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Variation in the hemoglobin glycation index

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ABSTRACT

A high hemoglobin glycation index (HGI) has been repeatedly associated with greater risk for hypoglycemia in people with diabetes and greater risk for chronic vascular disease in people with or without diabetes. This review explores how different sources of analytical and biological variation in HbA1c and blood glucose individually and collectively affect the clinical information value of HGI. We conclude that HGI is a complex quantitative trait that is a clinically practical biomarker of risk for both hypoglycemia and chronic vascular disease.

1. Introduction

The hemoglobin glycation index (HGI) can identify people with hemoglobin A1c (HbA1c) levels that are higher or lower than average compared to other people with the same blood glucose concentration.[1,2] Clinical studies confirm that people with a high HGI (higher HbA1c than predicted by blood glucose) have greater risk for chronic vascular disease in nondiabetic,[3–8] type 1 diabetic,[9] prediabetic[10] and type 2 diabetic[11–16] populations. High HGI diabetes patients also have greater risk for hypoglycemia caused by overtreatment with glucose-lowering drugs.[11,12,17,18] This review explores how different sources of variation in HbA1c and blood glucose affect the clinical information value of HGI for assessing risk and guiding treatment for both hypoglycemia and chronic vascular disease.

2. Calculating HGI

HGI is a linear regression residual that is derived in two steps. First, a predicted HbA1c is generated by inserting fasting plasma glucose (FPG) into a regression equation describing the linear relationship between HbA1c and FPG in a reference population. Predicted HbA1c is then subtracted from the individual's observed HbA1c (HGI = observed HbA1c – predicted HbA1c) to generate HGI. We proposed standardizing how HGI is calculated using a linear regression equation (predicted HbA1c = 0.024 FPG + 3.1) derived from a National Health and Nutrition Examination Survey (NHANES) mixed race, diabetes-treatment naïve reference population to generate predicted HbA1c.[19] Widespread standardization would make HGI measurements comparable between hospitals, clinics and research studies, including retrospectively.

Cohen et al.[20] developed a metric called the glycation gap (GG) that is like HGI except serum fructosamine replaces blood glucose in the regression equation. Fructosamine, glycated serum protein and glycated plasma protein assays are different techniques for assessing extracellular protein glycation that have been used to calculate GG. The fact that GG is strongly positively correlated with HGI[18,21] and associated with risk for chronic vascular disease indicates that HGI and GG measure the same phenotypic characteristic.

3. HGI variation in human populations

HGI was normally distributed in our demographically diverse NHANES reference population with a mean of 0.0 % and a 95 % confidence interval ranging from about −2.0 % to +2.0 %.[19] One way to look at HGI is that individuals with an HGI of −2.0 % or +2.0 % have 2.0 % less or more HbA1c than other people with the same blood glucose concentration. HGI tertile cutpoints were used to classify individuals as low (<−0.150), moderate (−0.150 to <+0.150) or high (≥ + 0.150) HGI. Clinical studies confirm that HGI is higher in people who are black, older or have diabetes.[22–24] High HGI has been associated with higher BMI in nondiabetic but not diabetic populations.[3,4,19,23] Familial

Abbreviations: HGI, hemoglobin glycation index; RBC, red blood cell; HbA1c, glycated hemoglobin A1c; FPG, fasting plasma glucose; MBG, mean blood glucose; BMI, body mass index; OGTT, oral glucose tolerance test; 2hPG, 2-hour plasma glucose.

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variation in HbA1c independent of blood glucose concentration[25] is evidence of genetic variation in HGI. So is a growing list of gene polymorphisms that have been associated with population variation in HbA1c, many of which have no clear role in hemoglobin glycation or glucose metabolism.[26–28]

Because HGI is calculated by linear regression, anything that affects HbA1c or FPG values could produce variation in HGI. Analytical variation in HbA1c or FPG is thus a potential source of HGI variation. Biological sources of HGI variation include genetic and environmental factors that affect person-to-person variation in HbA1c or blood glucose.

4. Analytical sources of HGI variation

4.1. Glycated hemoglobin assay variation

Clinical use of HbA1c evolved out of research showing that glycated hemoglobin assays offered a convenient way to indirectly assess mean blood glucose (MBG) for the diagnosis and management of diabetes. Clinical demand spurred development of glycated hemoglobin assays based on protein separation techniques like cation exchange chromatography, isoelectric focusing, boronate affinity chromatography, immunoassay and mass spectrometry. But different analytical methods gave different results for the same blood sample which meant glycated hemoglobin assay results were not always comparable between laboratories.

Hemoglobin glycation is a nonenzymatic process where open chain glucose molecules spontaneously react with deprotonated alpha and beta globin amino groups. Glycation of beta globin's N-terminal valine (βVal1) produces two cyclic complexes with different molecular stability and charge properties. One is the stable βVal1 fructosamine that elutes in cation exchange and isoelectric focusing A1c analytical fractions and is the intended target of clinical HbA1c tests. The other is βVal1 glucosylamine, or labile A1c, whose levels increase or decrease as blood glucose increases or decreases. But alpha and beta globins have other chemically modifiable amino groups and other chemical modifiers. The reason different glycated hemoglobin assays give different results for the same blood sample is simply because some include chemically modified hemoglobins besides βVal1 fructosamine in the measured analytical fraction.

Historically, lack of glycated hemoglobin assay specificity made analytical bias a confusing source of nonrandom variation in HbA1c measurements. This problem was largely resolved by national and international standardization programs that normalize commercial HbA1c assay measurements.[29] The current widespread use of standardized assays makes HbA1c assay variation a minor source of random variation in HGI.

4.2. Glucose assay variation

Glucose is typically measured enzymatically in plasma. Because red blood cells (RBC) continue to metabolize glucose during storage, poor sample storage conditions (long storage times, high storage temperatures) can falsely lower estimates of plasma glucose concentration.[30] This sample storage issue is well-known, however, and is routinely addressed by separating RBC from plasma, refrigerating samples or by adding a glycolytic inhibitor like sodium fluoride. Proficiency testing helps assure the accuracy and precision of clinical assays such that glucose assay variation is a minor source of random variation in HGI.

4.3. Patient compliance

One problem with using FPG to calculate HGI is that failure to comply with the fasting protocol can artificially lower HGI. Consider a person with an HbA1c of 5.0 % and an FPG of 80 mg/dl. By inserting 80 mg/dl into the proposed HGI standardization equation we derive a predicted HbA1c of 5.0 % which, when subtracted from the person's observed HbA1c, produces an HGI of 0.0 %. But what if this person ate a meal that raised blood glucose to 100 mg/dl at collection time? By inserting 100 mg/dl into the HGI standardization equation we derive a predicted HbA1c of 5.5 % which, when subtracted from the person's observed HbA1c, produces a falsely low HGI of −0.5 %. Noncompliance with the fasting protocol is thus a potential source of nonrandom analytical variation in FPG that artificially lowers HGI.

5. Biological sources of HGI variation

5.1. Factors associated with lower HGI

5.1.1. Shorter RBC lifespan

As RBC age they undergo physical and biochemical changes that cause programmed cell death (apoptosis) and recycling by the spleen. Every day in every person, millions of new RBC are produced and millions of old RBC are recycled. Average RBC lifespan is about 120 days for a healthy person. All blood samples thus contain a mixture of RBC that have been exposed to plasma glucose for as little as one day or as long as four months. Because βVal1 fructosamine is relatively stable it accumulates slowly over time and is more abundant in older RBC. Because the amount of βVal1 fructosamine in a blood sample is the average amount present in a collection of young and old RBC, anything that makes RBC lifespan shorter than average, like hemolytic anemia,[31] will result in lower than expected HbA1c levels.

Clinical use of the HbA1c test as a surrogate for average blood glucose is based on the assumption that average RBC lifespan is the same between individuals and within individuals over time. Cohen et al.[32] tested this hypothesis using biotin labeling to measure RBC lifespan in six diabetic and six nondiabetic subjects and concluded that "Red cell life span heterogeneity in hematologically normal people is sufficient to alter HbA1c". RBC age measured some years later using stable isotopes in nine of the same study subjects was correlated with RBC age determined using biotin labeling.[33] The use of two different techniques for measuring RBC age, the small number of observations, and the inclusion of both diabetic and nondiabetic subjects weakens the conclusion that RBC age is consistent within individuals over time.

If variation in RBC lifespan is significantly responsible for population variation in HGI, then it should also be associated with the same characteristics as HGI. We are unaware of any report that longer RBC lifespan is associated with greater risk for hypoglycemia or chronic vascular disease. RBC lifespan would also have to be longer than average in black, obese and older people to mechanistically explain why these groups have higher HGI. We conclude that although variation in RBC lifespan undoubtedly contributes to population variation in HGI, it is technologically challenging to measure and its relative contribution to person-to-person variation in HbA1c and HGI in human populations remains unclear.

5.1.2. Glucose-6-phosphate dehydrogenase deficiency

Glucose-6-phosphate dehydrogenase (G6PDH) deficiency has been associated with lower HbA1c levels in diabetes patients[34] and with lower glutathione recycling capacity and greater oxidative stress.[35] G6PDH introduces glucose into the pentose phosphate pathway to generate NADPH, a critical reducing agent that participates in many intracellular oxidation-reduction reactions.[36] The RBC enzyme glutathione reductase uses NADPH to recycle oxidized glutathione (GSSG) to its reduced form (GSH) which has a role in many intracellular redox reactions including carbonyl detoxification and recycling of vitamins C and E.

The gene for G6PDH is highly polymorphic, producing a range of enzyme activity in human populations. Common alleles that lower G6PDH activity tend to be associated with malaria resistance and more prevalent in blacks than whites.[37] A genetic study by Wheeler et al.[38] reported that "Among African Americans, the T allele at rs1050828 was associated with measured HbA1c that was lower than fructosamine-

predicted HbA1c (0.31%-units, 95% CI 0.25–0.37, $p=6.4\times10^{-19}$). Among men with the C allele, measured HbA1c was similar to fructosamine-predicted HbA1c (residuals, 0.04%-units, 95% CI −0.04 to −0.12, N= 351). This suggested that only the T allele was associated with markedly lower HbA1c than expected from glycemic measurements". Lower HbA1c than predicted by fructosamine is the hallmark of a low GG phenotype. It remains to be determined to what extent person-to-person variation in G6PDH activity might contribute to population and racial variation in HbA1c and HGI.

5.1.3. Human immunodeficiency virus (HIV) infection

Kim et al.[39] studied HbA1c and FPG in 100 HIV-infected adults with type 2 diabetes and 200 demographically matched non-infected diabetic controls. The authors reported that HbA1c underestimated FPG by 29 mg/dl in HIV-infected diabetes patients, the hallmark of a low HGI phenotype. Glycated albumin levels closely reflected blood glucose in both groups. Low prevalence makes HIV infection at most a minor contributor to population variation in HGI.

5.1.4. Aspirin use

Acetylsalicylate (aspirin), a cornerstone for the treatment of cardiovascular disease, has both biochemical and analytical effects on HbA1c. Biochemically, Rendell et al.[40] reported that aspirin competitively inhibits the reaction between glucose and βVal1 amino groups. Finamore et al.[41] reported that aspirin-mediated acetylation of hemoglobin amino groups decreases hemoglobin glycation. Analytically, aspirin reportedly interferes with HbA1c analysis by charge based protein separation techniques.[42] Anything that lowers the rate of hemoglobin glycation in vivo, or interferes with HbA1c measurement, could lower HGI. The extent to which aspirin affects HbA1c or HGI in human populations remains unclear.

5.2. Factors associated with higher HGI

5.2.1. Inflammation and obesity

Inflammation is an ordered sequence of events that evolved to maintain metabolic homeostasis in response to oxidative stress.[43] Obesity-mediated chronic inflammation is partly a consequence of excess lipid storage where fat cells release molecular mediators of inflammation which can produce a pro-inflammatory state and oxidative stress.[44] The term "inflamm-aging" has been used to describe low-grade chronic inflammation caused by slowly failing antioxidant systems during aging.[45]

High HGI is associated with higher BMI and older age in nondiabetic study populations but not in diabetes populations.[19] Multiple clinical studies show that people with normal glucose metabolism and high HGI have higher plasma levels of C-reactive protein (Crp)[3,10,19,23] and fibrinogen.[3] Crp and fibrinogen are secreted by the liver in response to factors released by macrophages and adipocytes during the acute phase of the inflammatory response. Higher levels of inflammatory biomarkers in nondiabetic people with high HGI could be a sign of 1) higher than average inflammatory burden due to obesity or other oxidative stress, or 2) lower than average reducing capacity due to lower antioxidant status.

5.2.2. Low iron status

Iron is the key element in hemoglobin and oxygen transport. Iron deficiency has been associated with higher HbA1c independent of blood glucose concentration or glycated serum albumin,[46–48] the hallmark of a high HGI phenotype. A study by Bae et al.[49] of 87,284 nondiabetic Koreans without anemia showed that men and women with lower hemoglobin levels had higher HbA1c compared to others with the same FPG. English et al.[50] reviewed 12 studies and concluded that "… the presence of iron deficiency with or without anemia led to an increase in HbA1c values compared with controls, with no concomitant rise in glucose indices". A consensus mechanistic explanation has not been forthcoming.

5.2.3. Lower fructosamine-3-kinase activity

The enzyme fructosamine-3-Kinase (FN3K) limits vascular accumulation of advanced glycation endproducts (AGE) that degrade blood vessels.[51] FN3K does this by catalyzing the removal of glucose from lysine amino groups of collagen and elastin thus promoting normal intermolecular linkages that provide structure and elasticity to the extracellular matrix. Dunmore et al.[52] reported that RBC from diabetes patients with high GG had lower RBC FN3K protein and activity levels. Conversely, Delpierre et al.[53] reported that "Interindividual variability of FN3K activity is substantial and impacts on the glycation level at specific sites of haemoglobin, but does not detectably affect the level of HbA1c or GHb [total glycated hemoglobin]". Unlike HGI, FN3K activity did not differ between nondiabetic and diabetic subjects. The extent to which person-to-person variation in FN3K activity explains population variation in HbA1c or HGI remains unclear.

5.2.4. Higher RBC glucose transport

Unlike muscle, fat and liver cells, RBC do not have insulin-dependent Glut4 glucose transporters. Instead, RBC have insulin-independent Glut1 which transports glucose bidirectionally by facilitated diffusion.[54] Glut1 also transports dehydroascorbic acid (DHA), the oxidized form of vitamin C, in competition with glucose. RBC have an important role in vitamin C recycling by DHA reductase which uses GSH to convert DHA to ascorbic acid which can be used in intraerythrocyte redox reactions like vitamin E recycling or exported for use in extracellular reactions or by other cells.

Khera et al.[55] used a glucose analog to assess glucose gradients across RBC membranes to see if differences in glucose transport could explain population variation in GG. Glucose transport was assessed in RBC from five nondiabetic, 10 type 1 diabetic and 11 type 2 diabetic subjects. The authors reported that the intracellular/extracellular glucose gradient "…did correlate with A1c (r^2=0.19) and with the glycation gap (r^2=0.20), consistent with a model in which differences in internal glucose concentration at a given mean plasma glucose contribute to differences in A1C for given level of glycemic control". This observation supports the hypothesis that person-to-person variation in glucose transport mechanistically contributes to population variation in GG, but only weakly due to the technologically challenging assay system, low correlation coefficients and the small number and metabolic heterogeneity of study subjects.

Other research into the relationship between glucose transport and HbA1c levels has been conflicting. Garg et al.[56] reported that compared to nondiabetic controls, pediatric type 1 diabetes patients had higher serum glucose and HbA1c levels but 50 % lower RBC Glut1 protein levels measured by ELISA. Harik et al.[57] used cytochalasin-B to measure Glut1 and conversely concluded that diabetes patients had 22 % higher RBC Glut1 protein levels compared to nondiabetic controls. Bistritzer et al.[58] used 3-*O*-methyl glucose in kinetic studies which showed that while maximum velocity (Vmax) for glucose transport was higher in RBC from diabetes patients, the glucose concentration required to achieve half Vmax (Km) was not different between diabetic and control subjects. Vmax reflects how fast glucose reaches equilibrium between intracellular and extracellular compartments, which in RBC is a matter of seconds. The fact that Km was not different means that, unlike HGI, the equilibrium distribution of glucose between extracellular and intracellular compartments was not different in diabetic and nondiabetic subjects.

Assessment of Glut1 transport kinetics is technologically challenging and research to date has not convincingly demonstrated a significant role for Glut1 in population variation in HbA1c. That said, it seems mechanistically feasible that person-to-person variation in RBC glucose transport could affect hemoglobin glycation in ways that contribute to population variation in HGI.

5.2.5. Higher intraerythrocyte pH and 2,3-BPG

Intraerythrocyte hydrogen ion concentration plays a major role in

how hemoglobin binds and releases oxygen. So does 2,3-bisphosphoglycerate (2,3-BPG) which is produced in RBC via the glycolytic pathway in proportion to intracellular pH. Erythrocyte 2,3-BPG and glycolytic intermediates like glucose-6-phosphate interact with βHis2 and βVal1 amino groups in ways that may influence glucose binding.[59,60] Kunika et al.[61] reported that inorganic phosphates accelerate HbA1c synthesis. Clark et al.[60] concluded that "The clinical difference between average blood glucose and predicted HbA1c, and the presence of unstable HbA-glucose complexes may be more fully explained by initial noncovalent binding interactions and different concentrations of BPG, Pi and $HCO3^-$ in serum vs. erythrocytes".

Yudkin and colleagues[62] introduced the terms "high glycator" and "low glycator" to describe nondiabetic people with higher or lower than expected glycated hemoglobin levels; where the expected level was based on the discrepancy between glycated hemoglobin rank level vs. postprandial glucose rank level during an oral glucose tolerance test (OGTT). A follow-up study by Gould et al.[63] of five of the same low glycators and seven high glycators compared demographic and biochemical features over time. The authors reported that "Mean centile glycated haemoglobin was positively correlated with intraerythrocyte pH (r=0.55; P<0.05)" and "Erythrocyte 2,3-bisphosphoglycerate, a catalyst of glycation, was elevated in high compared to low glycators". The authors concluded that "These data indicate that the intra-erythrocyte environment of high glycators favours glycation of haemoglobin". These reports were among the first to show that differences in the quantitative relationship between glycated hemoglobin and blood glucose were consistent within individuals over time. Within individual consistency of RBC pH and 2,3-BPG could not be assessed because these variables were not measured at the first study visit.

5.2.6. Higher postprandial glucose

The hypothesis that population variation in HGI is an artifact of person-to-person variation in postprandial glucose has been frequently advanced. Rationale supporting this hypothesis is that using FPG to calculate HGI ignores the contribution of postprandial glucose to variation in HbA1c. Direct evidence supporting a mechanistic role for postprandial glucose in HGI variation was reported by Ahn et al.[10] in a study of cardiovascular disease (CVD) in 1248 treatment-naïve Koreans with prediabetes or diabetes. The population was divided into HGI tertile subgroups which exhibited the characteristic stepwise increase in mean HGI going from low (−0.80 %) to moderate (−0.07 %) to high (+0.96 %) HGI. The authors concluded that "High HGI was independently associated with overall and individual CVDs. This result suggests that discrepancy between HbA1c and fasting glucose levels can reflect vascular health in subjects with impaired glucose metabolism." They also reported that postchallenge glucose was statistically different between the three HGI groups with mean two hour plasma glucose (2hPG) first decreasing then increasing going from low (11.9 ± 6.0 mmol/l) to moderate (11.1 ± 4.4 mmol/l) to high (15.3 ± 5.6 mmol/l) HGI. This up and down trend contrasts with the stepwise increase or decrease characteristically observed in variables associated with HGI.

Wang et al.[64] used a developmental database of 2734 subjects with no history of diabetes to generate a linear regression equation (predicted HbA1c = 0.029 FPG + 3.0) that is very similar to what we observed in NHANES. HGI was then calculated in a study population of 1381 outpatients with no history of diabetes who were diagnosed by OGTT as nondiabetic (42.3 %), prediabetic (41.3 %) or diabetic (16.4 %). Compared to study subjects with HGI ≤ 0 (mean HGI = −0.3 %), subjects with HGI > 0 (mean HGI = +0.4 %) had significantly 1) lower FPG (95.0 vs. 98.5 mg/dl), 2) higher HbA1c (6.1 vs. 5.5 %), 3) higher 2hPG (151.1 vs. 144.6) and 4) higher glucose increment (56.1 vs. 46.1 mg/dl). The authors concluded that "…postchallenge glucose increment was independently associated with HGI in subjects with no history of diabetes". The authors noted, however, that "The aforementioned findings were observed in those who had abnormal glucose regulation (diabetes or pre-diabetes) by OGTT, but not in those who had normal glucose tolerance". This suggests that person-to-person variation in postprandial glucose contributes to population variation in HGI in people with diabetes but not in those with normal glucose metabolism.

Marini et al.[3] also found no relationship between postprandial glucose and HGI in a study of 387 nondiabetic (HbA1c < 6.5 % and FPG < 126 mg/dl) white adults. There was a characteristic stepwise increase in mean HGI going from low (−0.50 %) to moderate (−0.1 %) to high (+0.40 %) HGI but no difference (p = 0.18) in mean 2hPG among subjects classified as low (113 ± 29 mg/dl) moderate (114 ± 31 mg/dl) or high (119 ± 34 mg/dl) HGI (because mean HGI were not reported, those listed here were calculated using the report's regression equation and group mean HbA1c and FPG). Hsia et al.[65] studied HGI and OGTT in a mixed race recruitment cohort of 3946 adults with FPG < 126 mg/dl participating in the Vitamin D and Type 2 Diabetes (D2d) study. The authors observed a characteristic stepwise increase in mean HGI going from low (−0.35 %) to moderate (−0.01 %) to high (+0.30 %) HGI, but no difference (p = 0.82) in mean 2hPG among subjects classified as low (138 ± 46 mg/dl) moderate (138 ± 45 mg/dl) or high (139 ± 44 mg/dl) HGI.

Blood glucose variables that have been used to calculate HGI or GG include 1) FPG, 2) MBG based on data from continuous glucose monitoring, patient glucose meters, or daily multipoint glucose profile sets, and 3) glycated serum protein. In study after study, regardless of how blood glucose was assessed, the same demographic and clinical features have been consistently associated with high HGI, including black race, obesity, diabetes, older age, higher Crp and greater risk for chronic vascular disease. If MBG and glycated serum protein comprehensively account for person-to-person variation in blood glucose, and high HGI calculated with FPG is associated with the same characteristics, then we can conclude that person-to-person variation in postprandial glucose is not a major source of HGI variation in nondiabetic populations. Collectively, these studies suggest that person-to-person variation in postprandial glucose contributes to HGI variation in people with diabetes but not in people with normal glucose metabolism.

5.2.7. Diabetes

We used the proposed standardization equation to compare HGI in diabetes treatment-naïve adult NHANES participants and those being treated for type 2 diabetes.[19] The results showed that participants with diagnosed type 2 diabetes had 0.6 % higher mean HGI compared to treatment naïve adults. The HbA1c vs. FPG linear regression equation derived from the diabetic participants had a lower slope (0.020) and higher intercept (4.2) compared to the slope (0.024) and intercept (3.1) of the treatment-naïve participants. We reviewed twelve other reports of linear regression slopes and intercepts in type 2 diabetes populations[11–14,17,18,66–71] and found that every one had a lower slope (mean 0.017, range 0.008 to 0.022) and higher intercept (mean 5.2, range 4.4 to 6.8) compared to treatment-naïve NHANES participants.

Higher HGI in diabetes patients could be a consequence of high blood glucose or of treatment for high blood glucose. To determine which, we graphically evaluated the quantitative relationship between HbA1c and FPG in our diabetes-treatment naïve adult NHANES population. The results showed that almost all participants with FPG greater than about 200 mg/dl had HbA1c levels that were above the population regression line and thus HGI > 0.[19] Graphical data presented by Rhee et al.,[4] Hsia et al.[65] and Wang et al.[64] similarly show that treatment-naïve people with higher than normal FPG tend to have HbA1c that are above the regression line and thus HGI > 0. These observations indicate that treated or not, hyperglycemia shifts the quantitative relationship between HbA1c and blood glucose in a way that characteristically increases HGI.

5.2.8. Statin use

Statins are cholesterol-lowering drugs that inhibit HMG-CoA reductase, the first and key rate-limiting enzyme in the cholesterol biosynthetic pathway.[72] Multiple studies have reported that statin use is

associated with higher HbA1c levels in both nondiabetic and diabetic study populations.[73,74] Liew et al.[75] reported that statin use was associated with significantly higher HbA1c levels even after statistically controlling for FPG, the hallmark of a high HGI phenotype. The observation that statin users have higher HbA1c levels than non-users generated widespread concern that statins promote new onset diabetes. This led the U.S. Food and Drug Administration to require that statin drug labels include a warning that higher HbA1c levels and impaired glycemic control have been reported in statin users. Despite incontrovertible evidence of a shift to higher HbA1c levels, the general clinical consensus has been that the benefits of statin use far outweigh suspected harms.[76] Although statin use clearly changes the quantitative relationship between HbA1c and FPG in a way that increases HGI, a mechanistic explanation has not been forthcoming.

5.2.9. Steroid use

Yucel et al.[77] compared HbA1c in 141 nondiabetic children with asthma and 52 control subjects and concluded that asthmatic children taking low doses of inhaled steroids have higher HbA1c levels than healthy children. Tillmann et al.[78] evaluated the effect of prednisolone on HbA1c in nondiabetic renal transplant recipients and reported that steroid use was associated with higher HbA1c levels and greater prevalence of prediabetes. Rambaran et al.[79] reported that a history of corticosteroid use was associated with higher HbA1c in nondiabetic subjects with chronic obstructive pulmonary disease. In contrast to what has been observed in nondiabetic people, Habib et al.[80] and Mizrachi et al.[81] reported no effect of steroid use on HbA1c in diabetes patients. These observations suggest that steroid use is associated with higher HbA1c in nondiabetic but not diabetic study populations.

5.2.10. Asthma

Asthma is a chronic inflammatory lung disease that affects 20 million people in the U.S. and >300 million people worldwide.[82] It is caused by a combination of genetic and environmental factors, including allergens and air pollution. There is increasing evidence that obesity, metabolic syndrome and asthma are epidemiologically and mechanistically linked.[83] Sathiyapriya et al.[84] reported significantly higher HbA1c levels in nondiabetic people with asthma compared to healthy people with similar FPG, the hallmark of a high HGI phenotype. None of the asthma patients in the study had received oral corticosteroids in the previous six months which suggests the difference was not related to steroid use. Lipid peroxides and serum fructosamine were also elevated in asthma patients leading the authors to conclude that "An increased glycation of proteins was found in asthma patients. These data also support the premise that lipid peroxides per se do have a role to play in glycation of hemoglobin and plasma proteins."

6. Conclusions

It is a fact of nature that genetic variation causes different people to have different characteristics when exposed to the same environment. Individuals can also express different characteristics over time or when exposed to different environments. Characteristics that have been consistently associated with a high HGI phenotype include greater morbidity and mortality, older age, black race, obesity, diabetes, specific genetic polymorphisms, and chronic inflammation. This report reviews multiple analytical and biological factors, from RBC lifespan to asthma, many of which clearly influence the quantitative relationship between HbA1c and blood glucose measured by HGI. We conclude that HGI is a complex quantitative trait that reflects the influence of genetic and environmental factors that collectively produce a wide range of HGI in human populations. The degree to which any single factor contributes to population variation in HGI remains unclear and difficult to determine. What is abundantly clear, however, is that HGI calculated using simple linear regression, *and without statistically controlling for any covariates*, is a clinically practical biomarker of 1) hypoglycemia risk in people with diabetes, and 2) risk for chronic vascular disease in people with or without diabetes.

Clinical use of standardized HGI is immediately warranted to help prevent hypoglycemia in high HGI diabetes patients. HbA1c and FPG tests performed by most clinical laboratories can be used to manually calculate HGI at routine medical visits using our proposed HGI standardization equation (HbA1c = 0.024 FPG + 3.1). Electronic medical records make it even easier for health care providers to automatically calculate and monitor HGI over time. Widespread standardization would make HGI comparable between research laboratories and facilitate basic research to mechanistically explain why higher than predicted HbA1c is associated with greater risk for chronic vascular disease. Standardization would also facilitate clinical research to determine how HGI can help personalize lifestyle modifications or guide pharmaceutical interventions to minimize chronic vascular disease. Many ongoing or completed large scale clinical trials have standardized HbA1c and FPG data needed to retrospectively study HGI and its relationship to diabetes, aging, obesity, asthma and other chronic conditions.

CRediT authorship contribution statement

JMH and DSH wrote the review and are responsible for the intellectual content and final approval of the published version.

Declaration of competing interest

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