Chapter 3

Biological correlates of blood pressure variability in elderly at high risk of cardiovascular disease

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ABSTRACT

Background

Visit-to-visit variability in blood pressure is an independent predictor of cardiovascular disease. This study investigates biological correlates of intra-individual variability in blood pressure in older persons.

Methods

Nested observational study within the PROspective Study of Pravastatin in the Elderly at Risk (PROSPER) among 3,794 male and female participants (range 70–82 years) with a history of, or risk factors for cardiovascular disease. Individual visit-to-visit variability in systolic and diastolic blood pressure and pulse pressure (expressed as 1 SD in mm Hg) was assessed using nine measurements over 2 years. Correlates of higher visit-to-visit variability were examined at baseline, including markers of inflammation, endothelial function, renal function and glucose homeostasis.

Results

Over the first 2 years, the mean intra-individual variability (1 SD) was 14.4 mm Hg for systolic blood pressure, 7.7 mm Hg for diastolic blood pressure, and 12.6 mm Hg for pulse pressure. After multivariate adjustment a higher level of interleukin-6 at baseline was consistently associated with higher intra-individual variability of blood pressure, including systolic, diastolic, and pulse pressure. Markers of endothelial function (Von Willebrand factor, tissue plasminogen activator), renal function (glomerular filtration rate) and glucose homeostasis (blood glucose, homeostatic model assessment index) were not or to a minor extent associated with blood pressure variability.

Conclusion

In an elderly population at risk of cardiovascular disease, inflammation (as evidenced by higher levels of interleukin-6) is associated with higher intra-individual variability in systolic, diastolic, and pulse pressure.

Blood pressure varies over time within individuals, resulting in variability in blood pressure.[1-4] Until the 1990s variability in blood pressure was merely regarded as a random phenomenon and an obstacle to determine the usual blood pressure.[1,5-7] Usual or average blood pressure was considered to be the main determinant in the cause of cardiovascular disease and accounting for the benefits of antihypertensive drugs. [1,5-7] However, recent data suggest that variability in blood pressure assessed across several visits (i.e., visit-to-visit variability) is reproducible and not a random phenomenon,[8,9] and that visit-to-visit variability in blood pressure itself is a predictor of incident cardiovascular disease.[10-18] Long-term visit-to-visit variability in systolic blood pressure has been claimed to be associated with increased risk of stroke,[10,12-14] coronary events[10,15,18] and all-cause mortality,[11,17,18] all in middle-aged persons. In older age, visit-to-visit variability blood pressure is associated with an increased long-term risk for cardiovascular and total mortality and cognitive decline.[16,19] In addition, variability in diastolic blood pressure is predictive of coronary events and heart failure hospitalization.[16]

Higher visit-to-visit variability in blood pressure might result from biological factors. Possible mechanisms underlying high levels of visit-to-visit-variability of blood pressure include impaired baroreceptor function,[5,20] arterial stiffness,[11,21] impaired endothelial function,[22,23] inflammation,[21,24,25] and renal impairment.[26] Identifying factors associated with intra-individual variability in blood pressure may help elucidate the underlying mechanisms. It is unknown which factors correlate most strongly with variability of blood pressure in older people. We hypothesized that inflammation, impaired endothelial function, renal function, and glucose metabolism might be important biological pathways underlying visit-to-visit variability of blood pressure in older age, based on previous studies on correlates of higher variability of blood pressure in middle-aged populations.[10,21-26] We tested these hypotheses in 3,794 subjects participating in the PROspective Study of Pravastatin in the Elderly at Risk (PROSPER).[27]

METHODS

Study design

Details of the design and outcome of PROSPER have been published elsewhere.[27-29] In short, between December 1997, and May 1999 a total of 5,804 individuals were screened and enrolled in Scotland (n = 2,520), Ireland (n = 2,184) and the Netherlands (n = 1,100). Men and women aged 70–82 years were recruited if they had either pre-existing vascular disease (coronary, cerebral, or peripheral) or increased risk of such disease because of smoking, hypertension, or diabetes. Their plasma total cholesterol was required to be 4.0–9.0 mmol/L and their triglyceride concentrations ≤6.0 mmol/L. Individuals with poor cognitive function (Mini-Mental State Examination score <24 points) were

excluded. Blood pressure was not part of the inclusion or exclusion criteria. In total, 5,804 participants were randomized to either placebo or pravastatin; 2,913 assigned placebo and 2,891 assigned pravastatin.[27] During the pre-randomization visits, baseline participant characteristics were collected.[28] The institutional ethics review boards of all centers approved the protocol, and all participants gave written informed consent.

Blood pressure measurements

Sitting blood pressure was measured once at baseline and at follow-up visits every 3 months during the trial (mean follow-up 3.2 years) with a fully automatic electronic sphygmomanometer (Omron M4).

Biomarkers

Markers of inflammation. Saved biobank of baseline K2EDTA samples were used to assay interleukin-6 in 2007 using a high-sensitivity enzyme-linked immunosorbent assay (R&D Systems) with inter- and intra-assay coefficients of variation of <6% and sensitivity of <0.16 pg/mL.[30] High sensitivity C-reactive protein levels were measured on stored K2EDTA (at −80 °C) baseline samples by automated particle-enhanced immunoturbidimetric assay (Roche UK, Welwyn Garden City, UK), with inter- and intra-assay coefficients of variation of <3% and a lower limit of sensitivity of 0.1 mg/L.[31] The laboratory participates in a national external quality control for highsensitivity C-reactive protein. All laboratory analyses were conducted by technicians blind to the identity of samples.

Markers of endothelial function. In baseline blood samples from the biobank (stored at <80 °C) tissue plasminogen activator antigen was measured in citrated plasma using an enzyme-linked immunosorbent assay (Biopool AB) and VonWillebrand factor antigen level was measured in citrated plasma using an enzyme-linked immunosorbent assay with rabbit antihuman polyclonal antibodies (Dako).[32]

Markers of renal function. Baseline serum creatinine levels were measured at central laboratories, one in each of the three participating countries. Glomerular filtration rate was estimated using the Modification of Diet in Renal Disease equation[33]:

eGFR=186 x serum creatinine level (mg dl)(-1.154) x age(-0.203) x 0.742 (if female)

Markers of glucose metabolism. Body mass index and detailed medical history was collected at baseline. Fasting glucose levels were assessed at baseline, using routine methods.[29] Diabetes at baseline was defined by self-reported history, a fasting blood glucose concentration of 7.0 mmol/L or greater or a blood glucose measurement of 11.1 mmol/L or greater when fasting status was uncertain, or self-reported use of antidiabetic drugs (any oral hypoglycemic agent or insulin). Data on fasting glucose and fasting insulin levels were used to calculate the degree of insulin resistance according to homeostatic model assessment (HOMA).[34] The HOMA score was calculated using

the HOMA index[34] by dividing the product of fasting levels of glucose and insulin by a constant.

Statistical analysis

Variability of blood pressure was defined as the SD of the mean per person over 9 visits (0–24 months). Variability was assessed for systolic blood pressure, diastolic blood pressure and pulse pressure. Participants with one or more missing blood pressure measurements in the first 24 months (n = 1,403); those who had a cardiovascular event in the measurement period (0–24 months, n = 316) and those with atrial fibrillation (n = 4) were excluded. A total of 487 participants had missing data for one or more of the biomarkers and were also excluded (some participants had more than one exclusion criteria) (Figure 1). Variability of systolic blood pressure was also quantified using the coefficient

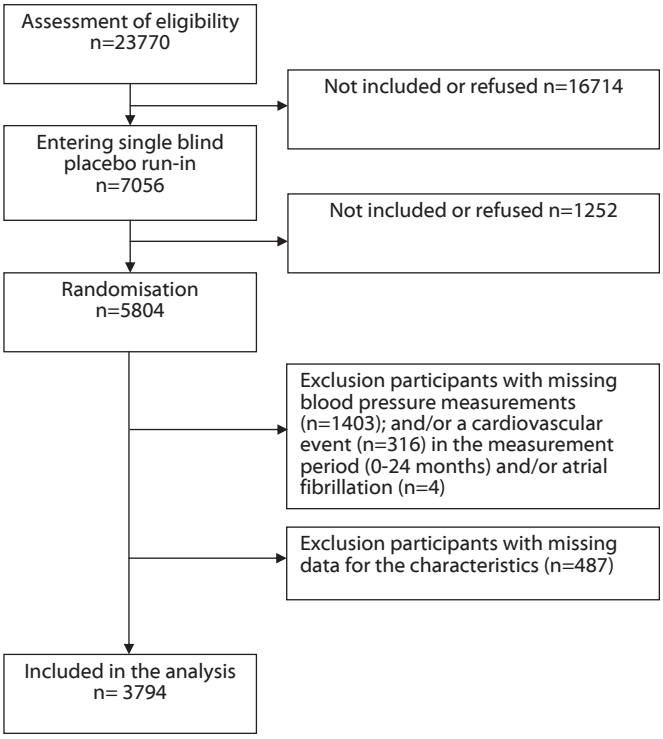

Figure 1. Flow chart

of variation (SD/ mean). The results for SD and coefficient of variation were qualitatively the same; therefore the results for SD are presented.

F-tests were used to test the variability in blood pressure variability between participants receiving pravastatin and those receiving placebo. Variability variables were split into quartiles. Baseline characteristics were calculated by quartile of SD of variability variables; they are reported as mean with SD for continuous variables and as numbers with percentage (%) for categorical variables. The association between the characteristics at baseline (independent variable) with the variability variables (dependent variable) was assessed using linear regression. The distribution of the variables C-reactive protein and interleukin-6 was skewed, therefore, these variables were log-transformed.

The markers of inflammation, endothelial function, renal function, and glucose metabolism were first analyzed separately, and then combined. The initial "minimally adjusted" regression model included adjustment for country and randomized treatment. In a subsequent model adjustment for age, gender, current smoking, average blood pressure during follow-up (systolic and diastolic blood pressure and pulse pressure), history of cardiovascular disease, history of hypertension, total cholesterol, high-density lipoprotein, and low-density lipoprotein was made. In the final model, the multivariable analysis, all biomarkers were combined to determine the subset of characteristics that were independently associated with variability in blood pressure. The beta's from the linear regression analyses for the variables C-reactive protein and interleukin-6 are not easy to interpret as these variables were log-transformed. Therefore, the change in SD per doubling of these variables was calculated. In addition, the *R*-squared values for the models were calculated. In an additional analysis we excluded interleukin-6 from the final model to assess what effect this would have on the parameter estimates of the other variables.

A change in antihypertensive drug treatment could have influenced the SD in blood pressure. We therefore performed a sensitivity analysis in all participants that remained on the same antihypertensive treatment throughout the first 24 months.

RESULTS

Table 1 presents the baseline characteristics for the participants. Of the 3,794 participants 1,810 (47.7%) were men, the mean age was 75.2 years (SD 3.3) and 1,603 (42.3%) had a history of cardiovascular disease. The mean intra-individual SD of 9 measurements of systolic blood pressure was 14.4 mm Hg; the mean SD of diastolic blood pressure was 7.7 mm Hg and the mean SD of pulse pressure was 12.6 mm Hg. There was no significant difference in variability in blood pressure between participants receiving pravastatin

and those receiving placebo (*P* values 0.31, 0.92, 0.15); therefore, data from both groups were combined and all analyses were adjusted for treatment.

Table 1. Characteristics of study participants (n=3,794)

Characteristic	
Categorical variates (n, %)	
Men	1,810 (47.7%)
Current smoker	937 (24.7%)
Country	
Scotland	1,582 (41.7%)
Ireland	1,437 (37.9%)
Netherlands	775 (20.4%)
History of cardiovascular disease[a]	1,603 (42.3%)
History of hypertension	2,412 (63.6%)
Continuous variates (mean, SD)	
Age (years)	75.21 (3.32)
Average systolic blood pressure (mmHg)	153.78 (16.32)
Variability in systolic blood pressure (mmHg)	14.38 (5.11)
Average diastolic blood pressure (mmHg)	83.25 (7.54)
Variability in diastolic blood pressure (mmHg)	7.68 (2.97)
Average pulse pressure (mmHg)	70.53 (12.96)
Variability in pulse pressure (mmHg)	12.64 (4.56)
Baseline total cholesterol (mmol/L)	5.69 (0.90)
Baseline Low-density lipoprotein cholesterol (mmol/L)	3.79 (0.80)
Baseline High-density lipoprotein cholesterol (mmol/L)	1.28 (0.35)

[a] Any of stable angina, intermittent claudication, stroke, transient ischemic attack, myocardial infarction, peripheral arterial disease surgery, or amputation for vascular disease more than 6 months before study entry.

Biological correlates of systolic blood pressure variability

Table 2 shows the baseline characteristics per quartile of variability in systolic blood pressure. Higher levels of C-reactive protein, interleukin-6 and creatinine and lower estimated glomerular filtration rate, all at baseline, were associated with higher quartiles of intra-individual variability of systolic blood pressure in the minimally adjusted analysis. Baseline levels of Von Willebrand Factor and tissue plasminogen activator were not associated with intra-individual variability in systolic blood pressure, nor was there an association between the glucose metabolism characteristics and intra-individual variability in systolic blood pressure.

Table 2. Baseline biomarkers by quartile of intra-individual systolic blood pressure variability (measured as SD of 9 measurements over 24 months)

			Quartile of SD of systolic blood pressure				
			Q1	Q2	Q3	Q4	*P* value minimally adjusted analysis[a]
Pathway	Baseline biomarker	All Subjects (n = 3794)	≤ 10.8 (n = 947)	>10.8 - ≤13.6 (n = 951)	>13.6 - ≤17.2 (n = 948)	>17.2 (n = 948)	
Inflammation	Log(C-reactive protein)	1.08 (1.10)	1.03 (1.07)	0.98 (1.09)	1.13 (1.12)	1.19 (1.11)	<0.001
	Log(Interleukin-6)	0.95 (0.65)	0.91 (0.66)	0.90 (0.62)	0.98 (0.65)	1.00 (0.66)	<0.001
Endothelial function	Von Willebrand factor	139.7 (45.80)	141.0 (45.87)	139.3 (45.18)	139.2 (45.71)	139.2 (46.46)	0.31
	Tissue plasminogen activator	10.93 (4.01)	10.91 (3.82)	10.92 (3.78)	10.92 (4.10)	10.97 (4.31)	0.15
Renal function	Creatinine	100.5 (21.66)	99.68 (20.17)	101.5 (21.77)	100.2 (22.03)	100.6 (22.59)	<0.01
	Estimated glomerular filtration rate	60.27 (14.14)	61.12 (13.37)	60.12 (14.26)	60.19 (14.18)	59.64 (14.69)	<0.001
Glucose metabolism	Body mass index	26.90 (4.08)	26.97 (4.07)	26.98 (4.07)	26.91 (4.15)	26.75 (4.02)	0.23
	Glucose	5.44 (1.38)	5.48 (1.40)	5.48 (1.39)	5.39 (1.35)	5.38 (1.39)	0.84
	History of diabetes	391 (10.3%)	104 (11.0)	100 (10.5)	95 (10.0)	92 (9.7)	0.67
	Log(HOMA)	0.65 (0.70)	0.67 (0.70)	0.69 (0.70)	0.61 (0.70)	0.61 (0.71)	0.26

Mean and corresponding SDs and numbers and corresponding percentage.
Abbreviation: HOMA, homeostatic model assessment.
[a]Adjusted for randomised treatment code and country by linear regression. *P* for trend across quartiles.

Biological correlates of diastolic blood pressure variability

Table 3 shows the baseline characteristics per quartile of variability in diastolic blood pressure. Higher levels of C-reactive protein and interleukin-6, were associated with higher quartiles of intra-individual variability of diastolic blood pressure. In contrast to systolic blood pressure variability, higher estimated glomerular filtration rate was associated with a higher variability in diastolic blood pressure. Higher levels of tissue plasminogen activator, higher body mass index and higher level of HOMA were all associated with higher variability in diastolic blood pressure in the minimally adjusted analysis.

Table 3. Baseline biomarkers by quartile of intra-individual diastolic blood pressure variability (measured as SD of 9 measurements over 24 months)

			Quartile of SD of diastolic blood pressure				
			Q1	Q2	Q3	Q4	*P* value minimally adjusted analysis[a]
Pathway	Baseline biomarker	All Subjects (n = 3794)	≤ 5.7 (n = 965)	>5.7- ≤7.2 (n = 916)	>7.2 -≤ 9.1 (n = 959)	> 9.1 (n = 954)	
Inflammation	Log(C-reactive protein)	1.08 (1.10)	0.99 (1.10)	1.07 (1.08)	1.09 (1.10)	1.18 (1.11)	<0.001
	Log(Interleukin-6)	0.95 (0.65)	0.89 (0.64)	0.92 (0.64)	0.93 (0.63)	1.04 (0.68)	<0.001
Endothelial function	Von Willebrand factor	139.7 (45.80)	139.4 (43.15)	138.1 (46.04)	140.0 (46.90)	141.1 (47.05)	0.04
	Tissue plasminogen activator	10.93 (4.01)	10.96 (4.11)	10.68 (3.75)	10.94 (4.07)	11.12 (4.07)	<0.01
Renal function	Creatinine	100.5 (21.66)	101.4 (21.15)	100.1 (21.35)	100.7 (21.63)	99.71 (22.49)	0.66
	Estimated glomerular filtration rate	60.27 (14.14)	60.18 (13.60)	60.07 (13.71)	60.30 (14.19)	60.52 (15.02)	0.04
Glucose metabolism	Body mass index	26.90 (4.08)	26.81 (3.96)	26.90 (4.08)	26.93 (4.04)	26.97 (4.24)	0.03
	Glucose	5.44 (1.38)	5.43 (1.27)	5.44 (1.42)	5.46 (1.31)	5.40 (1.52)	0.65
	History of diabetes	391 (10.3%)	100 (10.4)	98 (10.7)	95 (9.9)	98 (10.3)	0.51
	Log(HOMA)	0.65 (0.70)	0.65 (0.69)	0.63 (0.67)	0.64 (0.72)	0.65 (0.72)	0.03

Mean and corresponding SDs and numbers and corresponding percentage.
Abbreviation: HOMA, homeostatic model assessment.
[a]Adjusted for randomised treatment code and country by linear regression. *P* for trend across quartiles.

Biological correlates of pulse pressure variability

Higher levels of C-reactive protein ($P < 0.001$), interleukin-6 ($P < 0.001$) and tissue plasminogen activator at baseline ($P < 0.01$) were also associated with higher variability of pulse pressure in the minimally adjusted analyses (Supplementary Table 1). Moreover, lower estimated glomerular filtration rate ($P < 0.001$), higher body mass index ($P < 0.001$) and higher level of HOMA at baseline ($P < 0.01$) were also associated higher intra-individual variability in pulse pressure.

Adjusted multivariable analysis

In the adjusted multivariable analysis, the association between higher levels of interleukin-6 and higher variability of systolic and diastolic blood pressure and pulse pressure remained significant (all $P < 0.01$) (Table 4). Every doubling of interleukin-6 was associ-

ated with 0.26 higher intra-individual SD (i.e., variability) systolic blood pressure and 0.21 (higher intra-individual SD diastolic blood pressure. *R*-squared value for the adjusted multivariate model was 8.2% for systolic blood pressure variability and 4.8% for diastolic blood pressure, and 8.2% for pulse pressure variability.

Table 4. Change in intra-individual SD (of 9 measurements over 24 months) systolic and diastolic blood pressure per doubling of C-reactive protein and interleukin-6, multivariable analysis per pathway

	Systolic blood pressure				Diastolic blood pressure			
Baseline characteristic	**Change in SD[a]**	***P* value[a]**	**Change in SD[b]**	***P* value[b]**	**Change in SD[a]**	***P* value[a]**	**Change in SD[b]**	***P* value[b]**
C-reactive protein	0.10 (-0.01,0.22)	0.09	0.07 (-0.05, 0.18)	0.25	0.06 (-0.01, 0.12)	0.10	0.04 (-0.03, 0.11)	0.22
Interleukin-6	0.28 (0.08, 0.48)	<0.01	0.26 (0.06, 0.46)	0.01	0.20 (0.09, 0.32)	<0.001	0.21 (0.09, 0.33)	<0.001

[a]Adjusted for country and treatment by linear regression; *P* value calculated by linear regression.
[b]Adjusted for age, gender, country, treatment, current smoking, average SBP over 2 years for variability in SBP and average DBP over 2 years for variability in DBP, history of cardiovascular disease, history of hypertension, total, HDL- and LDL- cholesterol by linear regression; *P* value calculated by linear regression.

Table 5 shows that there was no independent association between tissue plasminogen activator and variability in systolic and diastolic blood pressure in the adjusted multivariable analysis. A higher level of tissue plasminogen activator was associated with higher pulse pressure variability, every point higher of tissue plasminogen activator was associated with 0.05 (95% CI 0.01–0.09) higher SD pulse pressure. *R*-squared value for the adjusted multivariable analysis was 8.0%.

The associations between renal function and variability in blood pressure disappeared when adjusted for age, gender, current smoking, average blood pressure during follow-up (systolic and diastolic blood pressure and pulse pressure), history of cardiovascular disease, history of hypertension, and cholesterol in the multivariable analysis.

The association between body mass index and pulse pressure variability remained significant in the multivariable analysis (parameter estimate 0.06, 95% CI 0.02–0.10). *R*-squared value for the multivariate model was 8.0%.

In the final step of the analysis, all biomarkers were included in the same adjusted multivariable model. In this model, only higher levels of interleukin-6 were associated with higher variability of systolic and diastolic blood pressure and pulse pressure (all $P < 0.02$). Every doubling of interleukin-6 was associated with 0.24 higher SD systolic blood pressure, 0.19 higher SD diastolic blood pressure and 0.23 higher SD pulse pressure. Moreover, every point higher of body mass index was associated with a 0.045 (95% CI 0.004, 0.087) higher SD pulse pressure. The *R*-squared value for this adjusted multivariable model including all biomarkers was 9.0% for systolic blood pressure variability, 5.0% for diastolic blood pressure variability and 8.7% for pulse pressure variability.

Table 5. Associations with systolic and diastolic blood pressure variability (intra-individual SD of 9 measurements over 24 months), multivariable analysis per pathway

		Systolic blood pressure				Diastolic blood pressure			
Pathway	**Baseline characteristic**	**Beta (95% CI)[a]**	***P* value**	**Beta (95% CI)[b]**	***P* value**	**Beta (95% CI)[a]**	***P* value**	**Beta (95% CI)[b]**	***P* value**
Endothelial function	Von Willebrand factor	-0.003 (-0.006, 0.001)	0.17	-0.003 (-0.006, 0.001)	0.14	0.002 (-0.001, 0.004)	0.14	0.002 (-0.001, 0.004)	0.16
	Tissue plasminogen activator	0.04 (-0.01, 0.08)	0.09	0.04 (-0.01, 0.08)	0.09	0.03 (0.01, 0.05)	0.01	0.02 (-0.01, 0.05)	0.07
Renal function	Creatinine	-0.004 (-0.016, 0.008)	0.47	0.024 (-0.002, 0.051)	0.07	-0.005 (-0.012, 0.002)	0.15	0.014 (-0.002, 0.030)	0.08
	Estimated glomerular filtration rate	-0.041 (-0.059, -0.022)	< 0.01	0.011 (-0.029, 0.051)	0.58	-0.014 (-0.025, -0.003)	0.013	0.020 (-0.004, 0.044)	0.10
Glucose metabolism	Body mass index	-0.02 (-0.06, 0.03)	0.46	-0.04 (-0.08, 0.01)	0.10	0.01 (-0.01, 0.04)	0.18	0.02 (-0.01, 0.04)	0.29
	Glucose	-0.01 (-0.18, 0.16)	0.88	0.00 (-0.17, 0.17)	1.0	-0.04 (-0.14, 0.06)	0.44	-0.01 (-0.11, 0.09)	0.84
	History of diabetes	-0.24 (-0.95, 0.46)	0.50	-0.29 (-0.98, 0.40)	0.41	-0.12 (-0.53, 0.29)	0.56	-0.27 (-0.68, 0.14)	0.20
	Log (HOMA)	-0.11 (-0.40, 0.19)	0.48	-0.16 (-0.46, 0.14)	0.30	0.12 (-0.05, 0.29)	0.18	0.08 (-0.10, 0.25)	0.41

Abbreviation: HOMA, homeostatic model assessment.

[a]Adjusted for country and treatment by linear regression.

[b]Adjusted for age, gender, country, treatment, current smoking, average SBP over 2 years for variability in SBP and average DBP over 2 years for variability in DBP, history of cardiovascular disease, history of hypertension, total, HDL- and LDL- cholesterol by linear regression.

In an additional analysis we excluded interleukin-6 from the final multivariable model. In this model, higher level of C-reactive protein was associated with higher systolic and diastolic blood pressure variability as well as higher pulse pressure variability (all $P < 0.01$).

In a sensitivity analysis we explored the effect of excluding those participants (n = 1,816) with a change in antihypertensive drug treatment (defined as a change in listed antihypertensive drugs), and repeating the analysis for only those participants (n = 1,978) who remained on the same antihypertensive drugs throughout the first 24 months; in these analyses there was no material change in the relationships observed in the whole cohort.

DISCUSSION

This study shows that higher interleukin-6 levels are associated with visit-to-visit variability in systolic and diastolic blood pressure as well as pulse pressure in an older population at high risk for cardiovascular disease. In addition, higher body mass index was associated with higher visit-to- visit variability in pulse pressure. These associations were independent of various cardiovascular risk factors and average blood pressure and remained present when all participants that had a change in antihypertensive drug treatment were excluded.

Previously it has already been suggested that visit-to-visit variability in blood pressure is not a random phenomenon.[8] Several underlying mechanisms have been proposed with regard to higher levels of visit-to-visit variability in blood pressure.[8,35,36] Some have suggested that higher blood pressure variability might identify people with subclinical inflammation.[25] In animal models, variability of blood pressure has been associated with higher levels of C-reactive protein.[21,24] In the present study elevated interleukin-6 was indeed associated with variability in systolic and diastolic blood pressure as well as pulse pressure. In contrast, elevated C-reactive protein was not independently associated with higher visit-to-visit variability in blood pressure, in line with other studies.[11] However, when we excluded interleukin-6 from the final multivariable analysis, higher level of C-reactive protein became associated with higher variability in systolic and diastolic blood pressure as well as pulse pressure. This effect is biologically plausible; adjusting for interleukin-6 implies adjusting in the causal pathway, since interleukin-6 stimulates the production of C-reactive protein. Interleukin-6 and C-reactive protein were measured at baseline, prior to the measure of variability, therefore, we could not assess causal relationships. We hypothesis that subclinical inflammation may lead to higher blood pressure variability and as a result, is associated with higher risk of cardiovascular disease. Another explanation is that subclinical inflammation itself

causes higher risk of cardiovascular disease and higher blood pressure variability. Higher blood pressure variability could then be regarded as a marker of the association between subclinical inflammation and cardiovascular disease.

Recent evidence in a small sample of African Americans indicates that higher blood pressure variability is linked with endothelial injury, decreased endothelial functioning and disturbances in vascular smooth muscle functioning.[22,23] It has been suggested that tissue plasminogen activator, which is synthesized mainly in the vascular endothelium, is a marker for endothelial function, with higher levels of tissue plasminogen activator indicating endothelial dysfunction.[37-39] In our study, the associations between tissue plasminogen activator and blood pressure variability was only observed for pulse pressure variability in the adjusted analysis. The evidence suggesting that impaired endothelial function underlies the higher blood pressure variability presented in our study is therefore only limited and less convincing as compared to the inflammation pathway. We have previously shown that, pulse pressure variability was somewhat associated with long-term stroke risk in an older population at risk for cardiovascular disease, while systolic and diastolic blood pressure variability where not.[16] This could indicate that different underlying mechanism might play a role. Endothelial injury, resulting in endothelial dysfunction could be a result of variability in pulse pressure. Whether higher levels of tissue plasminogen activator are just a marker of higher variability of pulse pressure or a true mediator in the relation between variability in pulse pressure and cardiovascular disease is uncertain. The association between estimated glomerular filtration rate and blood pressure variability disappeared when adjusted for age, gender, current smoking, average blood pressure, history of cardiovascular disease, history of hypertension, and cholesterol. This could indicate that impaired renal function does not underlie high levels of visit-to-visit-variability of blood pressure. The previously observed association between history of diabetes and blood pressure variability[10] was not replicated in our study. We only found a low parameter estimate between body mass index and variability in pulse pressure, suggesting that glucose metabolism might not be relevant in an older population at risk for cardiovascular disease. Furthermore, it has been suggested that higher blood pressure variability may be a manifestation of baroreflex regulation of blood pressure.[5,20] However, decreased heart rate variability is associated with an increased risk of mortality in a previous study,[40] suggesting that heart rate variability does not play a role in the association between variability in blood pressure and mortality.

Another explanation may lie in cognitive decline. Both interleukin-6 levels[41] and C-reactive protein levels[42] and blood pressure variability[19] have been associated with cognitive decline. A common explanation may be that either cardiovascular burden, as measured in an increased level of interleukin-6 and C-reactive protein as well as increased blood pressure variability cause cognitive decline. Conversely, cognitive

decline may cause increased inflammatory markers (as a result of altered lifestyle, such as eating) and blood pressure variability.

The differences found in the associations between the different biomarkers and the variability in systolic and diastolic blood pressure and pulse pressure might reflect the differences in the associations with cardiovascular disease which were previously shown.[16]

Identifying inflammation as a factor associated with intra-individual variability in blood pressure and excluding other factors such as glucose, may help elucidate the underlying mechanisms of intra-individual variability in blood pressure. The model explains up to 9% of the variability in blood pressure, this modest fit could be considered to be a weakness of the study, despite small *P* values that reflect the large sample size. However, since we have only one baseline measure of biomarkers, this could be an underestimation of the true association due to regression dilution bias. We argue that part of the observed association could also be attributed to the fact that blood pressure was measured once every 3 months and the time of the day could vary between these measurements. The season of measurement could also influence the variability in blood pressure. This exogenous introduced variability however is unlikely to be of any effect on the influence of blood pressure variability on the pathogenesis of cardiovascular disease. Inflammation is one of the possible biological pathways of higher variability in blood pressure not explained by these exogenous factors. Other potential biological mechanisms remain unknown up to now. The change in drugs used as antihypertensive treatment could have influenced the blood pressure variability. This is, however, not expected to influence the association between interleukin-6 and blood pressure variability. The results did not materially changed when all participant that had a change in antihypertensive drug treatment (defined as a change in listed antihypertensive drugs) were excluded. It would be interesting to know if baseline variability in blood pressure predicts subsequent changes in inflammation biomarkers using longitudinal data. Metabolomics might also be a potential direction for further research to explore novel mechanisms of blood pressure variability.

This study was embedded in the PROSPER trial, a large double-blinded randomized placebo-controlled trial in older persons. This landmark clinical trial with older participants was performed following guidelines of good clinical practice. Blood pressure was not part of the inclusion or exclusion criteria for the PROSPER trial, therefore, people with the full range of baseline blood pressure and variability in blood pressure were included. The estimation of visit-to-visit variability should be reasonably reliable because of the frequency of measurements (nine measurements). The large sample size is another strength of this study. This study has certain limitations. A potential limitation is that the participants were randomized to an intervention (pravastatin vs. placebo), however, we found no difference in variability in blood pressure between both ran-

domized groups and all analyses were adjusted for the randomized treatment. Second, given the cross-sectional design of the study, we cannot conclude whether variability in blood pressure lead to subclinical inflammation, or vice versa. Third, we were lacking measures of baroreflex sensitivity, therefore this potential biological mechanism underlying individual variability in blood pressure in older persons could not be analyzed in this study. In addition, some of the associations between variability in blood pressure and baseline biomarkers may not be linear, however, we do not have sufficient power in our data to explore this fully. The trend or slope of blood pressure over time may have influenced the SD of blood pressure and thus can be considered as a potential confounding variable. The analysis was not adjusted for the trend or slope in blood pressure as we believe that this is beyond the scope of this paper. The final analysis were adjusted for randomized treatment code, country, age, gender, current smoking, average blood pressure, history of cardiovascular disease, history of hypertension, and cholesterol. One could argue that when adjusting for all these additional variables one might have introduced confounding instead of the intended adjustment for confounding. The step wise analysis approach was chosen to provide the most transparent results. Finally, there was no correction for multiple testing. These corrections could have resulted in type II errors and therefore prematurely discarding potentially useful observations which may help to elucidate the underlying mechanisms of intra-individual variability in blood pressure.

For the interpretation of the results it is important to note that the excluded patients are intrinsically different due to the criteria used for selection. The excluded patients include those with events in the measurement period (0–24 months, n = 316) and therefore differed in a systematic manner. They likely include the "sicker" patients. Another of our criteria is the requirement to have blood pressure measured at each of the nine time points during the 24 months. This excludes the non-attenders.

In conclusion, in this study of older population at risk, higher levels of interleukin-6 were independently associated with visit-to-visit variability in systolic and diastolic blood pressure and pulse pressure. Additional research is needed to confirm these results and to evaluate their potential clinical significance and potential mechanisms to reduce visit-to-visit variability.

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APPENDIX

Supplemental Table 1. Baseline biomarkers by quartile of intra-individual pulse pressure variability (measured as SD of 9 measurements over 24 months)

			Quartile of SD of pulse pressure				
			Q1	Q2	Q3	Q4	*P* value minimally adjusted analysis[a]
Pathway	Baseline biomarker	All Subjects (n= 3794)	≤ 9.4 (n = 959)	>9.4 - ≤12.1 (n = 946)	>12.1 - ≤15.2 (n = 943)	>15.2 (n = 946)	
Inflammation	Log(C-reactive protein)	1.08 (1.10)	0.99 (1.08)	0.99 (1.10)	1.16 (1.12)	1.19 (1.09)	<0.001
	Log(Interleukin-6)	0.95 (0.65)	0.90 (0.65)	0.89 (0.64)	0.98 (0.66)	1.01 (0.64)	<0.001
Endothelial function	Von Willebrand factor	139.7 (45.80)	139.5 (45.13)	139.4 (46.16)	140.0 (45.62)	139.8 (46.34)	0.95
	Tissue plasminogen activator	10.93 (4.01)	10.77 (3.87)	10.94 (3.90)	10.84 (4.06)	11.17 (4.19)	<0.01
Renal function	Creatinine	100.5 (21.66)	100.2 (21.11)	100.6 (20.69)	101.1 (21.94)	100.1 (22.87)	0.10
	Estimated glomerular filtration rate	60.27 (14.14)	61.22 (13.66)	60.24 (13.67)	59.74 (14.13)	59.86 (15.03)	<0.001
Glucose metabolism	Body mass index	26.90 (4.08)	26.68 (3.88)	26.85 (4.17)	26.70 (3.94)	27.39 (4.27)	<0.001
	Glucose	5.44 (1.38)	5.46 (1.38)	5.47 (1.48)	5.40 (1.27)	5.41 (1.40)	0.20
	History of diabetes	391 (10.3%)	100 (10.4)	99 (10.5)	90 (9.5)	102 (10.8)	0.07
	Log(HOMA)	0.65 (0.70)	0.63 (0.71)	0.63 (0.68)	0.64 (0.70)	0.68 (0.71)	<0.01

Mean and corresponding SDs and numbers and corresponding percentage.
Abbreviation: HOMA, homeostatic model assessment.
[a]Adjusted for randomised treatment code and country by linear regression. P for trend across quartiles.