Non-diabetic Glucometabolic Status and Progression of Aortic Stiffness: the Whitehall II study

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Short title: Non-diabetic Glucometabolic Status and Aortic Stiffening

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Word count: 3869

Number of Tables: 3

Number of Figures: 1

Structured Abstract

Objective Aortic stiffness is an important predictor of future morbidity and mortality. Diabetes is associated with increased aortic stiffness, but the importance of non-diabetic glucometabolic status for accelerated aortic stiffening is unclear. We tested the hypothesis that adverse glucometabolic status is associated with accelerated aortic stiffening in non-diabetic individuals, independently of known risk factors for arterial stiffening.

Research Design and Methods Glucometabolic status and other cardiovascular risk factors were assessed at baseline in 2008/09, and carotid femoral pulse wave velocity (cfPWV) at baseline and follow-up in 2012/13, in 4386 non-diabetic participants of the Whitehall II Study.

Results The mean age of the cohort at cfPWV baseline was 60 years, and 74% were male. cfPWV increased from (mean±SE) 8.30±0.03 to 8.98±0.04 m/s over 4 years of follow-up. At baseline, cfPWV was associated with fasting and 2-hour postload glucose, HbA1c, and HOMA-insulin resistance (HOMA-IR). HbA1c and HOMA-IR were associated with progression of cfPWV after adjusting for physiological confounders and cardiovascular risk factors. A 1SD higher HbA1c and HOMA-IR were associated with greater increases in cfPWV (0.11m/s per 5 years, 95%CI 0.04, 0.18, P=0.003 and 0.09m/s per 5 years, 0.01, 0.17, P=0.03, respectively). Additional adjustment for BMI weakened the association with HOMA-IR but not with HbA1c.

Conclusions HbA1c is independently associated with accelerated progression of aortic stiffness in non-diabetic individuals. These findings suggest that long-term glucometabolic status, even in non-diabetic individuals, could be an important target for preventative strategies against vascular ageing.

Key words aortic stiffening, pulse wave velocity, glucometabolic status, cardiovascular risk factors

Introduction

Aortic stiffness provides important prognostic information on overall cardiovascular risk and mortality[1]. In the most recent meta-analysis, cfPWV, the current gold-standard measure of aortic stiffness, was an independent predictor of future cardiovascular events, improving risk classification beyond that provided by traditional risk factors[2]. This has added to the view that aortic stiffness is a measure of vascular health or vascular age[3, 4] and has created considerable interest in aortic stiffness as a novel therapeutic target.

The precise mechanisms underlying aortic stiffening remain poorly understood. The heterogeneity in the rate of stiffening suggests it is not an inevitable consequence of the ageing process[5]. A number of risk factors for accelerated aortic stiffening have been described, including elevated blood pressure, renal disease, systemic inflammation and adiposity [6-10]. Diabetes has been linked with increased vascular stiffness in several case-control studies[11-13], and HbA1c level is associated with an accelerated age-related increase in cfPWV in individuals with type 2 diabetes[14]. In non-diabetic individuals, cross-sectional studies suggest an association between aortic stiffness, glycaemia, and indices of insulin resistance[11, 15, 16]. However, a prospective analysis using Whitehall II data is equivocal, finding associations only in men [17]. The evidence from longitudinal studies is also conflicting. Only one of seven previous longitudinal studies identified plasma glucose as an independent predictor of the rate of progression of cfPWV after adjusting for confounders, but only in women[18]. The remaining longitudinal studies report either no independent association [19-22], or did not examine glucose as a risk factor for stiffening[23, 24]. None of the studies provide any information concerning HbA1c and aortic stiffening.

We hypothesized that impaired glycaemic control is associated with accelerated aortic stiffening in non-diabetic individuals, and that this would be independent of known risk factors for arterial stiffening. Our aim was to test this hypothesis in the Whitehall II longitudinal study. This cohort study provides data on progression of cfPWV over a 4 year interval, together with repeated assessments of glycaemia, insulin resistance, anthropometric parameters and cardiovascular risk factors. We excluded individuals with clinical or biochemical evidence of diabetes to reduce the confounding effects of treatment and diabetic complications such as renal disease which may themselves alter stiffness.

Research Design and Methods

Participants

All participants were drawn from the Whitehall II cohort, a longitudinal observational study of 10,308 civil servants recruited between 1985-8 when aged 35-55 years old[25]. Participants have been followed-up every 4-5 years, with detailed clinical examinations and self-administered questionnaires in 1991-94, 1997-99, 2003-04, 2008-09 and 2012-13. For each examination phase, Research Ethics Committee approval was obtained, and participants gave written informed consent.

A total of 4347 out of 6225 participants seen at the screening clinic in 2008-09, and 4485 out of 5660 seen at 2012-13, underwent cfPWV assessment. The present analysis was based on non-diabetic participants. Diabetes was defined by self-report/doctor diagnosed, the use of anti-diabetic medication, fasting glucose ≥7.0, a 2hr glucose ≥11.1 mmol/L during an oral glucose tolerance test or HbA1c ≥ 6.5% (48mmol/mol). 617 participants met these criteria at baseline and 582 at follow-up, and were therefore excluded from all analyses, together with a further 95 participants at baseline and 301 at follow-up who had missing data on covariates. This left 4386 non-diabetic participants who underwent cfPWV assessment during the 2008-9 (n=3685) and/or 2012-3 (n=3602) examinations who make up the analytic sample. 2901 participants underwent cfPWV assessments at both time points. The same measurement protocol was used at each examination.

Measurements

Pulse wave velocity

Aortic stiffness was assessed by cfPWV, the current non-invasive gold-standard[26]. Higher values of cfPWV indicate a faster speed of wave travel between the arterial sites

and, hence, a stiffer aorta., Measurements were made after 15 minutes supine rest, in duplicate, using the SphygmoCor system (AtCor Medical, Sydney), as previously described[27]. Briefly, brachial blood pressure was measured and then cfPWV assessed between the carotid and femoral sites. Path length was determined with a tape measure, by subtracting carotid-sternal notch distance from femoral-sternal notch distance. If the difference between repeated measurements was >0.5m/s, a third measurement was taken, with the average of measurements used in the analysis. Heart rate was derived from the SphygmoCor software and blood pressure was measured using a validated oscillometric device immediately prior to cfPWV. Mean arterial pressure (MAP) was calculated as diastolic pressure plus one third of the pulse pressure.

Vascular disease, diabetes and anti-hypertensive medication

Prevalent vascular disease status (myocardial infarction and/or stroke) at the 2008-09 assessment was determined using self-report doctor diagnosis and hospitalization with verification from medical records where available. Prevalent diabetes was determined by oral glucose tolerance test, self-report doctor diagnosis and/or medication[28].

Anthropometry and other covariates

Anthropometric measures and cardiovascular risk factors were measured in 2003-04 and 2008-09 to provide mean exposure in the 5 years before baseline cfPWV assessment in 2008-09. Weight, height, and waist and hip circumferences were measured using standard protocols[29]. Serum, fluoride plasma and EDTA blood was collected after overnight fast or ≥5 hours after a fat-free breakfast for participants presenting in the afternoon. Serum total cholesterol, high density lipoprotein cholesterol (HDL), triglycerides and plasma glucose, were measured.

Glucometabolic measures

Samples were handled according to standard protocols. Venous blood samples were taken in fasted individuals (≥8 h of fasting or ≥5 h for afternoon visits), before a standard 2-h oral glucose tolerance test was administered in all participants[29]. Glucose samples were drawn into fluoride monovette tubes and insulin samples into native tubes which were centrifuged on site within 1 h. Plasma or serum was immediately moved into microtubes and stored at –70^{o}C. Blood glucose was measured using the glucose oxidase method[30] on YSI model 2300 STAT PLUS analyser (2003-04, and 2007-09), mean CV 1·4–3·1%) (YSI Corporation, Yellow Springs, OH, USA), and serum insulin with a DAKO insulin ELISA kit (DakoCytomation Ltd, Ely, UK)[31] (2003-04, mean CV 4·2–9·3%, 2007-09). HOMA insulin sensitivity and HOMA β-cell function were calculated on the basis of model-derived estimates (rather than linear approximations) with the HOMA2 calculator version 2.2[32]. Haemoglobin A1c (HbA1c) was measured in whole blood, drawn into EDTA monovette tubes, using the validated[33] Tosoh G8 high performance liquid chromatography platform (Tosoh Bioscience, Belgium).

The exposures and covariates used in the analyses were the mean values of risk factors assessed in 2003-4 and 2008-9 since, compared to a single measurement at 2008-09, these provide more reliable estimates of exposure in the 5 years prior to the first cfPWV measurement.

Statistical analysis

Distributions of glucometabolic indices among people without diabetes were categorised in sex-specific quintiles and also expressed in standardised units. Linear mixed models were used to estimate the relation of glucometabolic indices with cfPWV in 2008-09 and change in cfPWV between 2008-09 and 2012-13. These models used all available

cfPWV data, including cases where only one cfPWV measurement was available, which reduced selection bias and allowed better estimates of the associations of potential confounding factors. The models also accounted for correlation between repeated measures within individuals. We fitted the models with a random intercept and slope to account for individual differences in cfPWV at baseline and rate of change over follow-up. From these models, the effect of each glucometabolic index on cfPWV at baseline (2008-2009) is estimated by the coefficient for the main effect of the glucometabolic index and the effect on progression of cfPWV between 2008-09 and 2012-13 is estimated by the interaction of the main effect with time. The longitudinal effects of the glucometabolic indices have been expressed as 5-year changes in cfPWV to allow direct comparisons with previous studies[18, 20, 24]. All estimates were initially adjusted for age, sex, ethnic group, heart rate and MAP at the time of cfPWV measurement. Baseline cfPWV and progression of cfPWV per 5 years were estimated from these models by quintile of each glucometabolic index distribution and per 1SD increment in each index. This allowed us to examine associations with cfPWV across the distribution of each glucometabolic index and whether the coefficients increased linearly across quintiles. Tests of heterogeneity were conducted using likelihood ratio tests that compared the fit of the models with and without the quintiles of each glucometabolic measure. Two further models cumulatively adjusted for: (i) systolic blood pressure, antihypertensive medication, lipid lowering medication, smoking status, prevalent MI or stroke and mean triglyceride and HDL-cholesterol between 2003-04 and 2008-09 and (ii) mean BMI between 2003-04 and 2008-09 and estimated glomerular filtration rate (eGFR) in 2008-09. Sensitivity analysis was conducted to compare characteristics of participants with and without measurements of cfPWV, in order to exclude the possibility of selection bias. Further sensitivity analyses used the glucometabolic measures from

baseline (2008-09) or pre-baseline (2003-04), rather than averaging the glycaemic measures across the two phases.

Results

The mean age of the cohort at the time of the baseline cfPWV assessment was 60 years, 74% were male, and predominantly of white ethnic origin. Fewer than 5% had chronic disease, and ~30% were taking antihypertensive medication. A comparison of participants with and without cfPWV assessments across the entire cohort revealed that those individuals who did not have cfPWV measured were more likely to be female and have generally poorer health, in terms of chronic disease and taking anti-hypertensive or lipid-lowering medication (supplementary **Table S1**). Detailed participant characteristics for the two-cfPWV examination time points are provided in **Table 1**. Measures of exposure that were averaged across the pre-baseline (2003-4) and baseline (2008-9) cfPWV visits are presented separately for each examination phase in supplementary **Table S2**.

There was no evidence that the associations of the glucometabolic indices with baseline cfPWV and progression of cfPWV differed between men and women (**Table S3**). Cross-sectional associations between cfPWV and glucometabolic indices are shown by quintile in **Table 2**; adjusted for age, gender, ethnicity, MAP and heart rate. There were significant positive associations with fasting glucose, 2hr glucose, HbA1c and HOMA-IR. cfPWV increased by between 0.19 and 0.40 m/s when moving from the lowest to highest quintile of each determinant. Additional adjustment for other potential confounders, including drug therapy and cardiovascular risk factors, only modestly attenuated these associations (supplementary **Table S4**). When glucometabolic measures were treated as continuous variables, glucose and HOMA-IR were strongly, and HbA1c weakly, associated with baseline cfPWV (**Table 3**). These associations were weakened with additional adjustment for potential confounders.

After adjustment for age, sex, ethnicity, MAP and heart rate, only HbA1c and HOMA-IR were significantly associated with progression of cfPWV. There was a 0.39 m/s greater increase in cfPWV over 5 years in individuals in the top quintile of either parameter, compared with those in the lowest quintile (**Table 2 and Figure 1**). Adjustment for treatment and other risk factors (supplementary **Table S5**) did not significantly attenuate these associations. However, after the addition of BMI to the models, there was no longer an association with HOMA-IR. Analyses using the continuous variables revealed similar findings – a 1SD higher HbA1c or HOMA-IR at baseline was associated with a ~0.12m/s greater increase in cfPWV over 5 years (**Table 3**). In the fully adjusted model, only HbA1c was associated with progression in cfPWV.

Repeating these analyses using glucometabolic measures from baseline (2008-09) or pre-baseline (2003-04), rather than averaging across the two phases did not meaningfully alter the results (supplementary **Tables S6** and **S7**, respectively). Similarly, excluding the 1.1% of individuals who developed diabetes during follow-up had no influence on the results (data not shown). In order to exclude the possibility of a selection bias influencing our results, since measurement of cfPWV was not undertaken in all participants, a separate analysis using the entire cohort, explored the relationship between HbA1c and blood pressure, which is closely related to cfPWV. At baseline and follow-up, HbA1c was ~3% higher in hypertensives than non-hypertensives, in those with and without cfPWV assessment, after adjusting for confounding factors (supplementary **Table S8**). This supports our observed association between HbA1c and cfPWV and argues against the possibility that selection bias might be influencing our findings.

Conclusions

Our main findings are that, glucose, HbA1c and insulin resistance are all cross-sectionally associated with aortic stiffness, and that in longitudinal analysis, HbA1c and HOMA-IR are associated with the progression of aortic stiffness over a 4-year period. These associations were independent of other cardiovascular risk factors. The association between HbA1c and aortic stiffening was also independent of BMI. These observations suggest that factors underlying glucometabolic status may affect aortic stiffening even within the normoglycaemic range, which may have important implications for developing anti-stiffening strategies and exploiting novel therapeutic targets.

Aortic stiffness, and cfPWV in particular, is an important risk factor for future cardiovascular disease, independently of other cardiovascular risk factors[2]. However, the biological processes underlying aortic stiffening are incompletely understood. Cross sectional analyses have provided inconsistent results[6], and are limited in their ability to attribute causality. Existing longitudinal data highlight the importance of age and blood pressure, but the importance of other potential risk factors, including indices of glucose homeostasis, is unclear, due to lack of replication studies, variable lengths of follow-up, poor availability of data on individual risk factors, and variation in adjustment for confounders. Whitehall II is a large cohort with prospective data on cfPWV and other risk factors including a variety of glucometabolic indices, making it well suited to examine the relationship between glycaemia and aortic stiffening in non-diabetic individuals.

As expected, we found significant cross-sectional relationships between indices of glucose homeostasis and insulin resistance with cfPWV. Importantly, this remained after

adjustment for physiological confounders of stiffness such as MAP and heart rate[34]. Similar results have been reported previously using a variety of study designs, indices of stiffness, and varying levels of adjustment for physiological confounders [11, 15, 16, 35, 36]. In the present study, adjustment for other potential risk factors for arterial stiffening or cardiovascular disease only modestly reduced the strength of association with glucose measures, but the associations with HOMA-IR and HbA1c were more markedly attenuated, with an approximate halving of the beta values. This contrasts with cross-sectional findings from a cohort of 263 African-Americans[16], in whom HbA1c, but not fasting, or 2–hr glucose levels remained independently associated with cfPWV. This disparity possibly reflects ethnic differences in the association between HbA1c and arterial stiffness, or may reflect a lack of appropriate statistical power in the African-American study. In addition, we *a priori* excluded diabetic individuals, which may have removed any association with HbA1c in our data. Alternatively, residual confounding may explain our observed associations with plasma glucose.

In contrast to the cross-sectional observations, fasting and 2-hr glucose were not associated with progression of aortic stiffening in the current study, which is consistent with previous observations[19-22]. However, HbA1c and HOMA-IR were associated with accelerated progression of cfPWV, independently of potential confounders. Previously, one small study reported no association between cfPWV progression and HOMA[19], but HbA1c has been associated with progressive carotid artery stiffening in the ARIC Study[37], although unfortunately, cfPWV was not assessed. In the current study, cfPWV increased by ~0.7m/s, which is consistent with previous longitudinal studies which have reported increases of between 0.2 and 0.6 m/s per 5 years, in participants aged ~60 years[18, 20, 24]. Moreover, modest differences of 0.07% in HbA1c or 0.67 units in HOMA-IR were associated with a ~0.1m/s greater increase in cfPWV over 5 years, equating to

~12-14% of the overall change in stiffness. While the rate of stiffening is strongly dependent on age, our data suggest that even in non-diabetic individuals, modest differences in glucometabolic status have a meaningful impact on arterial stiffening, consistent with our hypothesis of accelerated vascular ageing.

There is a strong mechanistic relationship between measures of adiposity, insulin resistance and glycaemia. We and others have previously reported that measures of adiposity including BMI are associated with accelerated aortic stiffening, independent from other risk factors[10, 18, 20, 22]. Therefore, we additionally adjusted for BMI. After this, HbA1c, but not HOMA-IR, remained predictive of aortic stiffening. These findings suggest that adiposity and insulin resistance share common pathway(s) leading to aortic stiffening, but that these pathways may be independent of glycaemia, although this hypothesis requires further examination. A number of mechanisms may be responsible, including visceral and perivascular fat accumulation, and the vascular effects of insulin. Indeed, fasting insulin concentrations are positively associated with cfPWV in the general population [38]. In addition, abdominal and vascular adiposity alter adipokine levels, increase circulating pro-inflammatory stimuli, and may directly inflame the vasculature [39-41]. Low adiponectin levels have previously been associated with obesity and increased cfPWV progression[19], and in the Whitehall II cohort we have previously shown that a panel of inflammatory markers are associated with increased cfPWV 16 years later[17].

Systemic inflammation is also associated with cfPWV[7, 42]. Interestingly, the peroxisome proliferator–activated receptor γ (PPAR-γ) agonist, pioglitazone, improves inflammation and glycaemic control in obese, diabetic patients[43]. Pioglitazone was also effective in preventing strokes[44] and in reducing the progression to diabetes and major

cardiovascular events[45] in a high-risk non-diabetic population. As such, PPAR-γ agonists may represent one potential therapeutic strategy to retard aortic stiffening in non-diabetic individuals. In addition, formation of advanced glycation end-products (AGEs), which accumulate in tissues over time and with increased plasma glucose levels[46], correlate with aortic stiffness in non-diabetic individuals[47, 48]. Experimental cross-link breakers reduce cfPWV and pulse pressure in animals[49] and humans[50] and our longitudinal finding for HbA1c suggests that AGEs may represent another novel anti-stiffening target, even in non-diabetic individuals. However, both of these hypotheses need to be tested in well-designed intervention studies.

The present study has a number of limitations. We were restricted to 4 year follow-up data, and cannot exclude the possibility that differences in relative strength of the glucometabolic risk factor effects may be observed with longer follow-up, or indeed in younger adults. However, our data are consistent with observations made in other cohorts with similar lengths of follow up. Moreover, we used the gold-standard method of cfPWV to assess aortic stiffness and an identical protocol at both examinations. However, use of a 5 hour fast for afternoon examinations may have meant that those participants were not truly fasted. We are unable to comment on the impact of diabetes *per se* because we *a priori* excluded diabetic individuals. This allowed us to minimize potential confounding influences, such as therapy. However, we were able to demonstrate meaningful differences in progression of aortic stiffness, even within what is considered a normal range of HbA1c. Given that glucometabolic indices determine the development of diabetes up to 15 years in advance[51] and cfPWV predicts future cardiovascular risk[2], we believe that our observations are clinically important and suggest that further mechanistic and intervention studies of arterial stiffening should examine factors related to longer-term glucometabolic status. These could involve

lifestyle approaches and/or trials of glucose-lowering therapies in non-diabetic individuals, which could ultimately influence public health strategies.

In summary, in non-diabetic individuals, a higher HbA1c and HOMA-IR is associated with increased aortic stiffening. This was independent of potential confounders, or other cardiovascular risk factors, and, in the case of HbA1c, also independent of BMI. In contrast, point-estimates of glucose, either fasting or 2-hours post a standard glucose tolerance tests, were not associated with progression of aortic stiffness. Our data suggest that higher average glucose levels may be causally related to accelerated vascular ageing through long-term mechanisms rather than short-term dynamic changes in the arterial wall. As such, improving glucometabolic status may represent a strategy to improve vascular health.

Acknowledgements

We thank all of the participating civil service departments and their welfare personnel and establishment officers; the Occupational Health and Safety Agency; the Council of Civil Service Unions; all of the participating civil servants in the Whitehall II Study; and all members of the Whitehall II Study team. The Whitehall II study team is composed of research scientists, statisticians, study coordinators, nurses, data managers, administrative assistants, and data entry staff, who make the study possible.
CMM drafted the manuscript; IBW conceived the idea for the manuscript and reviewed/edited the manuscript; NBJ reviewed/edited the manuscript; DRW contributed to the discussion and reviewed/edited the manuscript; ASM reviewed/edited the manuscript; MK contributed to the discussion and reviewed/edited the manuscript; AGT contributed to the discussion and reviewed/edited the manuscript; EJB contributed to the analysis and discussion and reviewed/edited the manuscript; MJS performed statistical analysis and reviewed/edited the manuscript. MJS is also the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Sources of Funding

This work was supported by the British Heart Foundation (RG/13/2/30098), British Medical Research Council (K013351), the British Department of Health, the British Stroke Association (TSA 2008/05), the US National Heart, Lung, and Blood Institute (R01HL036310), and the US National Institute on Aging (R01AG013196 and R01AG034454). C.M. McEniery and I.B. Wilkinson receive support from the National Institute for Health Research Cambridge Biomedical Research Centre, and the British Heart Foundation Centre of Excellence. I.B. Wilkinson is a British Heart Foundation Senior Fellow. Daniel R. Witte and Nanna B. Johansen are supported by the Danish

Diabetes Academy, which is funded by the Novo Nordisk Foundation. Mika Kivimaki is supported by the Medical Research Council, the Economic and Social Research Council and NordForsk, the Nordic Programme on Health and Welfare and Martin Shipley is partially supported by the British Heart Foundation.

Conflicts of Interest

None

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Figure Legends

Figure 1. The association between HbA1c and change in cfPWV

Plotted points show the cfPWV change per 5 years for each quintile of HbA1c, plotted at the median of each quintile. Solid line shows linear association, dashed lines show the 95% confidence interval. Values shown are adjusted to non-diabetic white men at age 65 with a mean arterial pressure of 90mmHg. Test for quadratic (nonlinear) effect gave a P-value=0.48.

Table 1. Characteristics of participants who were non-diabetic at baseline

Characteristic	Baseline (2008-2009) (N=3685)		Follow-up (2012-2013) (N=3602)	
	Mean (SD)	%	Mean (SD)	%
Age, y	60.1 (5.7)		65.0 (5.6)	
Female		25.6		25.7
Ethnic group: White		94.0		94.6
South Asian		3.2		2.8
Black		2.1		1.9
Other		0.7		0.7
Diabetes		0.0		1.1
MI or Stroke		4.9		4.5
Anti-hypertensive medication		29.1		36.0
Lipid lowering medication		26.7		36.6
Ex-smoker		45.2		48.8
Current smoker		5.2		3.1
Body mass index, kg/m^2	26.0 (3.7)		26.0 (3.9)	
Systolic blood pressure, mmHg	124.2 (15.3)		126.7 (15.9)	
Diastolic blood pressure, mmHg	70.8 (10.0)		70.8 (9.8)	
Triglyceride, mmol/L	1.16 (0.65)		1.13 (0.57)	
HDL cholesterol, mmol/L	1.63 (0.45)		1.68 (0.47)	
LDL cholesterol, mmol/L	3.14 (0.94)		2.98 (0.94)	
Fasting glucose, † mmol/L	5.05* (0.10)		5.16* (0.10)	
2-hour glucose, † mmol/L	6.23* (0.26)		Not done	
HbA1c, † %	5.58* (0.07)		5.64* (0.06)	
HbA1c, † mmol/mol	37.4* (0.07)		38.4* (0.06)	
HOMA-IR, †	1.39* (0.67)		1.68* (0.66)	
Heart rate, bpm	65.8 (11.3)		68.1 (11.6)	
cfPWV, m/s	8.30 (1.93)		8.98 (2.39)	
Mean arterial pressure, mmHg	89.4 (10.5)		93.8 (10.9)	

Characteristics of the participants included in the present analyses at the baseline (2008-2009) and follow-up (2012-2013) visits. For glucometabolic indices (†) the baseline values represent the average of values at the 2003-04 and 2008-09 assessments. The mean for all 4386 participants included in the analyses is shown in supplementary Table S2, together with the separate means for 2003-04 and 2008-09. *Geometric mean and SD of logged values.

Table 2. The association of glucometabolic indices at baseline with cfPWV measured at baseline and progression of cfPWV over the follow-up period

Glucometabolic measure[a]			cfPWV at baseline			Change in cfPWV (per 5 years)		
	Median	Person Obs	Mean[b]	Difference[c] (95% CI)	P-value	Mean[b]	Difference[c] (95% CI)	P-value
Fasting glucose, mmol/L		(7287)						
Q1 - lowest quintile	4.65	1512	8.30	Ref	-	0.58	Ref	-
Q2	4.93	1251	8.33	0.04 (-0.13, 0.21)	0.64	0.60	0.02 (-0.21, 0.25)	0.89
Q3	5.10	1521	8.39	0.11 (-0.05, 0.27)	0.18	0.59	0.01 (-0.20, 0.23)	0.91
Q4	5.35	1603	8.50	0.22 (0.06, 0.37)	0.007	0.57	0.00 (-0.21, 0.22)	0.99
Q5 - highest quintile	5.70	1400	8.52	0.27 (0.11, 0.43)	0.001	0.59	0.02 (-0.20, 0.24)	0.87
Heterogeneity (P-value)				0.005			1.0	
Per 1SD higher fasting glucose				0.08 (0.03, 0.13)	0.004		0.01 (-0.07, 0.08)	0.89
2-hour glucose, mmol/L		(7055)						
Q1 - lowest quintile	4.65	1468	8.15	Ref	-	0.53	Ref	-
Q2	5.55	1488	8.26	0.11 (-0.05, 0.27)	0.18	0.53	0.00 (-0.22, 0.21)	0.97
Q3	6.20	1379	8.32	0.17 (0.00, 0.33)	0.05	0.52	-0.01 (-0.24, 0.21)	0.90
Q4	6.90	1409	8.25	0.10 (-0.06, 0.26)	0.22	0.56	0.03 (-0.19, 0.26)	0.76
Q5 - highest quintile	8.25	1311	8.46	0.32 (0.15, 0.48)	<0.001	0.73	0.20 (-0.03, 0.43)	0.09
Heterogeneity (P-value)				0.006			0.36	
Per 1SD higher 2-hour glucose				0.11 (0.06, 0.16)	<0.001		0.07 (-0.01, 0.14)	0.07
HbA1c, %		(7283)						
Q1 - lowest quintile	5.0	1472	8.35	Ref	-	0.44	Ref	-
Q2	5.2	1197	8.26	-0.08 (-0.25, 0.09)	0.33	0.58	0.14 (-0.09, 0.37)	0.24
Q3	5.4	1930	8.39	0.04 (-0.11, 0.19)	0.60	0.49	0.05 (-0.15, 0.26)	0.62
Q4	5.6	1418	8.56	0.21 (0.04, 0.37)	0.01	0.55	0.11 (-0.11, 0.33)	0.34
Q5 - highest quintile	5.8	1266	8.54	0.19 (0.02, 0.36)	0.03	0.83	0.39 (0.15, 0.62)	0.001

Heterogeneity (P-value)				0.003			0.01	
Per 1SD higher HbA1c				0.05 (0.00, 0.11)	0.05		0.12 (0.04, 0.19)	0.002
HOMA-IR		(7189)						
Q1 - lowest quintile	0.73	1583	8.21	Ref	-	0.41	Ref	-
Q2	1.12	1480	8.24	0.03 (-0.12, 0.19)	0.67	0.47	0.06 (-0.16, 0.27)	0.61
Q3	1.53	1474	8.46	0.26 (0.10, 0.41)	0.002	0.60	0.19 (-0.03, 0.40)	0.09
Q4	2.12	1415	8.55	0.35 (0.19, 0.51)	<0.001	0.62	0.20 (-0.02, 0.42)	0.07
Q5 - highest quintile	3.52	1237	8.61	0.40 (0.23, 0.57)	<0.001	0.80	0.39 (0.15, 0.62)	0.001
Heterogeneity (P-value)				<0.001			0.01	
Per 1SD higher HOMA-IR				0.15 (0.10, 0.21)	<0.001		0.11 (0.04, 0.19)	0.004

[a] Values are the averages of measurements made at 2003-04 and 2008-09.
[b] Means are adjusted for age, sex, ethnic group, heart rate and mean arterial pressure at the time of the cfPWV measurement and are shown adjusted to non-diabetic white men at age 65 with a mean arterial pressure of 90mmHg.
[c] Differences are adjusted for age, sex, ethnic group and mean arterial pressure at the time of the cfPWV measurement.

Table 3. The associations of glucometabolic indices with cfPWV and progression of cfPWV after adjustment for confounding factors and other cardiovascular risk factors

Glucometabolic measure[a]	Model adjustments	cfPWV at baseline		Change in cfPWV (per 5 years)	
		Difference[b] (95% CI)	P-value	Increase[b] (95% CI)	P-value
Fasting glucose	Model 1[c]	0.08 (0.03, 0.13)	0.004	0.01 (-0.07, 0.08)	0.89
	Model 2[d]	0.05 (-0.01, 0.10)	0.08	-0.01 (-0.08, 0.07)	0.87
	Model 3[e]	0.05 (-0.01, 0.10)	0.09	-0.03 (-0.10, 0.04)	0.44
2-hour glucose	Model 1[c]	0.11 (0.06, 0.16)	<0.001	0.07 (-0.01, 0.14)	0.07
	Model 2[d]	0.06 (0.01, 0.12)	0.03	0.06 (-0.02, 0.13)	0.12
	Model 3[e]	0.06 (0.00, 0.11)	0.04	0.04 (-0.03, 0.12)	0.24
HbA1c	Model 1[c]	0.05 (0.00, 0.11)	0.05	0.12 (0.04, 0.19)	0.002
	Model 2[d]	0.04 (-0.02, 0.09)	0.19	0.11 (0.04, 0.18)	0.003
	Model 3[e]	0.03 (-0.02, 0.09)	0.22	0.10 (0.03, 0.17)	0.008
HOMA-IR	Model 1[c]	0.15 (0.10, 0.21)	<0.001	0.11 (0.04, 0.19)	0.004
	Model 2[d]	0.07 (0.01, 0.13)	0.03	0.09 (0.01, 0.17)	0.03
	Model 3[e]	0.07 (0.00, 0.13)	0.05	0.02 (-0.07, 0.11)	0.63

[a] Values are the averages of measurements made at 2003-04 and 2008-09.
[b] Differences and increases in pulse wave velocity are per 1SD higher value for each glucometabolic measure
[c] Model 1 is adjusted for age, sex, ethnic group, heart rate and mean arterial pressure at the time of the cfPWV measurement
[d] Model 2 is adjusted as for Model 1 + systolic blood pressure, antihypertensive medication, lipid lowering medication, prevalent MI or stroke, smoking status and mean triglyceride and HDL-cholesterol between 2003-04 and 2008-09
[e] Model 3 is adjusted as for Model 2 + mean BMI between 2003-04 and 2008-09

Figure 1