Contents lists available at ScienceDirect

American Journal of Preventive Cardiology

Haptoglobin phenotype and intensive glycemic control for coronary artery disease risk reduction in people with type two diabetes: The Veterans Affairs Diabetes Trial

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ARTICLE INFO

ABSTRACT

Background: Intensive glycemic control reduced the risk of coronary artery disease (CAD) events among White ACCORD study participants with the haptoglobin (Hp)2-2 phenotype, and not among participants without the Hp2-2 phenotype. It is unknown whether these results persist in a population with more severe diabetes.
Methods: Haptoglobin phenotype was measured in 1746 (97 %) samples from the Veterans Affairs Diabetes Trial (VADT) randomized controlled trial. Multivariable-adjusted Cox regression models assessed the effect of intensive therapy on CAD risk among participants with and without the Hp2-2 phenotype separately and when stratified within pre-specified race/ethnicity-based subgroups. Time-varying (achieved) HbA$_{1c}$ data (<7.0 % or ≥8.0 % compared to 7.0–7.9, updated every 3 months) were also analyzed in relation to CAD risk within each phenotype.
Results: 567 (32.5 %) participants had the Hp2-2 phenotype. Compared to standard therapy, intensive glycemic control was not associated with risk of CAD among participants with the non-Hp2-2 or the Hp2-2 phenotype or for any race/ethnicity-based group. Compared to HbA$_{1c}$ of 7.0–7.9 %, having HbA$_{1c}$ <7.0 % was not associated with CAD risk for either phenotype or among any race/ethnicity-based group. Having HbA$_{1c}$ ≥8.0 % was associated with an increased risk of CAD among Hispanic participants without the Hp2-2 phenotype (HR= 3.61, 95 % CI: 1.54–8.41, p-interaction=0.53).
Conclusion: The effect of intensive glycemic therapy on CAD events was not dependent on Hp phenotype in the VADT study of veterans with severe diabetes who may represent a population where Hp phenotype information would not be useful for personalizing diabetes management. However, further research is needed to determine if these results are conclusive.

1. Introduction

People with type 2 diabetes mellitus (T2DM) have a substantially higher risk of incident coronary artery disease (CAD, such as myocardial infarction) compared to people without T2DM [1]. However, large randomized controlled trials such as the Action to Control Cardiovascular Disease Risk in Diabetes (ACCORD) [2], and the Veterans Affairs

Diabetes Trial (VADT) [3] studies that aimed to lower blood glucose to near-normal (HbA$_{1c}$ <6.5 %) levels among people with advanced T2DM have not provided evidence for CAD risk reduction from intensive glycemic control compared to standard therapy, thus making clinical decision making for T2DM difficult. One potential explanation is that intensive glycemic control (targeting HbA$_{1c}$ levels of <6.5 %) may be more beneficial in a subset of people with T2DM. Unmeasured differences between individuals that affect the relationship between blood glucose and incident CAD could help to provide an explanation for lack of evidence supporting intensive glycemic control for CAD prevention previously reported.

A common variation in the gene that codes for the abundant plasma protein haptoglobin (Hp) is associated with CAD risk in people with hyperglycemia [4–8]. In people with the Hp2-2 phenotype (~40 % worldwide) and hyperglycemia, antioxidant capabilities of Hp are impaired and high-density lipoprotein (HDL) has been shown to be dysfunctional and pro-atherogenic with the potential to increase susceptibility to atherosclerosis, and ultimately CAD [5,9–11]. As a result, intensive glucose lowering may only be beneficial for CAD prevention among people with the Hp2-2 phenotype, whereas it may not be beneficial among people without the Hp2-2 phenotype among whom Hp and HDL function are better preserved in hyperglycemia.

In a re-analysis of the ACCORD glycemic control trial [2] incorporating Hp phenotype, we observed that intensive glycemic control (targeting glycated hemoglobin (HbA$_{1c}$) of <6.0 %) versus standard therapy (targeting HbA$_{1c}$ of 7.0–7.9 %) was effective at preventing incident CAD events among White study participants with the Hp2-2 phenotype (HR=0.71, 95 % CI: 0.55–0.91). No CAD benefit was observed among ACCORD participants without the Hp2-2 phenotype (0.95, 0.79–1.13); these non-Hp2-2 participants had an increased mortality risk from intensive therapy (1.40, 1.08–1.81) [12]. However, it remains unknown whether these findings are consistent across different populations. The VADT studied patients with long-standing and poorly controlled T2DM and reported that intensive glycemic control had no effect on risk of cardiovascular disease [3]. Whether the Hp phenotype determines the effect of intensive glycemic control on CAD risk among patients with severe diabetes is unknown and may provide an explanation of the null findings from the VADT if the CAD benefit from intensive glycemic control is only among participants with the Hp2-2 phenotype.

The objectives of the current study were to perform analyses within the Hp2-2 and non-Hp2-2 phenotypes separately to determine (1) whether the effect of intensive glycemic control treatment on risk of incident CAD is dependent on Hp phenotype in the VADT, and (2) if the association between glycated hemoglobin (HbA1c) concentration and risk of incident CAD is dependent on Hp phenotype in the VADT. The frequency of the Hp2-2 phenotype differs among race/ethnicity-based and geographic populations [6], and thus we aimed to examine our

results in each phenotype group overall and with further stratification by the three largest race/ethnicity-based groups of White, Black and Hispanic participants separately.

2. Methods

2.1. Study design and participants

A re-analysis of data from the VADT study with the addition of Hp phenotype data was undertaken to determine whether the association between intensive glycemic control and CAD events varies by Hp phenotype. The design, methods and major findings of the VADT study (ClinicalTrials.gov identifier: NCT00032487) have been reported previously [3,13]. Briefly, 1791 veterans with T2DM were enrolled in the study from December 1, 2000 to May 30, 2003, and were randomized to receive either intensive (with a target HbA1c of ≤6.0 %) or standard (with a target HbA1c of 8.0–9 %) glycemic therapy over a median follow-up of 5.6 years with follow-up ending on May 30, 2008. Participants were aged ≥41 with diagnosed T2DM and had to have HbA1c ≥ 7.5 % and had to be unresponsive to maximal doses of an oral agent or insulin therapy. Exclusion criteria included the occurrence of a cardiovascular event during the previous 6 months, advanced congestive heart failure, severe angina, a life expectancy of less than 7 years, a body mass index (BMI, the weight in kilograms divided by the square of the height in meters) of more than 40 kg/m^2, a serum creatinine level of more than 1.6 mg per deciliter (141 μmol per liter), and an alanine aminotransferase level of more than three times the upper limit of the normal range. Each participating center obtained ethical approval, and all participants provided written informed consent. The VADT study was completed in 2009 and the data that support the findings of this study may be made available from the corresponding author and the Department of Veterans Affairs (VA) Cooperative Studies Program through a Data Use Agreement.

2.2. Haptoglobin phenotyping

Available samples from the VADT study ($n = 1746$, 97 % of the VADT cohort) were used to determine the Hp phenotype of participants using a validated high-throughput enzyme linked immunosorbent assay (ELISA), which can distinguish the Hp2-2 phenotype from the non-Hp2-2 phenotypes based on stoichiometry with a sensitivity and specificity of 99 % and 98 % respectively [14]. The exclusion of the other 45 participants occurred because serum samples from these participants were not available. Hp phenotype does not change over time; therefore, a blood sample from any visit could be used for each participant.

2.3. Outcome

We report our primary outcome of CAD events defined as a composite of the following pre-specified VADT outcomes [3,13]: non-fatal MI, angina, inoperable CAD, invasive coronary revascularization procedure, and death from myocardial infarction, coronary revascularization, or sudden death. Although the mechanism is not well understood, stroke is an endpoint that has been associated with the Hp1–1 phenotype rather than the Hp2-2 phenotype [15,16], suggesting that CAD and stroke should be separated from a composite CVD outcome for analyses by Hp phenotype. Therefore, the present analysis studied the primary outcome of CAD events rather than the original VADT study composite major CVD primary outcome of myocardial infarction, stroke, death from cardiovascular causes, congestive heart failure, surgery for vascular disease, inoperable coronary disease, and amputation for ischemic gangrene. All VADT outcomes were adjudicated by an endpoint committee that was unaware of treatment assignment [3,13].

2.4. Statistical analysis

Statistical analyses were conducted using Stata/SE software version 17.0 (College Station, TX) at a 2-tailed alpha level of 0.05. Except for when testing for Hardy Weinberg Equilibrium (HWE), participants with the Hp2–1 or Hp1–1 phenotypes (those without the Hp2-2 phenotype) were combined to form a group, which is a common approach when studying the Hp phenotype because of the low frequency of the Hp1–1 phenotype (~15 %) and because the structure and function of the Hp2–1 and Hp1–1 proteins are similar in comparison to the Hp2-2 protein [7,8, 17,18].

Participants were grouped based on Hp phenotype and treatment, and characteristics were compared using *t*-tests and Kruskal-Wallis tests for continuous variables and chi-square tests for categorical variables. Less than 5 % of baseline variables were missing. Thirteen participants (0.007 %) had missing baseline HbA$_{1c}$ information and thus were excluded from the HbA$_{1c}$ analysis.

Multivariable adjusted Cox proportional hazards regression models were used to quantify the association between intensive glycemic control treatment and CAD stratified by Hp phenotype group according to the intention-to-treat principal (as in the original VADT [3]). Models were adjusted for age, sex, race/ethnicity, hospital, and the presence of a previous cardiovascular disease event at baseline. The presence of an interaction between treatment and Hp phenotype was tested in the full cohort (not stratified by Hp phenotype) by adding an interaction term to the adjusted model.

Multivariable adjusted Cox regression models with time-varying covariables were used to quantify the relationship between the time-dependent HbA$_{1c}$ categories (categorized as <7.0 % or ≥8.0 % compared to 7.0–7.9) and CAD in the two Hp phenotype groups separately. Time-varying covariables included total cholesterol, BMI, systolic blood pressure, and urinary albumin creatinine ratio (ACR). Time-

Table 1
Baseline characteristics* stratified by treatment group and Hp phenotype.

Characteristic	Non-Hp2-2 Phenotypes				Hp2-2 Phenotype				Overall P-value **
	All (n = 1179)	Standard (n = 578)	Intensive (n = 601)	P-value	All (n = 567)	Standard (n = 296)	Intensive (n = 271)	P-value	
Age — years	60.2 ± 8.7	60.1 ± 8.5	60.4 ± 8.8	0.49	60.8 ± 8.7	60.8 ± 8.8	60.8 ± 8.7	0.97	0.24
Male — n (%)	1153 (97.8)	565 (97.8)	588 (97.8)	0.92	542 (95.6)	283 (95.6)	259 (95.6)	0.98	0.01
Diabetes duration—years	10 (5–16)	10 (6–16)	10 (5–15)	0.40	10 (6–15)	10 (6–15)	10 (5–16)	0.82	0.66
Previous CVD event — n (%)	477 (40.5)	236 (40.8)	241 (40.1)	0.80	224 (39.5)	120 (40.5)	104 (38.4)	0.60	0.70
Hypertension — n (%)	856 (72.8)	414 (71.9)	442 (73.7)	0.49	397 (70.0)	215 (72.6)	182 (67.2)	0.16	0.23
Race — n (%)				0.29				0.37	<0.01
White	683 (57.9)	342 (59.2)	341 (56.7)		400 (70.6)	213 (72.0)	187 (69.0)		
Hispanic	196 (16.6)	84 (14.5)	112 (18.6)		86 (15.2)	47 (15.9)	39 (14.4)		
Black	248 (21.0)	127 (22.0)	121 (20.1)		45 (7.9)	18 (6.1)	27 (10.0)		
Unspecified	52 (4.4)	25 (4.3)	27 (4.5)		36 (6.4)	18 (6.1)	18 (6.6)		
Medications — n (%)									
Insulin	623 (52.8)	309 (53.5)	314 (52.3)	0.68	286 (50.4)	150 (50.7)	136 (50.2)	0.91	0.35
Metformin	816 (69.2)	403 (69.7)	413 (68.7)	0.71	388 (68.4)	210 (71.0)	178 (65.7)	0.18	0.74
Sulfonylurea	706 (59.9)	356 (61.6)	350 (58.2)	0.24	360 (63.5)	191 (64.5)	169 (62.4)	0.59	0.15
Thiazolidinedione	210 (17.8)	104 (18.0)	106 (17.6)	0.87	121 (21.3)	64 (21.6)	57 (21.0)	0.86	0.08
Beta-blocker	352 (29.9)	173 (29.9)	179 (29.8)	0.96	150 (26.5)	80 (27.0)	70 (25.8)	0.75	0.14
Angiotensin-converting-enzyme inhibitor	774 (65.7)	378 (65.4)	396 (65.9)	0.86	387 (68.3)	198 (66.9)	189 (69.7)	0.47	0.28
Angiotensin receptor blocker	79 (6.7)	35 (6.1)	44 (7.3)	0.39	31 (5.5)	18 (6.1)	13 (4.8)	0.50	0.32
Diuretic	366 (31.0)	167 (28.9)	199 (33.1)	0.12	162 (28.6)	85 (28.7)	77 (28.4)	0.94	0.29
Any anti-hypertensive medication use	990 (84.0)	486 (84.1)	504 (83.9)	0.92	472 (83.3)	247 (83.5)	225 (83.0)	0.89	0.70
Statins	685 (58.1)	341 (59.0)	344 (57.2)	0.54	336 (59.3)	166 (56.1)	170 (62.7)	0.11	0.65
Any lipid-lowering medication use	782 (66.3)	382 (66.1)	400 (66.6)	0.87	379 (66.8)	195 (65.9)	184 (67.9)	0.61	0.83
Glycated hemoglobin —%				0.42				0.69	0.18
Mean	9.5 ± 1.6	9.5 ± 1.6	9.5 ± 1.6		9.3 ± 1.4	9.4 ± 1.5	9.3 ± 1.4		
Median (IQR)	9.1 (8.3–10.3)	9 (8.2–10.3)	9.1 (8.3–10.3)		9.0 (8.3–10.0)	9.0 (8.3–10.2)	9.0 (8.3–9.9)		
Weight — kg	96.8 ± 16.1	96.9 ± 16.2	96.7 ± 15.9	0.78	95.4 ± 16.3	95.0 ± 16.2	95.8 ± 16.6	0.60	0.09
BMI — kg/m^2	31.3 ± 4.4	31.3 ± 4.5	31.3 ± 4.4	0.97	31.1 ± 4.6	31.0 ± 4.6	31.2 ± 4.5	0.44	0.39
Blood pressure — mmHg									
Systolic	131.3 ± 16.9	131.5 ± 17.1	131.0 ± 16.7	0.65	131.8 ± 15.9	131.8 ± 15.7	131.8 ± 16.1	0.99	0.54
Diastolic	76.3 ± 10.1	76.3 ± 10.1	76.4 ± 10.2	0.89	75.4 ± 10.4	75.5 ± 10.3	75.3 ± 10.6	0.82	0.08
Cholesterol — mg/dL									
Total	183.0 ± 44.1	183.6 ± 46.3	182.5 ± 41.9	0.69	183.8 ± 53.1	186.8 ± 64.4	180.6 ± 37.0	0.15	0.75
Low-density lipoprotein	110.6 ± 57.3	110.1 ± 51.6	111.0 ± 62.4	0.79	112.3 ± 74.7	111.6 ± 64.1	113.1 ± 84.8	0.82	0.63
High-density lipoprotein	36.0 ± 10.0	36.0 ± 10.1	36.0 ± 10.0	0.99	35.8 ± 10.4	35.5 ± 11.0	36.1 ± 9.7	0.45	0.64
Triglycerides — mg/dL (IQR)	161 (114–236)	159 (112–239)	162 (116–236)	0.92	163 (112–241)	160 (108–267)	165 (112–226)	0.68	0.60
Current smoking — n (%)	195 (16.6)	85 (14.7)	110 (18.3)	0.10	94 (16.6)	52 (17.6)	42 (15.5)	0.50	0.98
Creatinine — mg/dL	1.01±0.22	1.01±0.21	1.02±0.23	0.41	0.99±0.21	1.0 ± 0.22	0.99±0.20	0.58	0.05
Estimated GFR†	87.8 ± 24.1	88.2 ± 22.6	87.5 ± 25.5	0.61	86.6 ± 21.7	86.3 ± 22.8	86.9 ± 20.6	0.75	0.29
ACR — μ/mg	18 (6–65)	18 (7–61)	18 (6–71)	0.90	22 (7–75)	21.5 (7–82)	23 (7–61.5)	0.72	0.11

ACR=albumin creatinine ratio; CVD= cardiovascular disease; GFR= glomerular filtration rate; Hp= haptoglobin; IQR= interquartile range.

* Plus-minus values are means ± SD.

** *P*-value comparing characteristics between Hp phenotypes.

† The estimated glomerular filtration rate (GFR) was calculated with the use of the Modification of Diet in Renal Disease Study equation.

varying covariables were used to relate the most recent measure for each of those variables to incident outcomes at the time of an event to avoid potential bias from using a single baseline measurement. Cluster variance estimates accounting for within-subject correlation of repeated measures were used. Time-independent covariables recorded at baseline only included: age, sex, race/ethnicity (for models not stratified by race/ethnicity only), hospital, assignment to the intensive glucose therapy,

previous CVD, smoking status, diabetes duration, glomerular filtration rate (GFR), anti-hypertensive medication use, statin and any lipid lowering medication use. The presence of an interaction between the HbA_{1c} categories as a continuous variable and Hp phenotype was tested in the full cohort (not stratified by Hp phenotype) by adding an interaction term to the adjusted model.

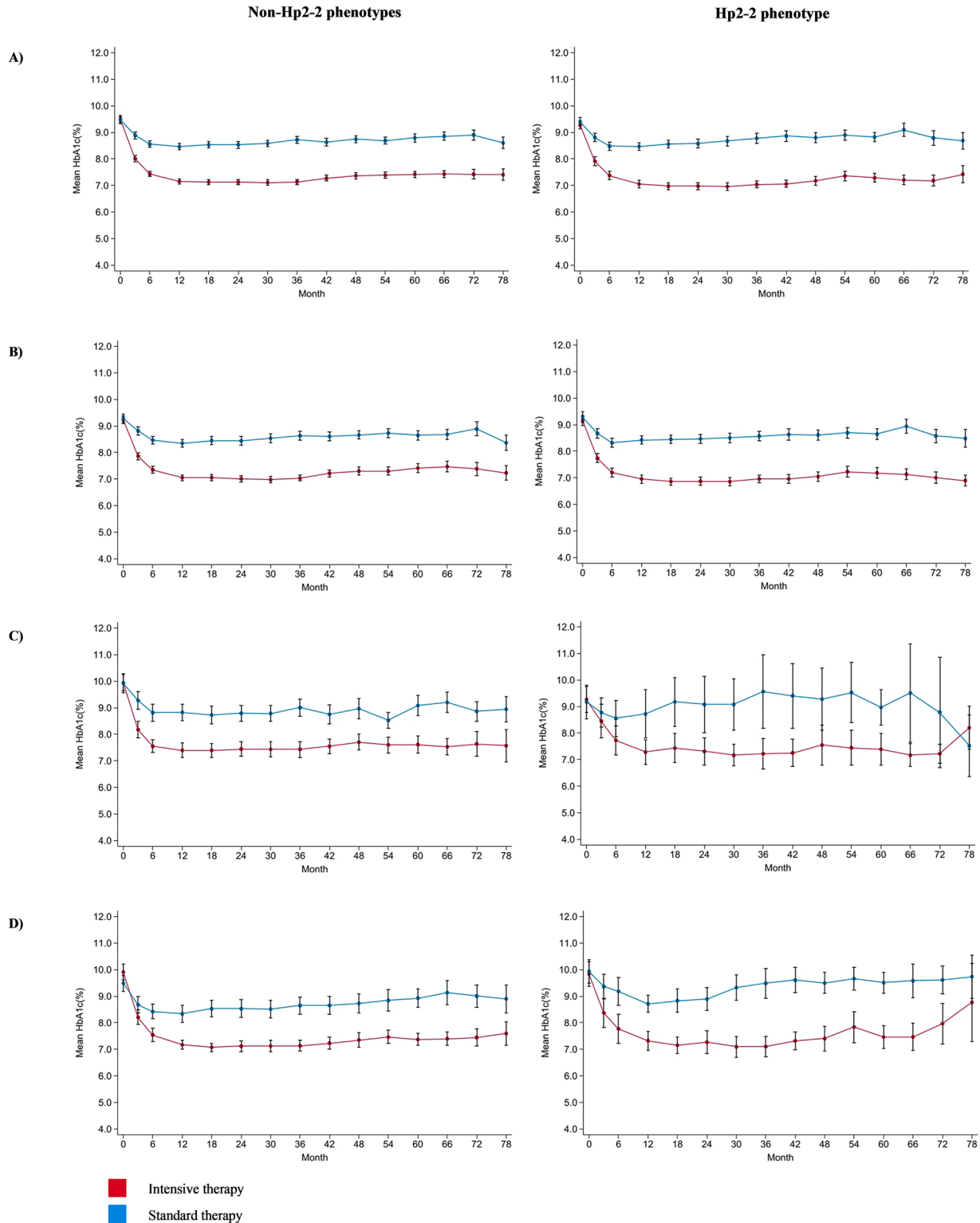Due to the differing phenotype frequencies by race/ethnicity and the

Fig. 1. Mean glycated hemoglobin levels by treatment group over study duration among (A) all participants, (B) White participants, (C) Black participants, and (D) Hispanic participants.

potential for subpopulation differences, we ran our analyses in each phenotype group overall (including all study participants) and then also further stratified within the three individual race/ethnicity-based groups in the study, which were White (62 %), Black (17 %) and Hispanic (16 %). We did not run the analysis in the "unspecified" race/ethnicity group (5 %, participants who did self-identified as belonging to a race/ethnicity other than one of the three largest race/ethnicity groups in this study) alone. When diverse populations are collapsed into a single group, racial/cultural relevance is lost and the results for this group cannot be interpreted as race/ethnicity-based. Further, collapsing race/ethnicity-based data is not consistent with current guidelines on reporting race/ethnicity-based data where specific racial categories are preferred over collective terms [19,20].

Follow-up time was defined as the time from randomization to date of documented outcome, or until a participant was censored if no event occurred.

3. Results

The distribution of Hp phenotype frequencies was 20.6 % Hp1–1, 46.9 % Hp2–1, and 32.5 % Hp2-2 and was not in HWE overall ($p = 0.04$) but was in HWE among White ($p = 0.35$), Black ($p = 0.31$) and Hispanic ($p = 0.33$) participants. Baseline characteristics by treatment group and phenotype group are found in Table 1. Characteristics that differed either between treatment groups or between phenotype groups included sex and race/ethnicity. Among participants without the Hp2-2 phenotype, the mean age was 60.2 years, 97.8 % were male, median diabetes duration was 10 years, mean glycated hemoglobin was 9.5 %, 40.5 % had a previous cardiovascular disease event and 52.8 % were taking insulin. Among participants with the Hp2-2 phenotype, the mean age was 60.8 years, 95.6 % were male, median diabetes duration was 10 years, 39.5 % had a previous cardiovascular disease event and 50.4 % were taking insulin. Mean HbA_{1c} over study duration in each treatment group for each phenotype group overall and further stratified by race/ethnicity is shown in Fig. 1. Mean HbA_{1c} differed between treatment groups throughout the study among both the non-Hp2-2 and Hp2-2 phenotype groups.

There were 435 CAD events (288 in the non-Hp2-2 phenotype group and 147 in the Hp2-2 phenotype group). The cumulative CAD incidence was 25.3 % and 24.3 % in the standard therapy arm and 23.6 % and 27.7

% in the intensive therapy arm for non-Hp2-2 and Hp2-2 phenotype groups respectively. When compared to standard therapy, intensive glycemic control was not associated with a significantly reduced risk of CAD events among participants with the non-Hp2-2 phenotype (hazard ratio (HR)=0.89, 95 % C.I.: 0.70–1.12) or with the Hp2-2 phenotype (1.22, 0.87–1.70, p-interaction=0.13) (Table 2). A sensitivity analysis excluding participants who had incident CAD within the first 12 months of the study resulted in materially unchanged results, as did a sensitivity analysis stratified by whether participants had a prior CVD event at baseline. Similar results were obtained among White, Black and Hispanic participants.

Compared to having HbA_{1c} 7.0–7.9 %, having HbA_{1c} <7.0 % was not associated with a reduced risk of CAD among participants without (0.73, 0.52–1.03) or with the Hp2-2 phenotype (1.25, 0.78–2.01), nor when the results were further stratified by race/ethnicity-based group for either phenotype group (Table 3). Compared to having HbA_{1c} 7.0–7.9 %, having HbA_{1c} ≥8.0 % was not associated with risk of CAD for either phenotype group overall or among White and Black participants. Having HbA_{1c} ≥8.0 % compared to 7.0–7.9 % was associated with an increased risk of CAD among Hispanic participants without the Hp2-2 phenotype (HR= 3.61, 95 % CI: 1.55–8.41) while no significant association was observed among Hispanic participants with the Hp2-2 phenotype (0.91, 0.31–2.68) (all p-interactions >0.05).

We performed sensitivity analyses of other outcomes including the VADT primary outcome of composite major CVD, and all-cause mortality (Supplementary Tables 1–4). In accordance with our CAD results, there was no evidence to suggest the effect of intensive glucose lowering on those events is dependent on Hp phenotype in the VADT study.

4. Discussion

We previously found that intensive glycemic control versus standard therapy was effective at preventing incident CAD events among White ACCORD study participants with the Hp2-2 phenotype while there was no association among participants without the Hp2-2 phenotype [12]. In the present study, we did not find sufficient evidence to suggest that the effect of intensive glycemic control on CAD risk is dependent on Hp phenotype in the VADT study. However, several factors that could have affected our results in the current study (including characteristics specific to the population studied and sample size) make it difficult to

Table 2

Multivariable adjusted hazard ratios (aHR) for CAD* events comparing assignment to intensive therapy versus standard therapy for each phenotype group separately, and further stratified by each of the three largest race/ethnicity groups (White, Black, Hispanic).

	Standard		Intensive		Hazard Ratios (HRs)	
	No. of events/n	Person-years	No. of events/n	Person-years	uHR (95 % CI)	aHR** (95 % CI)
Non-Hp2–2 Phenotype						
Overall	146/578	2467.11	142/601	2586.50	0.93 (0.74–1.17)	0.89 (0.70–1.12)***
White	101/342	1391.11	91/341	1393.19	0.90 (0.67–1.19)	0.87 (0.65–1.16)
Black	23/127	573.20	21/121	538.61	0.97 (0.53–1.75)	1.09 (0.55–2.17)
Hispanic	15/84	391.69	22/112	527.09	1.09 (0.56–2.10)	1.00 (0.49–2.06)
Hp2–2 Phenotype						
Overall	72/296	1324.12	75/271	1193.24	1.16 (0.84–1.60)	1.22 (0.87–1.70)***
White	57/213	930.41	54/187	804.68	1.10 (0.76–1.59)	1.19 (0.81–1.74)
Black	2/18	95.32	6/27	123.30	2.34 (0.47–11.60)	12.15 (0.58–255.87)
Hispanic	10/47	231.81	9/39	182.82	1.14 (0.46–2.80)	1.72 (0.61–4.88)

CAD= coronary artery disease; CI= confidence interval; Hp= haptoglobin; aHR= adjusted hazard ratio; uHR= unadjusted hazard ratio.

* The CAD event outcome is a composite of fatal and non-fatal MI, angina, inoperable CAD, invasive coronary revascularization procedure, and fatal CAD.

** Hazards ratios compared intensive therapy to standard therapy. Adjusted models were adjusted for age, sex, race/ethnicity (overall only), hospital, and the presence of a previous cardiovascular disease event at baseline.

P-values for the interaction between haptoglobin phenotype group and intervention in the adjusted model for the full cohort were 0.13 (overall), 0.17 (White), 0.62 (Black) and 0.52 (Hispanic).

*** A sensitivity analysis excluding participants who had incident CAD within the first 12 months of the study resulted in an aHR for intensive versus standard therapy of 0.90 (0.67–1.19) for non-Hp2–2 and 1.25 (0.86–1.83) for Hp2–2 (p-interaction=0.13). A sensitivity analysis stratified by prior CVD event at baseline was also conducted. For those without the Hp2–2 phenotype, the adjusted HR was 1.06 (0.71–1.57) for those without a prior CVD event at baseline and 0.80 (0.59–1.07) for those with a prior CVD event at baseline (p-interaction=0.29). For those with the Hp2–2 phenotype, the adjusted HR was 1.08 (0.62–1.86) for those without a prior CVD event at baseline and 1.27 (0.82–1.97) for those with a prior CVD event at baseline (p-interaction=0.52).

Table 3

Multivariable adjusted hazard ratios (aHR) for CAD* events comparing having time-varying achieved glycated hemoglobin (HbA$_{1c}$) of <7.0 % and ≥8.0 % to 7.0–7.9 % for each phenotype group separately, and further stratified within each of the three largest race/ethnicity groups (White, Black, Hispanic).

		HbA$_{1c}$ (%)						
		<7.0		7.0–7.9		≥8.0		
	No. of events/n	Person-years	aHR (95 %CI)	Person-years	aHR** (95 %CI)	Person-years	aHR (95 %CI)	
Non-Hp2–2 Phenotype								
Overall	287/1172	1478.70	0.73 (0.52–1.03)	1408.31	Ref.	2152.88	1.26 (0.96–1.66)	
White	191/678	797.62	0.74 (0.50–1.12)	818.38	Ref.	1159.49	1.09 (0.78–1.52)	
Black	44/247	316.31	0.59 (0.22–1.57)	253.70	Ref.	540.07	1.05 (0.55–2.00)	
Hispanic	37/195	295.81	0.84 (0.24–2.91)	260.02	Ref.	358.29	3.61 (1.55–8.41)	
Hp2–2 Phenotype								
Overall	146/561	621.80	1.25 (0.78–2.01)	717.17	Ref.	1160.79	1.18 (0.76–1.83)	
White	111/399	452.24	1.19 (0.69–2.03)	529.21	Ref.	758.42	1.33 (0.80–2.21)	
Black	8/45	61.17	0.70 (0.16–3.05)	61.76	Ref.	95.81	0.27 (0.04–1.90)	
Hispanic	18/84	74.90	0.74 (0.08–7.11)	86.47	Ref.	245.82	0.91 (0.31–2.68)	

CAD= coronary artery disease; CI= confidence interval; HbA$_{1c}$= glycated hemoglobin; Hp= haptoglobin; aHR= adjusted hazard ratio.

* The CAD event outcome is a composite of fatal and non-fatal MI, angina, inoperable CAD, invasive coronary revascularization procedure, and fatal CAD.

** Hazards ratios compared having HbA$_{1c}$ <7.0 % or ≥8.0 % to having HbA$_{1c}$ 7.0–7.9 %. Models were adjusted for age, sex, race/ethnicity (overall only), hospital, intensive therapy assignment, total cholesterol, body mass index (BMI), presence of a previous cardiovascular disease event at baseline, smoking status, systolic blood pressure, diabetes duration, statin use, any lipid lowering medication use, anti-hypertensive medication use, albumin creatinine ratio (ACR) and estimated glomerular filtration rate (GFR).

P-values for the interaction between haptoglobin phenotype group and HbA$_{1c}$ categories as a continuous variable in the adjusted model for the full cohort were 0.57 (overall), 0.98 (White), 0.21 (Black) and 0.53 (Hispanic).

determine whether the present findings are conclusive.

Participants in the VADT study had to have uncontrolled diabetes with a poor response to oral agents and insulin. Baseline insulin use was higher in the VADT study (52 % in VADT versus 35 % in ACCORD), and there was a higher proportion of patients with a previous CVD event (40 % in VADT, 35 % in ACCORD). Therefore, the VADT participants had more severe diabetes and cardiovascular disease compared to ACCORD that may not be able to be significantly altered by intensive glucose lowering. Further, blood glucose levels were higher in the VADT study compared to the ACCORD study (baseline median of HbA$_{1c}$ of 8.1 % in ACCORD versus 9.4 % in VADT). Although the two studies had the same intensive glycemic target (<6.0 %), similar pharmacological treatment strategies (multiple similar medications used in both arms of both studies), and the difference in HbA$_{1c}$ between treatment groups were similar, the end of study median HbA$_{1c}$ was higher in both treatment groups in the VADT study (6.4 % versus 7.5 % in ACCORD, 6.9 % versus 8.4 % in VADT) and participants in the VADT study may not have had sufficient glucose lowering to have a significant effect on CAD. In accordance with this hypothesis, in the ACCORD study, we also found that the reduced risk associated with intensive therapy among White and Black participants with the Hp2-2 phenotype was likely attributed to participants not having high HbA1c (≥8.0 %) rather than achieving strict glycemic control and did not support a glycemic target of <7.0 % for either phenotype group [21].

Another reason for the inconsistency between our present VADT results and our previous ACCORD study results may be related to HDL. Dysfunctional high-density-lipoprotein (HDL) is an important component in the mechanism linking hyperglycemia to CAD. In brief, it is well established that people with the Hp2-2 protein produce a Hp protein that is larger and less effective at removing oxidative hemoglobin (Hb) from the blood (a primary function of Hp) when compared to the Hp1–1 and Hp2–1 proteins. In hyperglycemic conditions (HbA1c ≥ 6.5 %), the function of Hp2-2 is further impaired, resulting in increased oxidative stress and oxidative modification of HDL (Hp can bind to HDL and

thereby tether Hb to HDL), which paradoxically renders HDL dysfunctional and makes it oxidative and pro-atherogenic and increases susceptibility to CAD in people with the Hp2-2 phenotype [5,9,11,22–26]. In accordance with the proposed biological mechanism, we previously demonstrated that compared to statin monotherapy, adding fenofibrate (HDL-cholesterol raising and triglyceride lowering drug) to statin therapy reduced the risk of CAD events among ACCORD lipid study participants with the non-Hp2-2 phenotype (0.74, 0.60–0.90) but not among those with the Hp2-2 phenotype (1.16, 0.87–1.56, p, interaction=0.009) [27]. This effect was pronounced in female participants (p-interaction=0.002) who have naturally higher HDL-cholesterol levels compared to male participants. We also demonstrated that Hp phenotype modified the relationship between HDL-cholesterol and CAD with higher HDL being CAD protective among ACCORD participants without the Hp2-2 phenotype but not those with the Hp2-2 phenotype [28]. The VADT study participants were predominantly (>95 %) male and had lower average HDL-cholesterol levels at baseline compared to the ACCORD study participants (47 mg/dL for women and 39 mg/dL for men in ACCORD versus 36 mg/dL in VADT) and the VADT study population had more advanced disease which can also lead to increased oxidative stress and affect HDL quality [29]. It is possible that serum levels and quality of HDL may affect the relationship between Hp phenotype and CAD in hyperglycemia which could help to explain why we did not find significant results in the present analysis of mostly male participants with lower HDL-cholesterol and more advanced disease.

Hp phenotype distribution varies according to race/geography [6] and in the current study, we saw that the two phenotype groups had different race/ethnicity distribution (Table 1). Further, HWE was not met when all participants were combined but was met in each of the three largest race/ethnicity-based groups in this study (White, Black and Hispanic participants separately) and the Hp2-2 phenotype frequency among White participants (37 %) was the same as what was observed in our previous ACCORD analysis [12]. We have also previously demonstrated that stratification by race/ethnicity-based group is an important

consideration in studies related to Hp phenotype [12,21] due to the potential for confounding related to systemic differences between populations. As such, we stratified our results by the three largest race/ethnicity-based groups in this study. This was also important because our previous significant findings related to intensive glycemic control and HbA$_{1c}$ were among the two largest race/ethnicity groups in the ACCORD study (White and Black participants separately) [12,21]. When stratified by race/ethnicity-based group, we did not find evidence to suggest that intensive glucose lowering reduced the risk of CAD for either phenotype group, although we were limited by sample size. Among Hispanic participants, we found that having HbA$_{1c}$ ≥8.0 % compared to 7.0–7.9 % was associated with an increased risk of CAD among participants without the Hp2-2 phenotype. The non Hp-2-2 phenotype is the largest group in this study (and therefore had the more power than the Hp2-2 group to detect a significant effect), and in the original VADT trial it was found that Hispanic participants had a greater response to treatment and a trend of a reduced CVD risk from intensive therapy [30]. The reason for the "enhanced cardiovascular response" of Hispanic participants to glucose lowering was unknown but the authors speculate that a lower burden of subclinical atherosclerosis in Hispanics contributed to reduced CVD outcomes in the intensive arm. As such, it is unlikely that our finding in Hispanic participants was related to Hp phenotype.

Some limitations of the current study deserve attention. We were limited by sample size in most of our subgroup analyses (Supplementary Table 5), which would hamper the ability to identify a significant effect, and ideally, we would have further restricted our results to participants without a history of CVD at baseline and conducted sex-stratified analyses had the sample size allowed. However, the hazard ratios observed in the present study were in the opposite direction of those previously observed in ACCORD and for this reason, the findings of this study should be viewed in the context of hypothesis-generation, and hopefully will stimulate further research in this area. Further, this post-hoc analysis provided an opportunity to examine the association between intensive glycemic control and CAD events by haptoglobin phenotype and among major race/ethnic groups in the context of a clinical trial and can be used in a meta-analysis with more power for subgroup analyses as the need to study the influence of Hp type on the relationship between glycemic control and risk of CAD in a more representative population remains a priority. Another limitation of this study was that participants were mostly older males with uncontrolled diabetes, limiting generalizability of the results (these results are not generalizable to populations with controlled diabetes and wider ranges of HbA$_{1c}$). There may be other unmeasured confounders that could have influenced our results such as HDL function, physical activity, or diet.

In summary, we did not find evidence to suggest the effect of intensive glucose lowering on CAD events is dependent on Hp phenotype in the VADT study of participants with severe diabetes and suboptimal response to diabetes therapy. The VADT study participants may represent a population where Hp phenotype information would not be useful for personalizing diabetes management and further research is needed to determine if these results are conclusive.
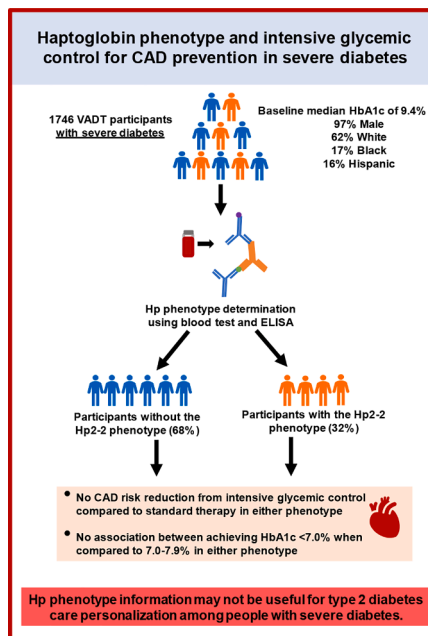
Funding

Disclosures

Dr. Levy is the author of a patent owned by his university regarding use of haptoglobin genotype to predict susceptibility to cardiovascular disease in individuals with diabetes. All other authors have nothing to disclose.

Central illustration

CRediT authorship contribution statement

Leah E Cahill: Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **Rachel A Warren:** Writing – review & editing, Writing – original draft, Software, Project administration, Methodology, Formal analysis, Data curation. **Gideon D Bahn:** Writing – review & editing, Resources, Methodology. **Allie S Carew:** Writing – review & editing, Validation. **Andrew P Levy:** Writing – review & editing, Resources, Methodology. **John Sapp:** Writing – review & editing. **Eric B Rimm:** Writing – review & editing. **Peter Reaven:** Writing – review & editing.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Leah Cahill reports financial support was provided by Canadian Institutes of Health Research. Andrew P. Levy has patent issued to Technion - Israel Institute of Technology. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work

reported in this paper.

Acknowledgements

The authors sincerely thank the Department of Veterans Affairs (VA) Cooperative Studies Program for providing access to the database of VADT data and frozen serum samples for haptoglobin phenotyping, as well as the staff and participants of the VADT study. All statements, opinions, or views are solely of the author(s) and do not reflect those of the US Department of Veterans Affairs.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ajpc.2024.100681.

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