbA$_{1c}$, independent of FPG and 2hPG, in the general population.

## Subjects, materials and methods

### Study population

The current study was performed using baseline data from the Hoorn Study, a population based cohort study conducted among men and women from the general population residing

in the city of Hoorn, the Netherlands [18]. For the first wave of the Hoorn Study a total of 3553 participants aged between 49 and 75 years were invited and 2540 agreed to participate in the study, of which 56 non-Caucasians were excluded. In the present study, we excluded 90 participants with known diabetes based on GP diagnosis and/or use of glucose-lowering medication at baseline and 15 participants with missing data on diabetes status. Furthermore, we excluded 69 participants without data on dietary intake variables and one participant with an extreme value for total energy ($<500$ or $>5000$ kcal/day), leaving 2309 participants for analysis. Baseline data were collected from October 1989 until February 1992.

The second wave was conducted July 2006 until November 2007 and a total of 6180 men and women aged 40–65 years were invited, of which 2807 agreed to participate in the study [18]. In the present study, we excluded 82 participants with known diabetes based on GP diagnosis and/or use of glucose-lowering medication at baseline and 54 participants with missing data on diabetes status. Furthermore, we excluded 43 participants without data on dietary intake variables and nine participants with extreme values for total energy ($<500$ or $>5000$ kcal/day), leaving 2619 participants for analysis. Informed consent was signed by all participants and both studies were approved by the Ethical Review Committee of the VU University medical center (VUmc).

## Baseline measurements

In the first wave, anthropometric measurements were taken at the first visit with participants being barefoot and wearing light clothes [18]. Data on dietary intake and alcohol consumption were obtained from a 75-item self-reported semi quantitative food frequency questionnaire (FFQ) [19]. Energy and nutrient intakes were estimated from FFQ through linkage with a computerized version of the Dutch Food Composition Table [20]. The FFQ was validated against a dietary history for total energy intake, all macronutrients, dietary fiber, alcohol, iron, vitamins B1, B6 and vitamin C. Pearson correlation coefficients were on average 0.71 (range: 0.65–0.78) for macronutrients, and 0.66 (range: 0.36–0.81) for vitamins and minerals [19]. Cigarette smoking status was self-reported.

In the second wave, the same methods as in the first wave were used for anthropometric measurements and cigarette smoking status [18]. Data on dietary intake and alcohol consumption were obtained from a 125-item self-reported quantitative FFQ. The Dutch Food Composition Table of 2006 was used to calculate energy and nutrient intake per day [21]. This FFQ was validated for total energy intake against actual energy intake needed to maintain stable body weights during 11 controlled dietary trials, with a Pearson correlation coefficient of 0.82 (95% CI, 0.80, 0.85) [22].

## Laboratory assays

In the first wave, an oral glucose tolerance test (OGTT) was performed after an overnight fast and blood samples were collected before and 2 hours after intake of the glucose load [18]. FPG and 2hPG levels were determined with the glucose dehydrogenase method (Merck, Darmstadt, Germany). HbA<sub>1c</sub> was assessed with an ion exchange high performance liquid chromatography (Modular Diabetes Monitoring System, BioRad Lab, Veenendaal, The Netherlands: normal range 4.3–6.1%). Alanine transaminase enzyme activity was measured in plasma as a marker of liver function [23]. This measurement was conducted according to the method of the International Federation of Clinical Chemistry from 1985 and expressed as IU/L. Serum creatinine was measured as indicator of renal function [24].

In the second wave, the same methods were used for the OGTT [18]. FPG and 2hPG levels were measured with the glucose oxidase method (Boehringer-Mannheim, Mannheim,

Germany). HbA$_{1c}$ was assessed using the Diabetes Control and Complications Trial (DCCT) standardized reverse-phase cation exchange chromatography (Menarini, Florence, Italy), for which the intra-assay coefficient of variation was 0.65% at a mean of 4.89%, and the interassay coefficient of variation was 1.55% at a mean of 5.52% [25]. In both studies, all analyses were performed at the clinical chemistry laboratory of the VUmc.

### Statistical analysis

Characteristics of the study populations are presented as mean ± SD for normally distributed continuous variables or median (25$^{th}$– 75$^{th}$ percentile) for positively skewed distributions. Categorical variables are presented as proportions. Missing data (0.1% of all observations in the first wave and 0.2% in the second wave) were imputed using multiple imputation, creating five imputed datasets. The Multivariate Imputation by Chained Equations (MICE) algorithm was used with the predictive mean matching method [26]. Results of the analyses were pooled using Rubin's rules [27].

Correlations between FPG, 2hPG and HbA$_{1c}$ were determined using Spearman correlations. Linear and quadratic associations of the determinants with HbA$_{1c}$ were added in the linear regression model to test for non-linear associations. Determinants for which the quadratic term was significant ($P < 0.05$) were divided into quartiles. Dietary intake variables were corrected for total energy intake by the residual method [28].

Using data from the first wave, a multivariate linear regression model with outcome HbA$_{1c}$ was created including FPG and 2hPG as fixed determinants, and age, sex, BMI, waist and hip circumference, heamaglobin, serum creatinine, serum alanine transaminase, smoking, alcohol consumption, carbohydrate intake, fiber intake, iron intake, vitamin b1 and b6 intake, vitamin C intake as potential determinants. Determinants in the multivariate model were included based on a stepwise backward selection procedure with $P < 0.10$ considered statistically significant. Continuous determinants were standardized into a Z-score. The set of significant predictors in the multivariate model constructed within the first wave was replicated as a multivariate model in the data of the second wave. Variables were considered as determinants of HbA$_{1c}$ if there was a significant association in the multivariable model in both cohorts. Effect-modification was investigated by including interaction terms between sex and other independent variables in the linear regression models. Interaction terms were considered statistically significant with $P < 0.10$. The explained variance of the models was estimated by the median R$^2$ of the imputed datasets [29]. All analyses were performed using IBM SPSS Statistics version 22 for Windows (SPSS, Chicago, IL, USA).

## Results

Table 1 presents the population characteristics of the first (1989) and the second (2006) wave of the Hoorn Study. At the time of examination, participants from the first wave were on average 7.5 years older (61.4 ± 7.3) than those of the second wave (53.9 ± 6.7). Furthermore, the proportion of current smokers was substantially greater in the first (31.5%) than in the second wave (21.0%), whereas the median alcohol consumption in the second wave (8.2 g/day) was approximately two times higher than in the first wave (4.3 g/day). The Spearman correlations (Table 1) of FPG with HbA$_{1c}$ were stronger in both waves of the Hoorn Study (first: $r = 0.32$; second: $r = 0.42$) than the correlations of 2hPG with HbA$_{1c}$ (first: $r = 0.22$; second: $r = 0.31$). Of all participants who were diagnosed with diabetes according to at least one of the screening tools (FPG $\geq$ 7.0 mmol/l or 2hPG $\geq$ 11.1 mmol/l or HbA$_{1c}$ $\geq$ 6.5% (48 mmol/mol)), 23.8% (first) and 12.1% (second) were diagnosed with diabetes according to all three screening tools (Fig 1).

**Table 1. Population characteristics and Spearman correlations of the participants of the Hoorn Study.**

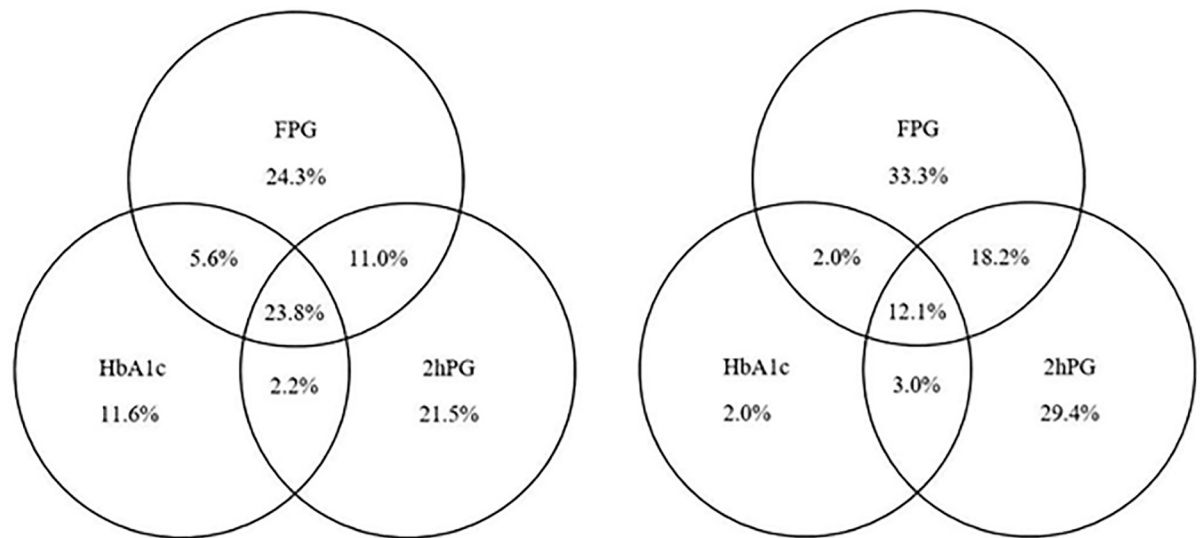|  | First wave | Second wave |
|---|---|---|
| *N* | 2309 | 2619 |
| Age (years) | 61.4 ± 7.3 | 53.9 ± 6.7 |
| Female sex (%) | 54.2 | 54.0 |
| BMI (kg/m$^2$) | 26.5 ± 3.5 | 26.1 ± 3.9 |
| Waist circumference (cm) | 90.6 ± 10.7 | 89.4 ± 11.4 |
| Hip circumference (cm) | 101.6 ± 6.7 | 100.3 ± 7.9 |
| Fasting plasma glucose (mmol/l) | 5.6 ± 1.0 | 5.5 ± 0.8 |
| 2h post-load glucose (mmol/l) | 6.1 ± 2.8 | 5.7 ± 2.0 |
| HbA$_{1c}$ (mmol/mol) | 36.0 ± 7.7 | 36.0 ± 4.4 |
| HbA$_{1c}$ (%) | 5.4 ± 0.7 | 5.4 ± 0.4 |
| Haemoglobin (mmol/l) | 9.0 ± 0.8 | N/A |
| Serum alanine transaminase (IU/l) | 11.0 (8.0–15.0) | N/A |
| Serum creatinine (μmol/l) | 90.1 ± 15.6 | N/A |
| Current smoker (%) | 31.5 | 21.0 |
| Total energy intake (kcal/day) | 2067.6 ± 581.5 | 2128.4 ± 665.0 |
| Alcohol consumption (g/day) | 4.3 (0.0–12.5) | 8.2 (2.1–19.6) |
| Carbohydrate intake (g/day) | 213.9 ± 65.8 | 243.6 ± 80.5 |
| Fiber intake (g/day) | 27.2 ± 7.9 | N/A |
| Iron intake (mg/day) | 12.5 ± 3.1 | 11.0 ± 3.0 |
| Vitamin B1 intake (mg/day) | 1.1 ± 0.3 | 1.2 ± 0.4 |
| Vitamin B6 intake (mg/day) | 1.4 ± 0.4 | 1.7 ± 0.5 |
| Vitamin C intake (mg/day) | 95.3 (67.2–134.1) | 92.0 (67.1–119.7) |
| FPG vs. HbA$_{1c}$[a] | 0.32[b] | 0.42[b] |
| 2hPG vs. HbA$_{1c}$[a] | 0.22[b] | 0.31[b] |
| FPG vs. 2hPG[a] | 0.42[b] | 0.37[b] |

N/A, not available; Values are means ± SD, median (25th–75th percentile) or percentage.

[a]Spearman correlations.

[b]Statistically significant ($P < 0.05$) correlation.

Table 2 shows the results of the multivariate linear regression models for both the first and the second wave (replication analysis). No interactions ($P > 0.10$ for interaction terms) were found between sex and other independent variables in the multivariate model. In the first wave, independent of glucose, significant associations were found for age, sex, BMI, waist circumference, hip circumference, serum creatinine, smoking, alcohol consumption, carbohydrate intake, fiber intake and vitamin C intake in the multivariate model with HbA$_{1c}$. Of these variables, age, sex, BMI, waist circumference, hip circumference, smoking, alcohol consumption and vitamin C intake were confirmed as statistically significant associations in the second wave. In the second wave, one SD increase in age (6.7 years) was associated with a 0.03% (95% CI, 0.03, 0.04) higher HbA$_{1c}$ level. On average females had a 0.08% (0.07, 0.09) higher HbA$_{1c}$ level than men. The quartiles of BMI and hip circumference showed a negative association with HbA$_{1c}$, while waist circumference was positively associated. Smokers had a 0.10% (0.09, 0.11) higher HbA$_{1c}$ level than non-smokers, whereas alcohol consumption was associated with a 0.07% (-0.07, -0.06) lower HbA$_{1c}$ level per one SD increase (15.1 g/day). Quartiles of vitamin C intake were inversely associated with HbA$_{1c}$. The multivariate association found in the first wave for carbohydrate intake was not confirmed in the second wave. Data on creatinine and fiber intake were not available in the second wave.

**Fig 1. Diagnosis of diabetes.** The percentage of participants diagnosed with diabetes in the first wave *(n = 181 out of 2309)* and the second *wave (n = 108 out of 2612)*. Diabetes mellitus was diagnosed when at least one of the following criteria were met: FPG ≥ 7.0 mmol/l; 2hPG ≥ 11.1 mmol/l; HbA$_{1c}$ ≥ 48 mmol/mol (6.5%).

https://doi.org/10.1371/journal.pone.0233769.g001

FPG alone explained 39.5% (first) and 51.2% (second) of the total variance in HbA$_{1c}$. FPG and 2hPG together explained 41.0% and 53.0% of variance in HbA$_{1c}$. Phenotypic and lifestyle determinants in combination with both FPG and 2hPG explained an additional 5.7% in the first wave and 3.9% in the second wave. Models containing phenotypic and lifestyle determinants without glucose explained 11.1% and 10.3% of variance in HbA$_{1c}$.

## Discussion

The current study investigated potential determinants of HbA$_{1c}$, independent of FPG and 2hPG. In two independent cohorts, we found that higher age, female sex, larger waist circumference and smoking were associated with a higher HbA$_{1c}$ level, whereas larger hip circumference, higher BMI, higher alcohol consumption and vitamin C intake were associated with a lower HbA$_{1c}$ level. The key finding of this study was that glycemic variables explained the vast majority of the variation in HbA$_{1c}$, while the contribution of phenotypic and lifestyle determinants was relatively small.

Our findings that age, sex, BMI, waist and hip circumference, smoking, alcohol consumption and vitamin C intake are associated with HbA$_{1c}$ in two independent cohorts is consistent with available evidence. In line with previous studies [13, 30, 31], we observed that age and smoking are associated with HbA$_{1c}$ independent of glucose. The current study reported a higher HbA$_{1c}$ level in women than in men, after correction of glucose levels. Previous studies that did not account for glucose reported higher levels of HbA$_{1c}$ in men, which is assumed to be the result of a longer erythrocyte lifespan in men [16, 32]. Similarly, we observed a higher HbA$_{1c}$ level in men when we did not correct for glucose. In the present study we found independent and opposite associations of waist and hip circumference with HbA$_{1c}$. Larger waist, and smaller hip circumference has previously been shown to be associated with risk for developing type 2 diabetes, independent of BMI [33, 34]. Our current findings showing associations between body composition and HbA$_{1c}$, independent of glucose measures are novel. The same is true for alcohol consumption which has been associated with a lower HbA$_{1c}$ level and improved insulin resistance in previous observational and intervention studies, but not

**Table 2. Multivariate linear regression models of associations of demographic, anthropometric, clinical and nutritional determinants with HbA1c independent of glucose in the Hoorn Study.**

| | | First wave (*n* = 2309) | | | Second wave (*n* = 2619) | | |
| --- | --- | --- | --- | --- | --- | --- | --- |
| | | HbA$_{1c}$ (% of Hb) | | | HbA$_{1c}$ (% of Hb) | | |
| | | Beta | 95% CI | *P*-value | Beta | 95% CI | *P*-value |
| FPG (mmol/l) per SD = 1.0 in first wave / 0.8 in second wave | | 0.353 | 0.299;0.358 | <0.001 | 0.272 | 0.266;0.278 | <0.001 |
| 2hPG (mmol/l) per SD = 2.9 / 2.0 | | 0.109 | 0.079;0.139 | <0.001 | 0.060 | 0.054;0.065 | <0.001 |
| Age per SD = 7.3 / 6.7 | | 0.052 | 0.031;0.073 | <0.001 | 0.031 | 0.027;0.036 | <0.001 |
| Sex F/M (ref) | | 0.096 | 0.036;0.155 | 0.002 | 0.081 | 0.069;0.092 | <0.001 |
| BMI (kg/m$^2$) | Q1 | 0 | | | 0 | | |
| | Q2 | -0.119 | -0.182;-0.057 | <0.001 | -0.015 | -0.029;-0.001 | 0.039 |
| | Q3 | -0.094 | -0.165;-0.022 | 0.011 | -0.016 | -0.033;0.001 | 0.068 |
| | Q4 | -0.135 | -0.228;-0.043 | 0.004 | -0.022 | -0.044;0.001 | 0.061 |
| Waist circumference (cm) per SD = 10.7 / 11.4 | | 0.047 | 0.012;0.081 | 0.009 | 0.015 | 0.006;0.024 | 0.001 |
| Hip circumference (cm) | Q1 | 0 | | | 0 | | |
| | Q2 | -0.058 | -0.117;0.002 | 0.059 | -0.013 | -0.027;0 | 0.057 |
| | Q3 | -0.002 | -0.068;0.065 | 0.953 | -0.029 | -0.045;-0.013 | <0.001 |
| | Q4 | -0.025 | -0.105;0.055 | 0.537 | -0.015 | -0.035;0.005 | 0.131 |
| Serum creatinine (μmol/l) | Q1 | 0 | | | N/A | | |
| | Q2 | 0.023 | -0.033;0.080 | 0.417 | N/A | | |
| | Q3 | 0.055 | -0.007;0.116 | 0.081 | N/A | | |
| | Q4 | 0.092 | 0.024;0.160 | 0.008 | N/A | | |
| Current smoking Y/N (ref) | | 0.236 | 0.191;0.281 | <0.001 | 0.101 | 0.090;0.112 | <0.001 |
| Alcohol consumption[a] per SD = 12.4 / 15.1 | | -0.086 | -0.108;-0.063 | <0.001 | -0.065 | -0.070;-0.059 | <0.001 |
| Carbohydrate intake[a] per SD = 65.8 / 80.5 | | -0.028 | -0.050;-0.005 | 0.018 | 0.000 | -0.005;0.005 | 0.996 |
| Fiber intake[a] per SD = 7.9 / NA | | 0.020 | -0.004;0.044 | 0.096 | N/A | | |
| Vitamin C intake[a] | Q1 | 0 | | | 0 | | |
| | Q2 | -0.057 | -0.114;-0.001 | 0.048 | -0.032 | -0.045;-0.020 | <0.001 |
| | Q3 | -0.075 | -0.134;-0.016 | 0.013 | -0.040 | -0.053;-0.027 | <0.001 |
| | Q4 | -0.113 | -0.175;-0.051 | <0.001 | -0.038 | -0.052;-0.025 | <0.001 |

N/A, not available; Q, Quartile (range); First wave; BMI, Q1 (<24.1) Q2 (24.1;26.1) Q3 (26.2;28.3) Q4 (>28.3); Hip circumference, Q1 (<97.5) Q2 (97.5;101.9) Q3 (102.0;106.7) Q4 (>106.7) Serum creatinine, Q1 (<79.0) Q2 (79.0;88.0) Q3 (88.1;98.0) Q4 (>98.0); Vitamin C, Q1 (< -0.7) Q2 (-0.7;-0.2) Q3 (-0.1;0.6) Q4 (>0.6). Second wave; BMI, Q1(<23.4) Q2 (23.4;25.6) Q3(25.7;28.2) Q4 (>28.2); Hip circumference, Q1 (<95.4) Q2 (95.4;99.8) Q3 (99.9;105.2) Q4 (>105.2); Vitamin C, Q1 (< -0.7) Q2 (-0.7; -0.1) Q3 (0.0; 0.6) Q4 (>0.6); Multivariate linear regression models regarding potential determinants and outcome HbA$_{1c}$, in addition to fasting plasma glucose and 2h post-load glucose.

First wave complete model; R$^2$ 46.7%. FPG + 2hPG; R$^2$ 41.0%. (non-glycemic additional 5.7%) FPG; R$^2$ 39.5%. 2hPG; R$^2$ 29.7%. Phenotypic and lifestyle determinants; R$^2$ 11.1%. Second wave complete model; R$^2$ 56.9%. FPG + 2hPG; R$^2$ 53.0%. (non-glycemic additional 3.9%) FPG; R$^2$ 51.2%. 2hPG; R$^2$ 27.9%. Phenotypic and lifestyle; R$^2$ 10.3%.

[a]Corrected for total energy intake by the residual method.

https://doi.org/10.1371/journal.pone.0233769.t002

particularly with HbA$_{1c}$ independent of glucose [35–37]. The mechanism by which alcohol would improve insulin sensitivity is currently not resolved. Similarly, our finding that vitamin C intake was associated with a lower HbA$_{1c}$ level has been reported in previous observational studies, but not independent of glucose [38, 39]. As suggested by Davie et al. [40], this relationship is possibly not mediated by glucose but caused by a competition between vitamin C and glucose to react with the protein amino group, thereby reducing glycation of haemoglobin.

In both the first (46.7%) and second wave (56.9%) model we found a substantially higher explained variance in HbA$_{1c}$ as compared to a multivariate model created by Jansen et al. (26.2%), which contained some phenotypic, clinical, lifestyle and genetic determinants, in

addition to FPG [13]. This difference is already apparent in a model with FPG alone, which explained 10.9% of the variance in HbA$_{1c}$ in the study by Jansen et al., whereas it explained 39.5% (first) and 51.2% (second) in the current study. One reason for this difference may be the larger variation in HbA$_{1c}$ in our study compared to Jansen et al. The standard deviation of HbA$_{1c}$ was 0.7% and 0.4% in the Hoorn Study, while this was only 0.3% in the study by Jansen et al.

The present study and previous studies indicate that HbA$_{1c}$ may reflect in part aging processes, body composition, smoking and dietary habits. These findings contribute to better understanding of the relatively poor correlations between glucose and HbA$_{1c}$ observed in a previous investigation in the second wave [25] and other population-based studies [7]. In individuals with levels of HbA$_{1c}$ that are higher or lower than expected based on glucose levels, the present data may provide clues for explaining such disagreement. Furthermore, presently identified determinants of HbA$_{1c}$ might explain the stronger relationship with CVD observed in previous studies [4–6]. Future prospective studies are needed to better understand which factors explain or mediate the relationship between HbA1c and CVD.

The main strength of our study is the investigation of a combined set of potential determinants of HbA$_{1c}$, in addition to both FPG and 2hPG, which provides a better reflection of diurnal glucose than FPG alone. Another strength of this study is the selection of potential determinants identified by previous studies, and the replication of the analyses in independent data, both limiting the probability of chance-findings. Our study also has some limitations. Firstly, several potential determinants of HbA$_{1c}$, such as erythrocyte lifespan, vitamin B12 and E, genetic factors, rheumatoid arthritis and antiretrovirals could not be taken into account. Particularly erythrocyte lifespan could be of significant influence on HbA$_{1c}$ levels because the glycation of haemoglobin partially depends on it [11]. Secondly, data on serum creatinine and fiber intake were not available in the second wave. Therefore, the model of the first wave could not be exactly replicated in the second wave.

In summary, the current study shows that, in addition to both FPG and 2hPG, age, sex, BMI, waist and hip circumference, smoking, alcohol consumption and vitamin C intake are associated with HbA$_{1c}$ in the general population. Although the variation in HbA$_{1c}$ explained by these factors is relatively low, it suggests that variation in HbA$_{1c}$ levels apart from glucose may also be partially determined by phenotypic and lifestyle factors. These findings contribute to better understanding of the low correlation between glucose levels and HbA$_{1c}$ in the general population.

## Acknowledgments

## Author Contributions

**Conceptualization:** Willem Wisgerhof, Carolien Ruijgrok, Petra J. M. Elders, Joline W. J. Beulens, Marjan Alssema.

**Data curation:** Nicole R. den Braver, Karin J. Borgonjen—van den Berg, Amber A. W. A. van der Heijden, Petra J. M. Elders, Joline W. J. Beulens.

**Formal analysis:** Willem Wisgerhof, Carolien Ruijgrok.

**Funding acquisition:** Joline W. J. Beulens.

**Investigation:** Carolien Ruijgrok, Petra J. M. Elders, Joline W. J. Beulens.

**Methodology:** Willem Wisgerhof, Carolien Ruijgrok, Joline W. J. Beulens, Marjan Alssema.

## References

1. Nathan DM, Turgeon H, Regan S (2007) Relationship between glycated haemoglobin levels and mean glucose levels over time. Diabetologia 50: 2239–2244 https://doi.org/10.1007/s00125-007-0803-0 PMID: 17851648

2. Kilpatrick ES, Bloomgarden ZT, Zimmet PZ (2009) International Expert Committee report on the role of the A1C assay in the diagnosis of diabetes: response to the International Expert Committee. Diabetes Care 32: e159; author reply e160 https://doi.org/10.2337/dc09-1231 PMID: 19940222

3. American Diabetes A (2009) Diagnosis and classification of diabetes mellitus. Diabetes Care 32 Suppl 1: S62–67

4. Zhang YR, Hu G, Yuan ZY, Chen LW (2012) Glycosylated Hemoglobin in Relationship to Cardiovascular Outcomes and Death in Patients with Type 2 Diabetes: A Systematic Review and Meta-Analysis. Plos One 7

5. Khaw KT, Wareham N, Bingham S, Luben R, Welch A, Day N (2004) Association of hemoglobin A1c with cardiovascular disease and mortality in adults: the European prospective investigation into cancer in Norfolk. Ann Intern Med 141: 413–420 https://doi.org/10.7326/0003-4819-141-6-200409210-00006 PMID: 15381514

6. Di Angelantonio E, Gao P, Khan H, et al. (2014) Glycated Hemoglobin Measurement and Prediction of Cardiovascular Disease. Jama-J Am Med Assoc 311: 1225–123

7. Collaboration NCDRF (2015) Effects of diabetes definition on global surveillance of diabetes prevalence and diagnosis: a pooled analysis of 96 population-based studies with 331,288 participants. Lancet Diabetes Endocrinol 3: 624–637 https://doi.org/10.1016/S2213-8587(15)00129-1 PMID: 26109024

8. Genuth S, Lachin JM, Nathan DM (2005) Biological variation in HbA(1c) predicts risk of retinopathy and nephropathy in type 1 diabetes—Response to McCarter et al. Diabetes Care 28: 233–233 https://doi.org/10.2337/diacare.28.1.233 PMID: 15616268

9. Yudkin JS, Forrest RD, Jackson CA, Ryle AJ, Davie S, Gould BJ (1990) Unexplained Variability of Glycated Hemoglobin in Nondiabetic Subjects Not Related to Glycemia. Diabetologia 33: 208–215 https://doi.org/10.1007/BF00404798 PMID: 2347434

10. Florez JC (2010) A genome-wide association study of treated A1C: a genetic needle in an environmental haystack? Diabetes 59: 332–334 https://doi.org/10.2337/db09-1636 PMID: 20103712

11. Gallagher EJ, Le Roith D, Bloomgarden Z (2009) Review of hemoglobin A(1c) in the management of diabetes. J Diabetes 1: 9–17 https://doi.org/10.1111/j.1753-0407.2009.00009.x PMID: 20923515

12. Welsh KJ, Kirkman MS, Sacks DB (2016) Role of Glycated Proteins in the Diagnosis and Management of Diabetes: Research Gaps and Future Directions. Diabetes Care 39: 1299–1306 https://doi.org/10.2337/dc15-2727 PMID: 27457632

13. Jansen H, Stolk RP, Nolte IM, Kema IP, Wolffenbuttel BH, Snieder H (2013) Determinants of HbA1c in nondiabetic Dutch adults: genetic loci and clinical and lifestyle parameters, and their interactions in the Lifelines Cohort Study. J Intern Med 273: 283–293 https://doi.org/10.1111/joim.12010 PMID: 23121487

14. Ziemer DC, Kolm P, Weintraub WS, et al. (2010) Glucose-independent, black-white differences in hemoglobin A1c levels: a cross-sectional analysis of 2 studies. Ann Intern Med 152: 770–777 https://doi.org/10.7326/0003-4819-152-12-201006150-00004 PMID: 20547905

15. Hong JW, Noh JH, Kim DJ (2016) Association between Alcohol Intake and Hemoglobin A1c in the Korean Adults: The 2011–2013 Korea National Health and Nutrition Examination Survey. Plos One 11

16. Selvin E, Zhu H, Brancati FL (2009) Elevated A1C in Adults Without a History of Diabetes in the US. Diabetes Care 32: 828–833 https://doi.org/10.2337/dc08-1699 PMID: 19196895

17. Monnier L, Lapinski H, Colette C (2003) Contributions of fasting and postprandial plasma glucose increments to the overall diurnal hyperglycemia of type 2 diabetic patients: variations with increasing levels of HbA(1c). Diabetes Care 26: 881–885 https://doi.org/10.2337/diacare.26.3.881 PMID: 12610053

18. Rutters F, Nijpels G, Elders P, et al. (2018) Cohort Profile: The Hoorn Studies. Int J Epidemiol 47: 396–396j https://doi.org/10.1093/ije/dyx227 PMID: 29244153

19. Grootenhuis PA, Westenbrink S, Sie CM, de Neeling JN, Kok FJ, Bouter LM (1995) A semiquantitative food frequency questionnaire for use in epidemiologic research among the elderly: validation by comparison with dietary history. J Clin Epidemiol 48: 859–868 https://doi.org/10.1016/0895-4356(95)00013-t PMID: 7782793

20. NEVO Tabel: Dutch Food Composition Table 1989/1990. The Hague (NL): Voorlichtingsbureau voor de Voeding 1989. In:

21. Nevo (2006) Dutch Food Composition Database 2006. The Hague: Stichting Nevo. In:

22. Siebelink E, Geelen A, de Vries JH (2011) Self-reported energy intake by FFQ compared with actual energy intake to maintain body weight in 516 adults. Br J Nutr 106: 274–281 https://doi.org/10.1017/S0007114511000067 PMID: 21338536

23. Schindhelm RK, Dekker JM, Nijpels G, et al. (2007) Alanine aminotransferase predicts coronary heart disease events: A 10-year follow-up of the Hoorn Study. Atherosclerosis 191: 391–396 https://doi.org/10.1016/j.atherosclerosis.2006.04.006 PMID: 16682043

24. Henry RMA, Kostense PJ, Bos G, et al. (2002) Mild renal insufficiency is associated with increased cardiovascular mortality: The Hoorn Study. Kidney Int 62: 1402–1407 https://doi.org/10.1111/j.1523-1755.2002.kid571.x PMID: 12234312

25. van 't Riet E, Alssema M, Rijkelijkhuizen JM, Kostense PJ, Nijpels G, Dekker JM (2010) Relationship Between A1C and Glucose Levels in the General Dutch Population The New Hoorn Study. Diabetes Care 33: 61–66 https://doi.org/10.2337/dc09-0677 PMID: 19808928

26. White IR, Royston P, Wood AM (2011) Multiple imputation using chained equations: Issues and guidance for practice. Stat Med 30: 377–399 https://doi.org/10.1002/sim.4067 PMID: 21225900

27. Rubin DB (2004) Multiple Imputation for Nonresponse in Surveys., New York: John Wiley and Sons

28. Willett W (2012) Overview of Nutritional Epidemiology Nutritional Epidemiology, pp 1–16

29. Marshall A, Altman DG, Holder RL, Royston P (2009) Combining estimates of interest in prognostic modelling studies after multiple imputation: current practice and guidelines. BMC Med Res Methodol 9: 57 https://doi.org/10.1186/1471-2288-9-57 PMID: 19638200

30. Pani LN, Korenda L, Meigs JB, et al. (2008) Effect of Aging on A1C Levels in Individuals Without Diabetes. Diabetes Care 31: 1991–1996 https://doi.org/10.2337/dc08-0577 PMID: 18628569

31. Nakashima K, Nishizaki O, Andoh Y (1993) Acceleration of Hemoglobin Glycation with Aging. Clin Chim Acta 215: 111–118 https://doi.org/10.1016/0009-8981(93)90254-2 PMID: 8513562

32. Yang YC, Lu FH, Wu JS, Chang CJ (1997) Age and sex effects on HbA(1c)—A study in a healthy Chinese population. Diabetes Care 20: 988–991 https://doi.org/10.2337/diacare.20.6.988 PMID: 9167111

33. Seidell JC, Perusse L, Despres JP, Bouchard C (2001) Waist and hip circumferences have independent and opposite effects on cardiovascular disease risk factors: the Quebec Family Study. Am J Clin Nutr 74: 315–321 https://doi.org/10.1093/ajcn/74.3.315 PMID: 11522554

34. Snijder MB, Zimmet PZ, Visser M, Dekker JM, Seidell JC, Shaw JE (2004) Independent and opposite associations of waist and hip circumferences with diabetes, hypertension and dyslipidemia: the AusDiab Study. Int J Obes Relat Metab Disord 28: 402–409 https://doi.org/10.1038/sj.ijo.0802567 PMID: 14724659

35. Harding AH, Sargeant LA, Khaw KT, et al. (2002) Cross-sectional association between total level and type of alcohol consumption and glycosylated haemoglobin level: the EPIC-Norfolk Study. Eur J Clin Nutr 56: 882–890 https://doi.org/10.1038/sj.ejcn.1601408 PMID: 12209377

36. Gulliford MC, Ukoumunne OC (2001) Determinants of glycated haemoglobin in the general population: associations with diet, alcohol and cigarette smoking. Eur J Clin Nutr 55: 615–623 https://doi.org/10.1038/sj.ejcn.1601233 PMID: 11464236

**37.** Schrieks IC, Heil AL, Hendriks HF, Mukamal KJ, Beulens JW (2015) The effect of alcohol consumption on insulin sensitivity and glycemic status: a systematic review and meta-analysis of intervention studies. Diabetes Care 38: 723–732 https://doi.org/10.2337/dc14-1556 PMID: 25805864

**38.** Boeing H, Weisgerber UM, Jeckel A, Rose HJ, Kroke A (2000) Association between glycated hemoglobin and diet and other lifestyle factors in a nondiabetic population: cross-sectional evaluation of data from the Potsdam cohort of the European Prospective Investigation into Cancer and Nutrition Study. Am J Clin Nutr 71: 1115–1122 https://doi.org/10.1093/ajcn/71.5.1115 PMID: 10799373

**39.** Shoff SM, Maresperlman JA, Cruickshanks KJ, Klein R, Klein BEK, Ritter LL (1993) Glycosylated Hemoglobin Concentrations and Vitamin-E, Vitamin-C, and Beta-Carotene Intake in Diabetic and Non-diabetic Older Adults. Am J Clin Nutr 58: 412–416 https://doi.org/10.1093/ajcn/58.3.412 PMID: 8237854

**40.** Davie SJ, Gould BJ, Yudkin JS (1992) Effect of Vitamin-C on Glycosylation of Proteins. Diabetes 41: 167–173 https://doi.org/10.2337/diab.41.2.167 PMID: 1733805