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ORIGINAL RESEARCH

Glucose-6-Phosphate Dehydrogenase Modifies the Impact of Glucose on Arterial Aging in A Sex-Specific Manner

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BACKGROUND: An association between glucose-6-phosphate dehydrogenase deficiency (G6PDdef) and arterial stiffness has cross sectionally been reported in diabetic, but not in nondiabetic, subjects in Sardinia. The aims of the present longitudinal study were to evaluate: (1) sex differences in the influence of G6PDdef on trajectory of arterial stiffness; (2) the impact of hyperglycemia on trajectory of arterial stiffness; and (3) mechanistic pathways underlying this influence.

METHODS: In the Sardinia Study, repeated measurements of pulse-wave velocity were analyzed to characterize trajectories of arterial stiffness in >4000 men and women, aged 20 to 100 years, during a 9.4-year follow-up.

RESULTS: In men with high glucose, G6PDdef was associated with a steeper longitudinal increase in pulse-wave velocity (yearly change: 12.5 versus 6.9 cm/s in those without G6PDdef). In contrast, in women with high glucose, the longitudinal change is not influenced by the presence or absence of G6PDdef (yearly change: 4.5 cm/s in both groups). With high glucose, men with G6PDdef showed similar trends in fat deposition, but no substantial increase in triglycerides, over time compared with those without G6PDdef. Conversely, women with G6PDdef showed a greater gluteofemoral fat deposition over time, with no increase in visceral fat deposition, and a similar trajectory in triglycerides compared with those without G6PDdef.

CONCLUSIONS: Lower G6PD activity is associated with an accelerated increase in arterial stiffness in men, but not in women. This effect is accompanied by sex-specific changes in metabolic factors. Future studies will characterize the link between metabolic reprogramming and changes in arterial stiffness.

Key Words: central arterial stiffness ■ glucose-6-phosphate dehydrogenase ■ longitudinal study ■ pulse-wave velocity ■ sex differences

Carotid-femoral pulse-wave velocity (PWV) is a reliable and reproducible index of central arterial stiffness (CAS) in clinical settings.[1] Greater PWV values have been associated with multiple organ damage,[2] increased cardiovascular morbidity and mortality,[3] and cognitive impairment.[4] It has also emerged as a proxy of arterial aging, capturing the continuum from early (accelerated) vascular aging[5] to healthy vascular aging (ie, "slower than average" arterial aging),[5] and being a significant determinant of age-associated central arterial remodeling.[6]

The search for determinants of CAS suggested a complex framework: genetic loci,[7] blood composition,[8] inflammatory markers,[9,10] and metabolic alterations.[11] Gaps remain in the comprehension of the heterogeneity of the mechanisms linking metabolic alterations to CAS.

The island of Sardinia is characterized by having the highest prevalence (~8%–15%) in glucose-6-phosphate

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CLINICAL PERSPECTIVE

What Is New?

- Glucose-6-phosphate dehydrogenase activity is associated with a steeper increase in the trajectory of central arterial stiffness that is significant in men, but not in women.
- This effect becomes clinically relevant with concomitant hyperglycemia.

What Are the Clinical Implications?

- A deeper characterization of the observed sex-specific impact of reduced glucose-6-phosphate dehydrogenase activity on the trajectory of central arterial stiffness can contribute to a better understanding of sex differences in cardiovascular risk.

Nonstandard Abbreviations and Acronyms

CAS	central arterial stiffness
G6PD	glucose-6-phosphate dehydrogenase
G6PDdef	glucose-6-phosphate dehydrogenase deficiency
LME	linear mixed-effects (model)
MBP	mean blood pressure
PP	pulse pressure
PWV	pulse-wave velocity
SBP	systolic blood pressure

dehydrogenase (G6PD) deficiency (G6PDdef) worldwide.[12] Notably, the *G6PDX* gene, whose mutations cause G6PDdef, maps on the X chromosome.[13] G6PD contributes to the metabolism of glucose-6-phosphate, representing the rate-limiting enzyme in the pentose phosphate pathway. In normal conditions, ≈10% of glucose physiologically enters the pentose phosphate pathway.[14]

To date, the clinical relevance of G6PD deficiency has largely focused on its association with acute hemolytic anemia induced by exposure to fava beans or to selective drugs.[12] A few studies reported a higher risk for diabetes[15,16] and opposite effects on cardiovascular mortality and cardiovascular risk profiles[17,18] in G6PDdef subjects. Little is known about any clinical impact of G6PD activity on the vascular system in humans. We have previously reported that G6PDdef is associated with accelerated arterial aging (greater PWV) in diabetic, but not in not diabetic, subjects participating the Sardinia Study.[19]

Using data from the Sardinia Study, we sought to determine whether participants can be classified into distinct PWV trajectory groups based on G6PDdef and hyperglycemia and whether these trajectories differed in men and women. We additionally sought to describe the concomitant changes in fat distribution and metabolism associated with lower G6PD activity on the trajectory of CAS.

METHODS

Study Population

The Sardinia Study was approved by the Sardinian Regional Ethics Committee (protocol No. 2171/CE) and data safety commissioner before study initiation. All procedures were performed in accordance with the principles of the Declaration of Helsinki as well as the tenets of Good Clinical and Epidemiological Practice. Participants provided written informed consent before study enrollment.

Based on the written consent of the study participants, the data will only be made available locally within the framework of the access procedure specified in the rules of procedure of the research project. Interested researchers should direct their enquiries to the principal investigators.

The Sardinia Study investigates the genetics and epidemiology of complex traits/phenotypes, including cardiovascular risk factors and arterial properties, in a community-dwelling Sardinian founder population.[2] The subset of the Sardinia population for the present analysis consists of 4947 volunteers (2143 men and 2804 women) who entered the study over of a wide age range (20–101 years). See Figure 1 for details. Sex assignment at birth was used to stratify the cohort.

All participants signed informed consent to study protocols approved by the Sardinian Regional Ethics Committee (protocol No. 2171/CE).

G6PD Activity

Red blood cell G6PD activity was determined using a quantitative assay. G6PD deficiency (G6PDdef) was defined as G6PD activity <0.8 UI/g hemoglobin,[20] corresponding to a 80 value in the metric adopted in the present study. This threshold value had been chosen to identify individuals at greater risk of hemolytic anemia because of the genetic inherited deficit in G6PD.

Longitudinal Analysis: Trajectories of Outcome Variables Measured Over Time

The following variables were measured serially over time following a baseline measurement with participants having 1, 2, or 3 repeated measurements (Figure 1).

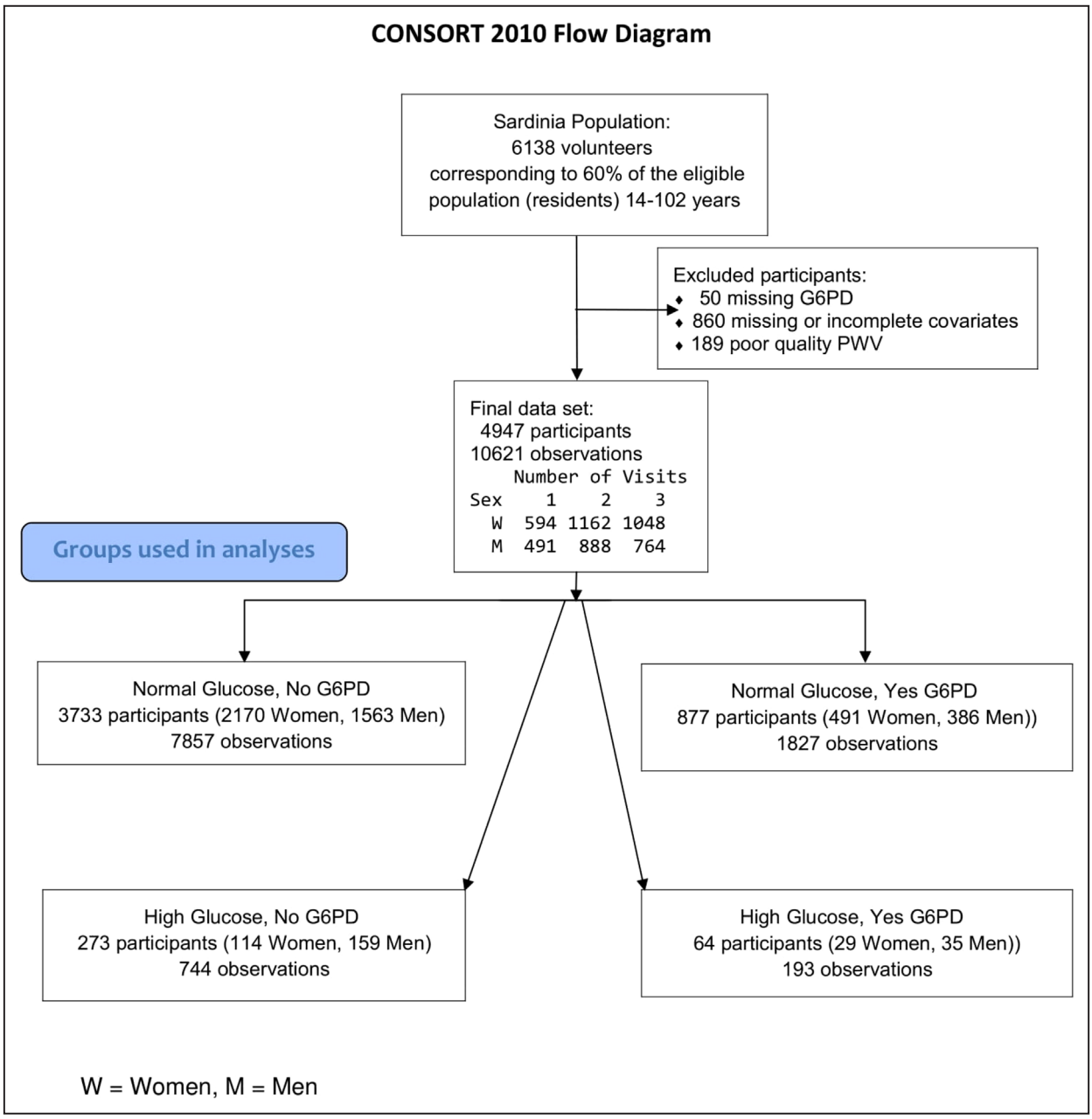

Figure 1. CONSORT-style flow diagram describing study population and follow-up completion.
G6PD indicates glucose-6-phosphate dehydrogenase; M, men; PWV, pulse-wave velocity; and W, women.

Blood Pressure

Blood pressure was measured in both arms with a mercury sphygmomanometer using an appropriately sized cuff after 15-minute rest in a dark, quiet room. Values for systolic blood pressure (SBP) and diastolic blood pressure (BP) were defined by Korotkoff phases I and V, respectively. The average of second and third measurements on both the right and left arm were used in the analysis. Pulse pressure (PP) was computed as PP=(SBP−diastolic BP); mean BP (MBP) was computed as MBP=diastolic BP+(PP/3).

Anthropometry

Height, weight, and waist circumference were determined for all participants. Body mass index was calculated as body weight (kg)/height (m^2).

According to the World Health Organization's data gathering protocol,[21] the waist circumference should be measured at the midpoint between the lower margin of the last palpable ribs and the top of the iliac crest, using a stretch-resistant tape. Hip circumference should be measured around the widest portion of the buttocks, with the tape parallel to the floor.

Fasting Glucose and Lipids

Blood samples were drawn from the antecubital vein between 7 and 8 AM after an overnight fast. Subjects were not allowed to smoke, engage in significant physical activity, or take medications before the collection of the samples. Plasma triglycerides and total cholesterol were determined by an enzymatic method (Abott Laboratories ABA-200 ATC Biochromatic Analyzer, Irving, TX). High-density lipoprotein cholesterol was determined by a dextran sulfate-magnesium precipitation. Low-density lipoprotein (LDL) cholesterol concentrations were estimated by the Friedewald formula. Fasting plasma glucose concentration was measured by the glucose oxidase method (Beckman Instruments Inc, Fullerton, CA).

Aortic Stiffness

Carotid-femoral PWV was measured as previously described[9] using nondirectional transcutaneous Doppler probes (model 810A, 9- to 10-Mhz probes; Parks Medical Electronics, Inc, Aloha, OR).

Statistical Analysis

Analyses were performed using SAS on Demand for Academics–SAS Studio version 9.04 (SAS Institute Inc, Cary, NC) or R (version 4.3.3). Data are presented as mean±SD unless otherwise specified.

For baseline data, ANOVA was used for numerical variables to test for interaction between sex and G6PD activity on PWV and established cardiovascular risk factors. Logistic models are used for categorical variables.

To identify predictors of the trajectory of CAS, we run 2 sets of linear mixed-effects (LME) models on this repeated-measures data, separately for men and women. Both sets of LME models initially included interaction terms between glucose, G6PDdef, and time to determine if the longitudinal trajectories vary depending on the combination of G6PDdef and glucose level.

The first set of models aimed at investigating the possible impact of G6PDdef according to glucose levels on the trajectory of CAS, indexed as PWV, after accounting for established cardiovascular risk factor (SBP, body mass index, LDL cholesterol, and glucose) levels and their interaction with time and G6PDdef. The second set of models aimed at investigating the possible impact of G6PDdef on the trajectory of CAS, independently of the known role of distending BP on CAS. Therefore, PWV normalized for MBP was the dependent variable in the second set of LME models.

The LME model contains a random effect for subject that allows the level of the longitudinal trajectories to vary among participants. Unfortunately, given the small number of repeated measurements, the data would not support the inclusion of a random effect for time. Fortunately, because the focus of the article is on average differences among the 4 groups, using a single random effect will not adversely affect the estimates and significance of the fixed effects. We consider the marginal and conditional R^2 values as a way of assessing the utility of the models.

Average model predicted yearly changes and percentage changes were calculated from the fixed effects of the linear mixed effect models over a 6-year period.

To evaluate the impact of glucose levels on PWV trajectory in men and women without or with G6PDdef, we calculated trajectory of PWV from the estimates of the LME models containing glucose and its interaction terms. For the purposes of estimating the group-specific slopes, high glucose was defined as a fasting glucose of 120 mg/dL and low glucose as a fasting glucose of 80 mg/dL.

RESULTS

Using 10 621 observations of data spanning 13 years with a median follow-up of 9.4 years, 2143 men and 2804 women were classified into 4 distinct groups based on sex and G6PDdef.

The identification of these 4 groups, based on biological plausibility, is further supported by the analysis of G6PD activity distribution in men and women, showing the presence of 2 separate groups of individuals, one showing extremely low G6PD activity (the G6PDdef group) and the remainder showing a more symmetric distribution centered in the 130s (Figure S1).

The Table summarizes baseline characteristics of study variables in the 4 groups. Both men and women with reduced G6PD activity had lower LDL cholesterol levels compared with men and women without reduced G6PD activity. Men presented higher glucose levels at baseline than women, but there was no observable difference in the trajectory of glucose levels by sex or by G6PDdef status (Figure S2). In addition, vascular stiffness, as captured by PWV, does not differ by sex or G6PD groups at baseline (). PWV did not differ between men or women at any age according to their G6PDdef status (Figure S3).

Tables S1 and S2 illustrate the validity of the ANOVA model assumptions, contain the median and quartiles for the study variables, and include a test of normality of the residuals from the ANOVA models using the Shapiro-Wilk statistic. The Shapiro-Wilk statistic shows that the transformed data are closer to normal. A test that uses robust variances using the 2-way function from the R package WRS2 has been included. The

Table Baseline Characteristics in Men and Women With or Without G6PDdef

Characteristic	Men (n=2143)		Women (n=2804)		*P* value*		
	19.6		18.5				
	(95% CI, 18.0–21.4)		(95% CI, 17.1–20.0)				
	No G6PDdef (n=1722)	Yes G6PDdef (n=421)	No G6PDdef (n=2284)	Yes G6PDdef (n=520)	Sex main effect	G6PD main effect	Sex×G6PD interaction
Age, y	43.4±16.9	42.3±17.8	43.8±16.8	44.3±16.6	0.042	0.573	0.189
G6PD activity, UI/g hemoglobin	134.6±19.5	10.3±10.2	130.5±20.5	28.0±24.4	<0.0001	<0.0001	<0.0001
Hypertension, %	35.0	31.8	22.9	23.8	<0.0001	0.628	0.223
Diabetes, %	5.0	3.8	3.1	3.5	0.002	0.644	0.287
Obesity, %	12.9	10.5	12.3	10.4	0.607	0.206	0.857
BMI, kg/m^2	26.0±3.4	25.5±3.4	24.8±4.1	24.5±3.9	<0.0001	0.225	0.276
Waist circumference, cm	89.6±9.6	88.5±9.9	80.5±11.0	80.4±10.9	<0.0001	0.752	0.236
Hip, cm	97.1±6.0	96.8±6.2	97.8±7.4	97.2±6.8	0.001	0.082	0.624
WHR, %	92.1±6.8	91.3±6.8	82.2±8.0	82.5±8.3	<0.0001	0.361	0.037
SBP, mm Hg	130±17	129±16	122±18	122±18	<0.0001	0.980	0.316
DBP, mmHg	79.7±10.9	77.9±10.5	75.3±10.2	74.9±9.7	<0.0001	0.538	0.039
MBP, mm Hg	96.4±11.8	94.7±11.4	90.7±12.1	90.5±11.5	<0.0001	0.708	0.089
PP, mm Hg	50.6±12.8	51.1±12.5	46.9±12.6	47.2±12.7	<0.0001	0.638	0.752
HR, bpm	64.4±11.3	64.2±10.6	68.7±10.7	68.4±10.3	<0.0001	0.563	0.782
Fasting glucose, mg/dL	93.2±25.3	92.7±23.4	86.7±20.4	87.7±21.3	<0.0001	0.403	0.383
Total cholesterol, mg/dL	210±44	200±42	210±40	208±41	0.623	0.212	0.015
LDL cholesterol, mg/dL	131±36	123±34	127±34	124±36	0.001	0.065	0.066
HDL cholesterol, mg/dL	58.7±13.1	57.4±12.5	68.1±14.7	69.0±15.0	<0.0001	0.191	0.029
Triglycerides, mg/dL	98.0±62.5	92.8±56.7	76.4±41.6	73.4±39.6	<0.0001	0.226	0.539
PWV, cm/s	680±224	662±210	666±225	659±201	0.050	0.524	0.481
PWV/MBP, cm/s per mm Hg	703±210	697±195	730±206	725±180	<0.0001	0.607	0.912

Data are presented as mean±SD unless otherwise specified. BMI indicates body mass index; bpm, beats per minute; DBP, diastolic blood pressure; G6PD, glucose-6-phosphate dehydrogenase; G6PDdef, G6PD deficiency; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MBP, mean blood pressure; PP, pulse pressure; HR, heart rate; PWV, pulse-wave velocity; SBP, systolic blood pressure; and WHR, waist/hip ratio.

*For numerical variables, *P* values are obtained from 2-way ANOVA models, whereas for categorical variables, a logistic generalized linear model is used.

3 tests yield almost identical results for almost all variables.

Sex Differences on the Impact of G6PD Activity and Glucose Levels on the Longitudinal Trajectory of Central Arterial Stiffness

To model the longitudinal trajectories, we fit LME models using a random effect for subject that allows the level of the longitudinal trajectories to vary among participants. This repeated-measures model examined relations of the dependent variables with concurrent age and risk factor levels across the repeated measurements. All evaluations at each examination cycle with a valid assessment of PWV and covariates were included in these analyses. Covariates included age at baseline, time (linear and quadratic terms as needed), G6PDdef, established cardiovascular risk factor levels (fasting glucose, BP, body mass index, and LDL cholesterol), and their interactions with time and with G6PDdef. This method has been explained previously.[22] Interactions are entertained to allow the longitudinal trajectories to vary depending on G6PDdef and glucose level. Backward elimination from a proposed full model is used to obtain the final model. Models are constrained to be well formulated.[23] The marginal and conditional R^2 values[24] are compared between the full and final models. In all cases, these measures of fit did not decline substantially from the full to the final model.

The final LME models obtained after backward elimination of not significant terms are summarized in Tables S4 and S5.

Figure 2 presents the smoothed average longitudinal trajectories of PWV as a function of the presence of G6PDdef and high glucose levels for 2143 men and 2804 women over a median follow-up of 7 years (maximum, 13 years) for a total of 10 621 observations.

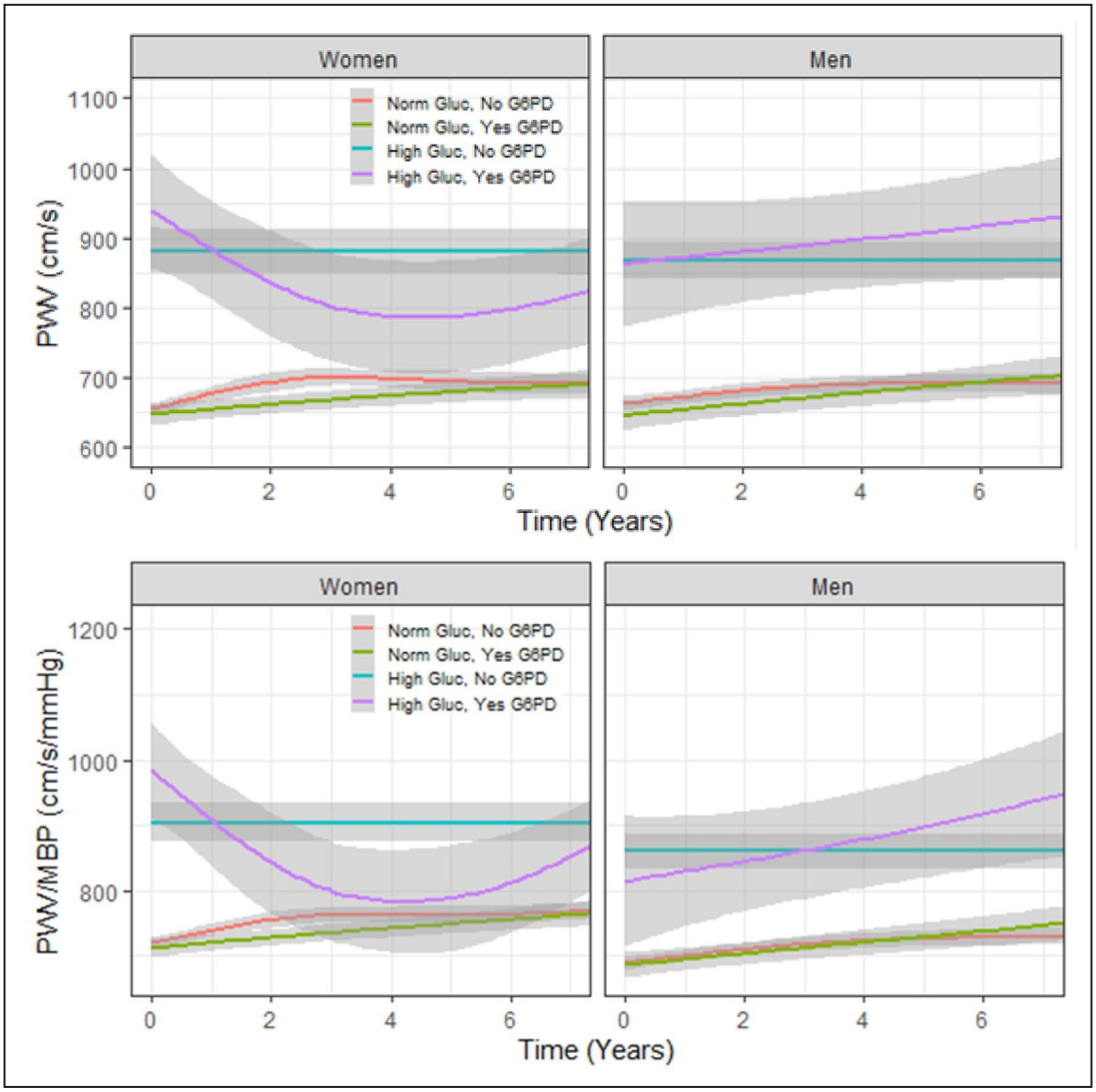

Figure 2. Smoothed trajectories of PWV in men and women.
Solid lines represent participants with normal glucose levels and without G6PDdef. Dashed lined represent participants with normal glucose levels and with G6PDdef. Dotted lines represent participants with high glucose levels and without G6PDdef. Dashed-dotted lined represent participants with high glucose levels and with G6PDdef. G6PD indicates glucose-6-phosphate dehydrogenase; G6PDdef, G6PD deficiency; Gluc, glucose; Norm, normal; and PWV, pulse-wave velocity.

With normal glucose levels, PWV slightly increased over time in a similar manner in subjects with or without G6PDdef both in men and in women (Figure 2A and 2B). In men, hyperglycemia was associated with a steeper trajectory in PWV compared with those without G6PDdef. From baseline to year 7, on average, PWV increased from 890 to 950 cm/s in men with G6PDdef, whereas there was a flat trend in those without G6PDdef (Figure 2B). Conversely, in women with high glucose levels, G6PDdef was associated with a possible U-shaped trajectory in PWV, with values always lower than in women without G6PDdef (Figure 2A).

The predicted longitudinal rates of change of PWV for subjects with normal or high glucose levels with or without G6PDdef are illustrated in Figure 3 by the yearly average rate of change over 6 years of PWV during the observation period within the same group of participants. In men, G6PDdef was accompanied by a steeper increase in PWV over time, with normal glucose levels (average yearly change: 11.7 versus 6.1 cm/s), that was marginally greater with hyperglycemia (average yearly change: 12.5 versus 6.9 cm/s). In women, no difference in the trajectory of PWV was associated with G6PDdef with normal (average yearly change: 8.9 cm/s in both groups) or with high glucose levels (average yearly change: 4.5 cm/s in both groups).

The graphs in Figure 4 illustrate predicted longitudinal changes of PWV/MBP for subjects with normal

		MEN	WOMEN
Norm Gluc	noG6PD	6.1	8.9
Norm Gluc	yesG6PD	11.7	8.9
High Gluc	noG6PD	6.9	4.5
High Gluc	yesG6PD	12.5	4.5

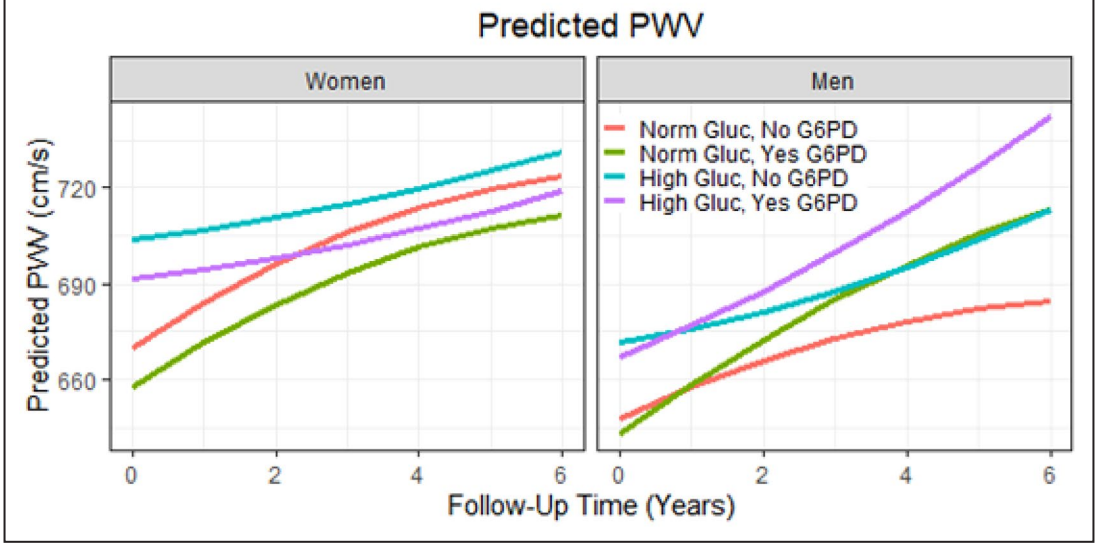

Figure 3. Model-predicted trajectories of PWV of the 4 different groups, labeled as in Figure 1, in men and women.

Refer to the model in Table S4 for statistics on the significance of differences in slopes. Significant interactions include: men: time^2 with glucose (P=0.0103), time with G6PD deficiency (P=0.0048); women: time^2 with glucose (P=0.0134). The longitudinal plots of the modeled data are constructed for population age=40 years, systolic blood pressure=120 mm Hg (when applicable), body mass index24 kg/m^2 (when applicable), normal glucose=80 mg/dL, and high glucose=120 mg/dL. G6PD indicates glucose-6-phosphate dehydrogenase; Gluc, glucose; MBP, mean blood pressure; Norm, normal; and PWV, pulse-wave velocity.

or high glucose levels with or without G6PDdef. The trends are similar to those observed for PWV.

Sex Differences on the Impact of G6PD Activity and Glucose Levels on the Longitudinal Trajectory of Adiposity and Triglycerides

We then explored the sex-specific impact of G6PD activity and glucose levels on longitudinal changes in BP, established cardiovascular risk factors known to be associated with stiffer arteries.

In men with high glucose levels, the trajectories of SBP, MBP, and PP indicated a marginally larger decrease when high glucose levels were accompanied with G6PDdef. This trend was not observable in the other groups of men nor in women (Figure S4).

To identify the possible contribution of metabolic changes to the observed sex differences in the trajectory of CAS with G6PDdef and higher glucose levels, waist and hip circumferences (and the waist/hip ratio) were selected as indexes of energy (fat) storage and deposition; triglyceride levels were selected as an index of de novo lipogenesis.

In men, no substantial differences in the trajectory of waist or hip circumferences, and waist/hip ratio, were observed between subjects with or without G6PDdef in normal or with high glucose levels (Figure 5A–C). Conversely, men with G6PDdef and high glucose levels did not show any substantial increase in triglyceride levels over time (Figure 5D).

In women, the combination of G6PDdef and higher glucose levels was associated with a slight decrease over time in waist/hip ratio compared with women with high glucose but no G5PDdef, suggesting a relatively higher increase in hip than in waist circumference, indicating a tendency toward a preferential storage of excess of energy (glucose) availability in the gluteofemoral rather than in the visceral fat (Figure 5A–C). No meaningful difference in the trajectory of triglycerides was observed between women with or without G6PDdef, in the presence of normal or higher glucose levels (Figure 5D).

	MEN	WOMEN
Norm Gluc noG6PD	7.6	9.7
Norm Gluc yesG6PD	12.9	9.7
High Gluc noG6PD	7.5	4.1
High Gluc yes G6PD	12.8	4.1

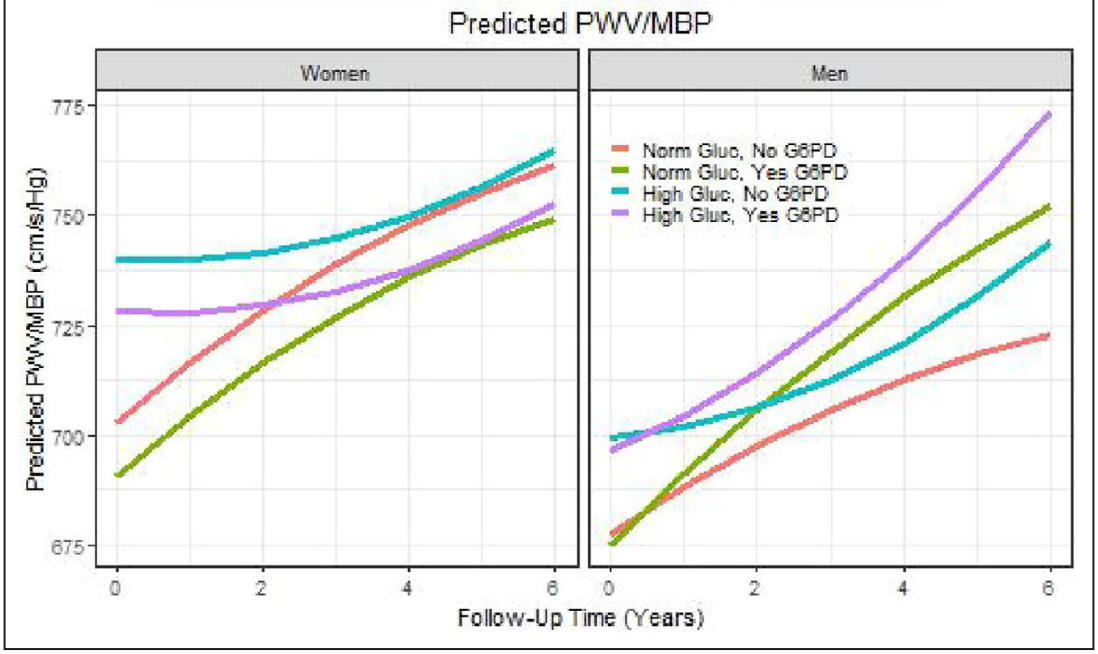

Figure 4. Model-predicted trajectories of PWV normalized for MBP (PWV/MBP) of the 4 different groups, labeled as in Figure 1, in men and women.
Refer to the model in Table S5 for statistics on the significance of differences in slopes. Significant interactions include: men: time^2 with glucose (P=0.0042), time with G6PD deficiency (P=0.0132); women: time^2 with glucose (P=0.0061). The longitudinal plots of the modeled data are constructed for population age=40 years, systolic blood pressure=120 mm Hg (when applicable), body mass index=24 kg/m^2 (when applicable), normal glucose=80 mg/dL, and high glucose=120 mg/dL. G6PD indicates glucose-6-phosphate dehydrogenase; Gluc, glucose; MBP, mean blood pressure; Norm, normal; and PWV, pulse-wave velocity.

No sizeable difference in high-density lipoprotein trajectory was observable in the 4 groups in both men and women (Figure 5E).

DISCUSSION

The present study, conducted in a large population of men and women over a broad age range, suggests that: (1) in the presence of higher glucose levels, lower G6PD activity is associated with a steeper longitudinal increase in CAS; (2) this trend is significant in men, but not in women; and (3) metabolic changes accompany the sex differences in the trajectory of CAS in G6PDdef and high glucose levels: in men, there were no changes in the waist or hip trajectory with a reduced increase in triglycerides; women exhibited a decreased waist/hip ratio.

The observation that the steeper increase in PWV in men with high glucose and G6PDdef is paralleled by a marginally larger decrease in SBP and PP seems counterintuitive. Previous studies reported a positive association between higher PWV and longitudinal changes in SBP[25,26] as well as between higher SBP and longitudinal changes in PWV.[27] However, other studies with repeated measurements of both PWV and BP showed a dissociation between SBP and the trajectory of PWV: a lower increase in systolic pressure over time for higher PWV in men after the age of 50 years[28] or among middle-aged adults.[29]

The observed dissociation between the trajectory of PWV and of SBP supports the hypothesis that the concomitant occurrence of high glucose and G6PDdef in men may act through changes in the arterial wall. We have recently found that carotid artery remodeling

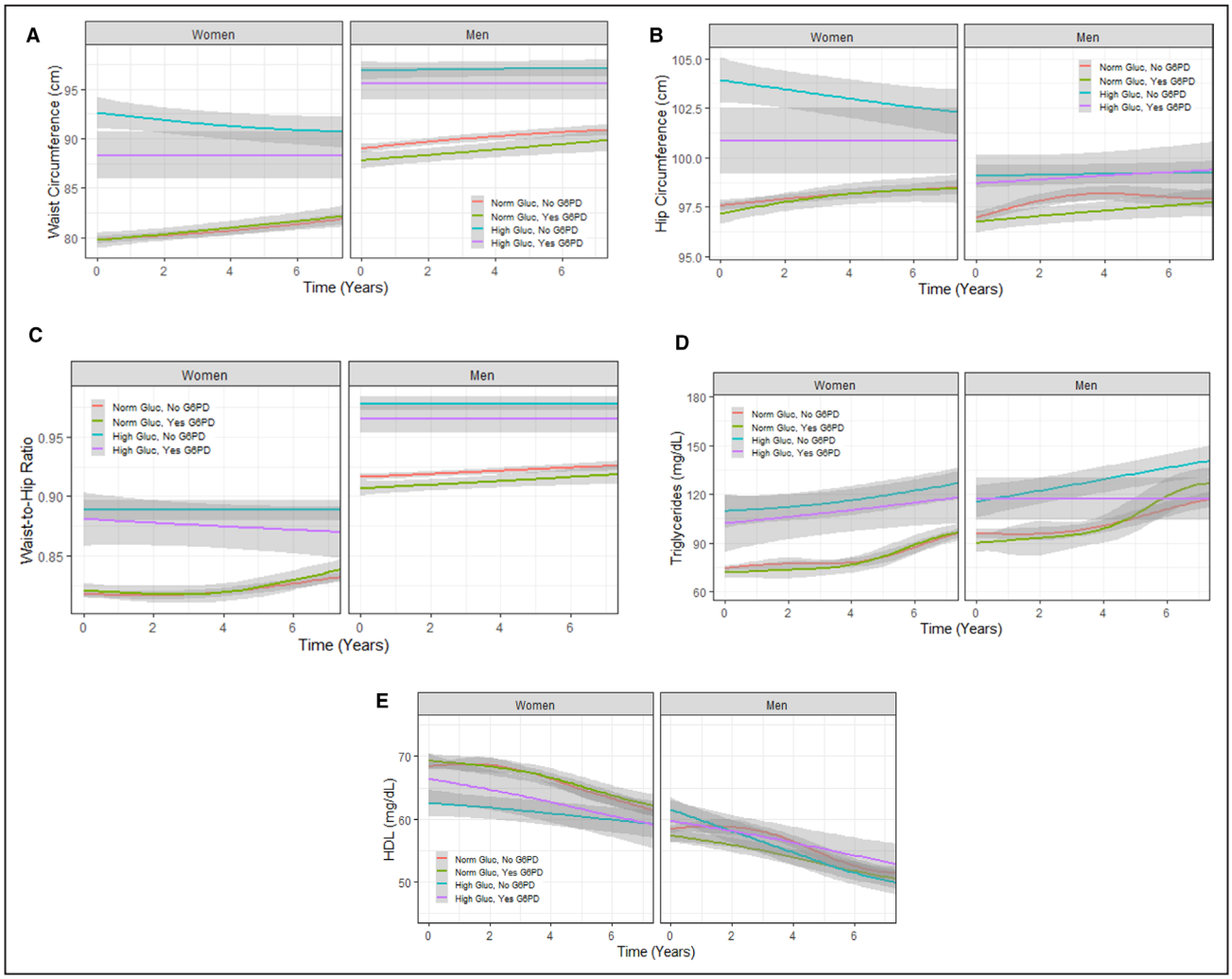

Figure 5. Longitudinal smoothed trajectories of variables versus follow-uptime by sex and G6PD and glucose group. **A**, waist; **(B)** hip circumferences; **(C)** waist-to-hip ratio; **(D)** triglycerides; and **(E)** HDL cholesterol. Group identification is as indicated in Figure 1. G6PD indicates glucose-6-phosphate dehydrogenase; Gluc, glucose; HDL, high-density lipoprotein; and Norm, normal.

was associated with a greater increase in PWV over time that was independent of changes in BP.[6] Notably, the impact of vascular remodeling was stronger in men than in women.[6] The present study supports the possible role of a "metabolic component" contributing to remodeling and, eventually, to greater rate of change in PWV (see discussion below).

The gene whose mutations causes G6PDdef maps onto the X chromosome.[13] G6PD deficiency represents the most common enzyme deficiency in humans.[12] To date, the clinical relevance of G6PD deficiency has mainly been related to its association with acute hemolytic anemia induced by exposure to fava beans or to selective drugs.[12]

Little is known about the clinical impact of G6PD activity on the vascular system in humans. Most of our knowledge on the (potential) impact of G6PD activity on vasculature comes from animal models. A G6PD-deficient rat model showed a lesser increase in CAS compared with wild-type rats.[30] Increased G6PD activity in the aorta from Zucker obese rats was accompanied by a greater superoxide production,[18] decreased nitric oxide bioavailability,[31] and a severe impairment in endothelial function.[32]

Therefore, animal models support the hypothesis that lower G6PD activity is protective for vasculature. However, our results in a large population of men and women of a broad age range suggest that, in the presence of high glucose, lower G6PD activity is associated with greater changes in CAS and, under these conditions, a reduced G6PD activity accelerates the longitudinal changes in PWV levels in men.

We cannot rule out that the effect of G6PD activity on the protection against oxidative stress plays a role in the accelerated CAS over time observed in men, but not in women.

However, the observed sex differences in the trajectory of adiposity and triglycerides in the presence of lower G6PD activity opens the door to alternative

mechanisms contributing to the impact of this enzyme on the trajectory of CAS.

Briefly, in the presence of high glucose, compared with subjects with normal G6PD activity, men with G6PDdef showed similar trends in fat deposition, but no substantial increase in triglyceride levels over time. Conversely, compared with subjects with normal G6PD activity, women with lower G6PD activity showed a relatively greater gluteofemoral fat deposition over time, with no increase in visceral fat deposition, and a similar trajectory in triglyceride levels.

These findings suggest that, in the presence of high glucose levels, women preferentially store excess energy as fat in the gluteofemoral rather than in the visceral area (greater hip and similar waist circumferences compared with peers with normal G6PD activity).[33] Men preferentially store excess energy in visceral adipose tissue, that releases free fatty acids directly into the portal vein, activating de novo lipogenesis in the liver and increased circulating triglyceride levels.[33,34]

Whether it has conferred a reproductive advantage under evolutionary selection pressure or not, there is a sexual dimorphism in energy metabolism.[35] Women have more fat mass, but lower energy expenditure than men, even after adjusting for differences in body composition.[36] Under conditions of energetic stress (such as starvation or prolonged physical exercise), men preferentially rely on carbohydrate and women on fatty acid as a primary fuel source.[37]

Fat is preferentially stored in visceral adipose tissue in men, whereas women tend to store fat in the lower body subcutaneous fat, primarily in the gluteofemoral area.[33,34] Visceral adipose tissue is more metabolically active and delivers fatty acids to the liver via the portal circulation, supporting gluconeogenesis and ketogenesis during starvation.[33,34] Conversely, gluteofemoral fat deposit in women is less responsive to catecholamine-stimulated lipolysis,[38] which enables long-term fat storage.

Sex hormones exert a relevant control over this sexual dimorphism in energy metabolism. Estrogens promote subcutaneous fat accumulation at the expense of visceral adiposity.[39] In men, aromatization of testosterone into estradiol prevents an accumulation of fat in the visceral district.[40]

Notably, in the presence of high glucose levels, men with lower G6PD activity did not show any increase in triglyceride levels compared with peers with normal G6PD activity. Therefore, the excess of glucose available likely has an alternative metabolic fate in men with lower G6PD activity.

The present study did not allow testing glucose metabolism products and, thus, we cannot demonstrate the metabolic fate of glucose in G6PDdef subjects. However, we may speculate about these metabolic pathways and propose the visual illustrated in Figure 6 as a hypothesis-generating tool.

Under normal conditions, ≈10% of glucose physiologically enters the pentose phosphate pathway, where G6PD catalyzes the conversion of glucose-6-phosphate to 6-phosphogluconolactone with the subsequent formation of ribulose and nicotinamide adenine dinucleotide phosphate reduced form (NADPH).[12]

However, in the presence of deficient G6PD activity, the reduced amount of glucose-6-phosphate entering the pentose phosphate pathway will result in its greater concentration in the cytoplasm. As illustrated in Figure 6, glucose-6-phosphate will enter many metabolic pathways: (1) activation of glycogen synthase to increase hepatic glycogen[41] or lipogenesis,[42] thus increasing intrahepatic triglyceride content; (2) metabolization to glyceraldheyde-3-phosphate and glycerol-3-phosphate with promotion of hepatic gluconeogenesis[43,44]; (3) increased oxidative stress via activation of polyol pathways (in vasculature via glyceraldheyde-3-phosphate)[45]; and (4) stimulation of the hexosamine pathway, producing glycosylation products, eventually resulting in loss of function of proteins,[46] shown to be associated with greater CAS.[47]

Therefore, increased glucose-6-phosphate availability may represent a key step in high glucose–mediated metabolic reprogramming (or remodeling) associated with greater longitudinal increase in CAS.

Strengths and Limitations

One of the strengths of this study is the use of longitudinal data with repeated measurements to assess the relationship between the longitudinal trajectory of CAS and G6PDdef, glucose, and other metabolic factors over time.

One important limitation of our study is that, because of the limited availability of cohorts with longitudinal data on CAS, glucose, and metabolic factors, as well as G6PD activity data, replication possibilities were limited, which could affect the generalizability of our findings.

Another issue is related to deaths and dropouts during the study. Only 383 or 7.7% of participants have died, a relatively small proportion. The proportion with G6PD deficiency is not statistically significantly different between those who died (6.3%) and those who were still alive (8.1%) (P=0.0703). Among those who died, 49.3% have only a single visit (versus 19.6% for alive participants) and few who died have 3 visits (17.7% versus 38.2% for those alive). So, not surprisingly, the amount of follow-up is different between those who died and those who were still alive (Table S3). Participants in the Sardinia study are volunteers who may or may not return for repeat visits. We assume that the reason participants do not return (and this includes death) is unrelated to the unmeasured values and, therefore, the data are missing at random. In this

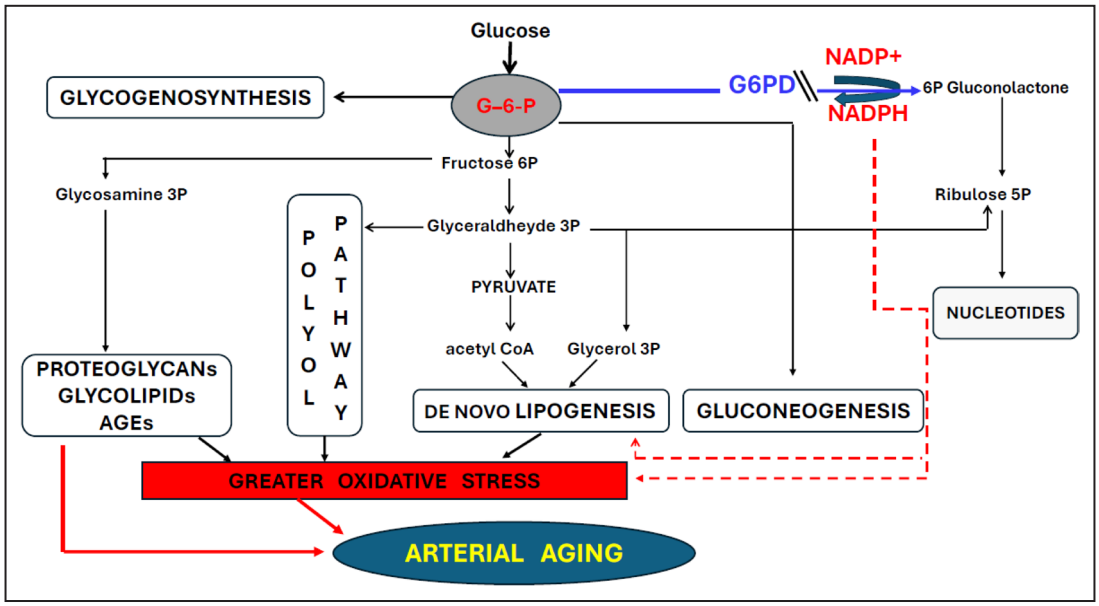

Figure 6. The complex possible alternative metabolic fates of high glucose in the presence of G6PDdef. G6PD indicates glucose-6-phosphate dehydrogenase; and G6PDdef, G6PD deficiency. AGE indicates advanced glycation endproduct; NADP, nicotinamide adenine dinucleotide phosphate; and NADPH, nicotinamide adenine dinucleotide phosphate reduced form.

case, likelihood-based approaches, like LME models, are appropriate.

Additionally, our results are based on association, thus not allowing conclusions about causation. Despite these limitations, our study provides valuable insights into the longitudinal relationship between CAS, metabolic factors, and G6PDdef in men and women, and highlights the need for further research in this area.

Modeling of repeated measurements over time with LME analyses was adjusted for established cardiovascular risk factors (body mass index, BP, and LDL and high-density lipoprotein cholesterol levels) and their change over time. However, we did not adjust for smoking, medication use (eg, antihypertensives, statins, and diabetes therapies), and menopausal status in women. Investigating the impact of menopausal status in women participating in the study could have provided further mechanistic insight.

No detailed information about the diet type of each participant was available. This could have contributed to better understand differences in triglyceride levels in men with or without G6PDdef.

An additional potential limitation of the current study is represented by the fact that we measured G6PD activity, without further characterization of allele profile in women. In fact, the *G6PDX* gene whose mutations cause the recessive G6PDdef condition maps on the X chromosome. Therefore, this population of women includes heterozygotes with partial G6PD enzyme activity.

Conclusions

G6PD activity may represent a modulator of systemic response to metabolic stressors, such as high glucose levels. When subjects with reduced G6PD activity are exposed to these stressors, the metabolic reprogramming (or remodeling) driven by increased glucose-6-phosphate availability, deriving from both high glucose levels and lower G6PD activity, impacts on accelerating age-associated CAS. This effect might be more relevant than any possible effect mediated by alteration in intracellular NAD(P)H balance.

Based on the findings of the present study, the hypothesis that low G6PD activity reroutes glucose into harmful pathways (polyol, hexosamine, and glycation) is plausible but remains merely speculative.

Future studies are needed to validate this hypothesis, by experiments aimed at measuring byproducts of the hypothesized glucose harmful pathway, such as sorbitol, O-linked N-acetylglucosamine, or oxidative stress markers.[48–50]

Additionally, future studies will better characterize the contribution of reduced G6PD activity to the excess cardiovascular risk characterizing men, compared with women, and to identify early interventions for these high-risk individuals.

ARTICLE INFORMATION

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Disclosure

The authors declare no competing interests.

Supplemental Material

Tables S1–S5
Figures S1–S4

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