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SHEAR STRESS IN THE COMMON CAROTID ARTERY IS RELATED TO BOTH INTIMA-MEDIA THICKNESS AND ECHOGENECITY

- The Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) study

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Short title: Shear stress and vascular wall

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Abstract. It has previously been shown that the degree of shear stress (SS) in the carotid artery is related to both plaque occurrence and intima-media thickness (IMT). Since the echogenecity also is an important feature of plaques, we investigated if a reduced shear stress also is related to the echolucency of plaque and the intima-media complex. In the Prospective Study of the Vasculature in Uppsala Seniors (PIVUS) study, a population-based study of 1016 subjects aged 70, left common carotid artery diameter, IMT, the grey scale median (GSM) of the intima-media complex (IM-GSM) and the blood flow velocity were measured by ultrasound. Occurrence of plaque was noted, and the echogenecity of the plaques was visually estimated by the Gray-Weale classification. Shear stress was inversely related to both IMT and IM-GSM (p=0.0084 and p=0.003, respectively), independently of gender and coronary risk, estimated by the Framingham risk score. Shear stress was lower in subjects with carotid plaque (44% of the sample) than in those without (p=0.0013), and was inversely related to the echogenecity in the subjects with plaque (p= 0.0092), independently of gender and coronary risk. A low shear stress in the common carotid artery was associated with both a thick IMT and an echolucent intima-media complex. A similar picture was seen when overt plaques were evaluated, suggesting that shear stress is of importance for both the extent and composition of atherosclerosis.

Keywords: Blood viscosity; intima-media thickness, plaques, shear stress.

1. Introduction

  A low shear stress in the carotid artery has previously been associated with atherosclerotic plaques and a thick intima-media thickness (IMT) at carotid artery ultrasound [1,7-9,12-15,26,27]. Several different mechanisms might be involved in shear stress-induced atherosclerosis: increased fluid residence time and transport of atherogenic particles [3,24], increased platelet and macrophage adhesion to the arterial wall [22], modulation of the transcription of genes for nitric oxide [4-6], platelet-derived growth factor and transforming growth factor-ß1 [21-23], and increased local production of mitogenic substances.
  Apart from the size of the atherosclerosis or IMT, tissue characterization by use of the degree of echogenecity of the plaque might provide important information of plaque vulnerability. Recent studies in the carotid and femoral artery have shown echolucent plaques to predict future cardiovascular events [20,25]. An own recent study showed the echogenecity, measured as the grey scale median, of the intima-media complex (IM-GSM), to be closely related to the echogenecity of overt plaques [17], suggesting that also measurements of IM-GSM could be of value for characterisation of the vascular wall. Furthermore, we showed that an echolucent IM-GSM was related to an atherogenic lipid profile and markers of oxidative stress [18], further exemplifying that IM-GSM could be an important biomarker in the evaluation of subclinical atherosclerosis.
  The present study aim to investigate if shear stress of the common carotid artery is related to the echogenecity of the intima-media complex and overt plaques, with the hypothesis that a low shear stress is associated with an echolucent vascular wall and echolucent plaques. We used data from the Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS)

study, a population-based cohort study initiated in more than 1000 subjects aged 70 years living in the community of Uppsala, Sweden [19] in whom a detailed characterisation of the carotid arteries was performed by ultrasound.

2. Materials and methods

2.1 Subjects

Eligible were all subjects aged 70 living in the community of Uppsala, Sweden. The subjects were chosen from the register of community living and were invited in a randomized order from the start of the study in April 2001 to the last included subject in June 2005. The subjects received an invitation by letter within 1 month of their 70th birthday in order to standardize for age. Of the 2025 subjects invited, 1016 subjects were investigated giving a participation rate of 50.1% [19].

The study was approved by the Ethics Committee of the University of Uppsala and the participants gave informed consent.

2.2 Baseline investigation

The participants were asked to answer a questionnaire about their medical history, smoking habits and regular medication.

All subjects were investigated in the morning after an overnight fast. No medication or smoking was allowed after midnight. During the investigation, the subjects were supine in a quiet room maintained at a constant temperature. After recordings of height, weight, abdominal and hip circumference, an arterial cannula was inserted in the brachial artery for blood sampling and later regional infusions of vasodilators. Lipid variables and fasting blood glucose were measured by standard laboratory techniques.
Characteristics of the sample are given in Table 1.

Approximately 10% of the cohort reported a history of coronary heart disease, 4% reported stroke and 9% diabetes mellitus. Almost half the cohort reported any cardiovascular medication (45%), with antihypertensive medication being the most prevalent (32%). Fifteen percent reported use of statins, while insulin and oral antiglycemic drugs were reported in 2 and 6%, respectively (see [19] for details).

As the participation rate in this cohort was only 50%, we carried out an evaluation of cardiovascular disorders and medications in 100 consecutive subjects who were invited to the study, but denied participation. The prevalences of cardiovascular drug intake, history of myocardial infarction, coronary revascularization, antihypertensive medication, statin use and insulin treatment were similar to those in the investigated sample, while the prevalences of diabetes, congestive heart failure and stroke tended to be higher among the non-participants (see [19] for details).

Table 1.
Multiple regression model with measured whole blood viscosity as dependent variable and haematocrit, gender (coded 1 for female and 0 for men), CRP, height, HDL-cholesterol, serum triglycerides, creatinine, albumin and ALT as independent variables.

Independent Variables	Mean value in the sample (SD in parenthesis)	Regression coefficient	Partial correlation	p-value
Gender (% female)	50.2	-0.066	-0.07	0.035
Haematocrit (%)	41 (3.0)	0.099	0.76	<0.0001
CRP (mg/L)	3.2 (4.7)	0.011	0.09	0.0002
Serum creatinine (μmol/L)	80 (19)	0.002	0.08	0.0014
Serum albumin (g/L)	40.3 (3.7)	0.008	0.05	0.028
ALT (μkat/L)	0.34 (0.23)	0.12	0.07	0.0032
Height (cm)	169 (9.0)	-0.004	-0.09	0.012
HDL-cholesterol (mmol/l)	1.5 (0.4)	-0.13	-0.12	0.0001
Serum triglycerides (mmol/l)	1.3 (0.6)	0.052	0.08	0.0033

2.3 Carotid artery ultrasound evaluation

The carotid artery was assessed by external B-mode ultrasound imaging (Acuson XP128 with a 10 MHz linear transducer, Acuson Mountain View, California, USA). The common carotid artery (CCA), the bulb and the internal carotid artery (ICA) were visualised and the occurrence of plaque was recorded on both sides. The IMT was evaluated in the far wall in the CCA 1-2 cm proximal to the bulb. The peak blood velocity of the common carotid artery was measured with pulsed Doppler with the sample volume placed in the middle of the vessel at the same distance from the bulb as the measurement of IMT.
The images were digitised and imported into the AMS (Artery Measurement Software) automated software [16] for dedicated analysis of IMT, GSM and plaque size. A maximal 10 mm segment with good image quality was chosen for IMT-analysis from the CCA. The programme automatically identifies the borders of the IMT of the far wall and the inner diameter of the vessel and calculates IMT and the diameter from around 100 discrete measurements through the 10 mm long segment. This automated analysis could be manually corrected if not found appropriate at visual inspection. The same analysis also calculated the carotid artery inner diameter in a similar way.
  A region of interest (ROI) was placed manually around the intima-media segment that was evaluated for IMT and the programme calculates the intima-media GSM (IM-GSM) from analysis of the individual pixels within the ROI on a scale from 0 (black) to 256 (white). The blood was used as the reference for black and the adventitia was the reference for white. The mean length of the evaluated intima-media segments was 9.0 (SD 2.1) mm when subjects with a segment recording less than 5 mm were excluded, leaving 946 subjects with valid recordings.
  The measurements of IMT were repeated in 30 random subjects giving a coefficient of variation of carotid artery IMT of 7.2 % and 7.5 % for IM-GSM.

A small plaque was considered to be present if the IMT was locally thickened more than 50% compared to the surrounding IMT. The carotid plaques were visually inspected and graded by three observers to reach a consensus according to the Gray-Weale classification [11]. According to this classification, the plaques were divided into four groups, echolucent, mainly echolucent, mainly echogenic and echogenic.

Since blood flow measurements were only performed in the left common carotid artery, we only used data on other characteristics from the same artery.

Shear rate was determined by the formula: 4*peak blood velocity/carotid artery diameter in diastole. Shear stress was calculated as shear rate times whole blood viscosity.

2.4 Assessment of whole blood apparent viscosity (WBV)

In order to calculate the wall shear stress, the whole blood apparent viscosity at a high shear rate was assessed.

Blood samples were collected in heparin-coated tubes and tested within one hour. Apparent WBV was analysed at 37°C in a low shear rotational viscometer (LS 30, Contraves AG, Zurich, Switzerland), at a shear rate of 100 s^{-1} (interpolated from measurements at 128.5 and 94.5 s^{-1}) at native haematocrit.

Whole blood viscosity was for practical reasons only measured on average every second day throughout the study, resulting in 571 evaluated subjects. In order to achieve a value on whole blood viscosity in every subject, we performed a stepwise multiple regression analysis with a number of biochemical variables that were measured and theoretically could influence whole blood viscosity (haematocrit, gender, CRP, height, weight, HDL-cholesterol, serum triglycerides, creatinine, albumin, ALT, sodium, potassium, urea, uric acid, LDL-cholesterol, leukocyte count).

2.5 Biochemical analyses

Serum alanine aminotransferase (ALT, EC 2.6.1.2, reagent: 8D36-30), albumin (Bromocresol purple, reagent: 7D54-20), alkaline phosphatase (EC 3.1.3.1, reagent: 7D55-30), creatinine (reagent: 14.3600.01, Synermed International, Westfield, IN, USA), C-reactive protein (CRP, reagent: 6K2601), potassium (ion selective electrode: 9D28), sodium (ion selective electrode: 9D28), triglycerides (reagent: 7D74-20), urea (reagent: 7D75-20) and uric acid (reagent: 7D76-20) were analyzed on an Architect Ci8200 (Abbott Laboratories, Abbott Park, IL, USA) and reported using S.I. units. If not stated otherwise within the brackets, the reagents were all obtained from Abbot Laboratories. The total coefficients of variation (CV) for the Architect methods were all below 3% except the total CV for creatinine which was 4.8%.

2.6 Statistics

Using a backwards stepwise regression model, whole blood viscosity was found to be significantly related to the haematocrit, gender, CRP, height, HDL-cholesterol, serum triglycerides, creatinine, albumin and ALT (see Table 1 for details). The total correlation coefficient for this model was 0.84. As could be seen in Table 1, the haematocrit was the major determinant of whole blood viscosity with a partial correlation coefficient of 0.76.

From the regression coefficients presented in Table 1 and the intercept of 0.611 whole blood viscosity was calculated in the total cohort. When the measured whole blood viscosity was

plotted vs calculated whole blood viscosity, the correlation coefficient was 0.85, p<0.0001 (Fig. 1).

Relationships between continuous variables were evaluated by Pearson's correlation coefficient and multiple regression analysis. Differences between groups were evaluated by ANOVA and ANCOVA. Skewed values were ln-transformed to obtain a normal distribution before analysis. Two-tailed significance values were given with p< 0.05 regarded as significant.
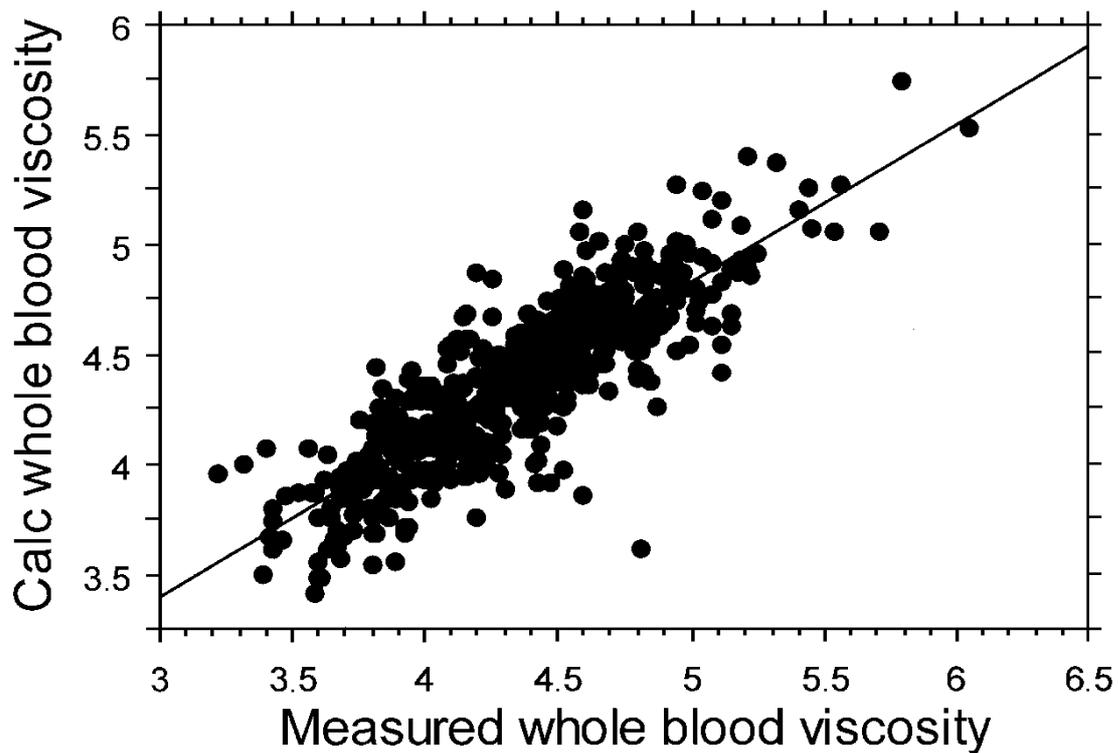

Fig. 1. Relationship between measured whole blood viscosity and calculated (calc) whole blood viscosity (r= 0.85, p<0.0001). The unit is mPa*s.

Table 2.
Data on left carotid artery atherosclerosis and shear stress and rate

Parameter	Mean (SD)
Wall shear stress (calculated) (dyne/cm^2)	21.4 (6.0)
Wall shear rate (calculated) (s^{-1})	493 (135)
IMT (mm)	0.89 (0.21)
IM-GSM (units)	79 (26)
Carotid diameter (mm)	6.3 (0.8)
Calculated whole blood viscosity (mPa*s)	4.3 (0.4)
Measured whole blood viscosity (mPa*s)	4.3 (0.4)
Peak blood flow velocity (m/s)	0.97 (0.24)

Notes: IMT- artery intima-media thickness, IM-GSM - the grey scale median of the intima-media complex, *n* = 1016. Prevalence of plaque 44%.

3 Results

3.1 Wall shear stress and wall shear rate vs IMT and IM-GSM

Data on left carotid artery atherosclerosis and rheology are presented in Table 2.

Wall shear stress was significantly related to IMT in an inverse way (r= -0.09, p=0.0084). This relationship was still significant after adjustment for gender and Framingham risk score in multiple regression analysis (p=0.036). Also wall shear rate was significantly related to IMT in an inverse way (r= -0.14, p<0.0001).

Wall shear stress was also significantly related to IM-GSM in an inverse way (r= -0.12, p=0.0003). This relationship was still significant after adjustment for gender, IMT and Framingham risk score in multiple regression analysis (p<0.0001). Also wall shear rate was significantly related to IM-GSM in an inverse way (r= -0.12, p=0.0003).

Shear stress
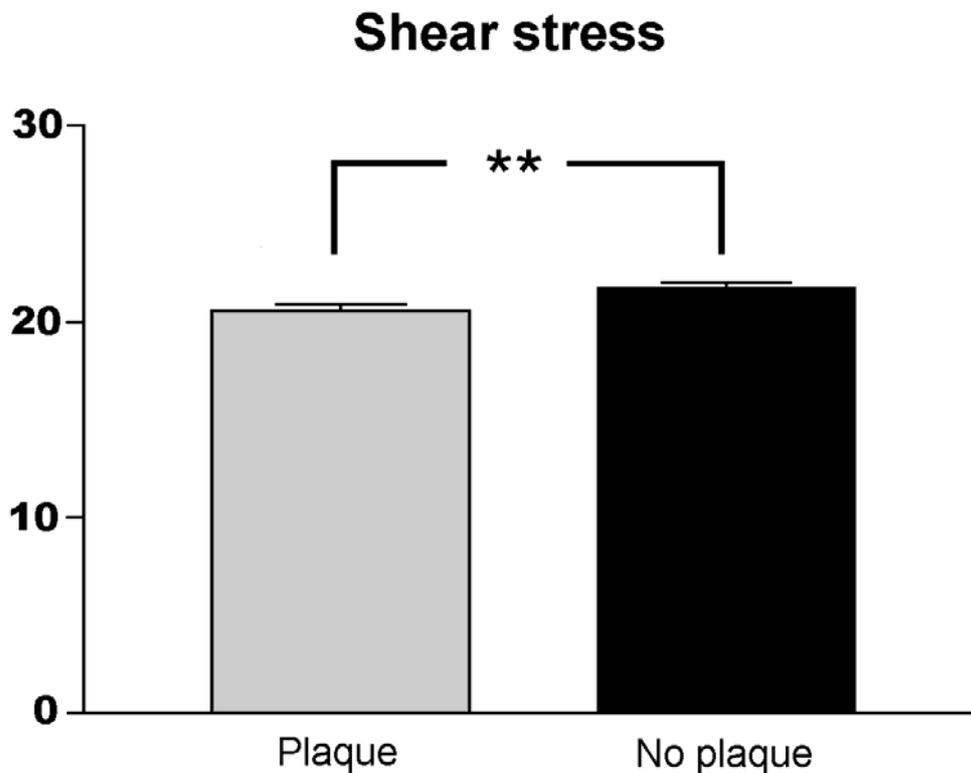

Fig. 2. Means and SEM for shear stress (dyne/cm^2) in subjects with (44% of the population) or without plaques in the left carotid artery.

3.2 Wall shear stress and wall shear rate vs plaque occurrence

Forty-four percent of the sample showed a plaque in the left carotid artery. Both wall shear stress (p=0.0013, Fig. 2) and wall shear rate (476 ± 139 SD s^{-1} in those with plaque vs 507 ± 128 s^{-1} in those without, p= 0.0032) were reduced in subjects with plaque compared with those without plaque in the left carotid artery.

Both wall shear stress (p=0.0080, see Table 3 for details) and wall shear rate (p=0.026) were still significantly related to the occurrence of plaque after adjustment for gender and Framingham risk score in two different logistic regression analysis models.

Table 3.
Multiple logistic regression model with occurrence of plaque in the left carotid artery as dependent variable and wall shear stress, gender (coded 1 for female and 0 for male) and Framingham score as independent variables.

Independent variable	Odds ratio	95%CI	p-value
Wall shear stress	0.96	0.94-0.99	0.0080
Gender	0.69	0.52-0.92	0.012
Framingham risk score	1.065	1.020-1.11	0.0047

3.3 Wall shear stress and wall shear rate vs plaque echolucency

  Both wall shear stress (p=0.0092 for trend, see Fig. 3 for details) and wall shear rate (p=0.033 for trend) were related to plaque echolucency, so that the highest wall shear stress or rate were seen in subjects with the most echolucent plaque. This pattern was still significant for wall shear stress (p=0.012), but not for wall shear rate (p=0.10), after adjustment for gender and Framingham risk score in two different ANCOVA models.
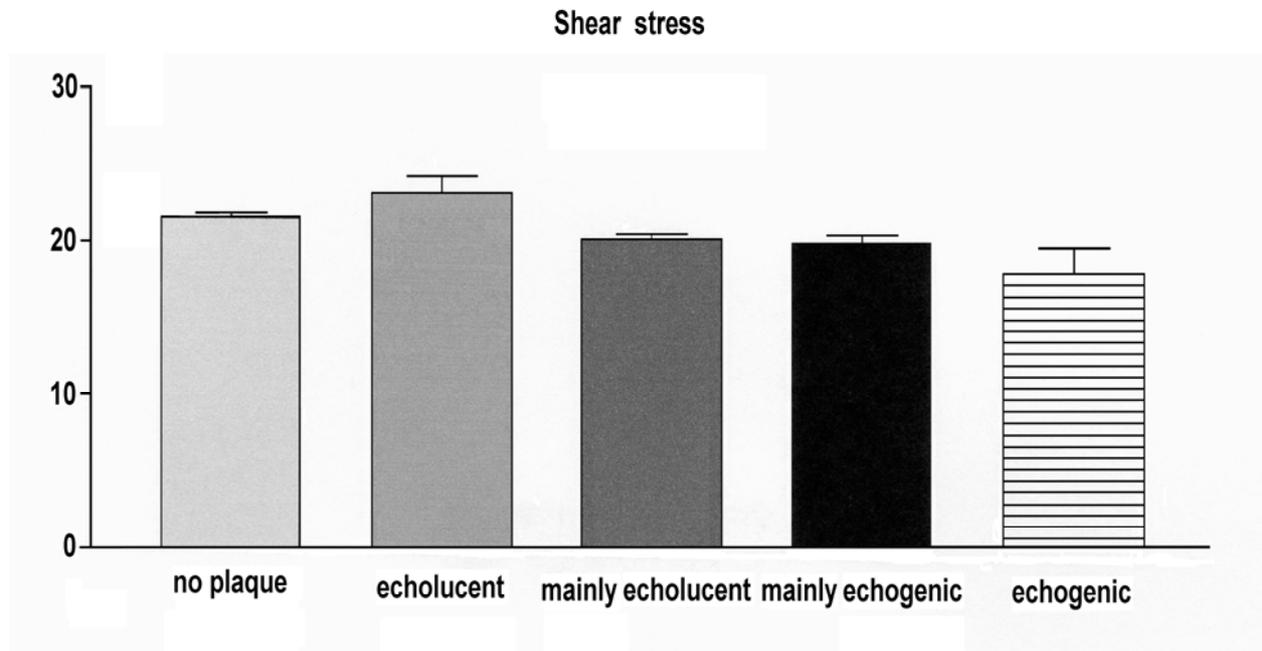

Fig. 3. Means and SEM for shear stress (dyne/cm^2) in subjects with no plaques (56%) in the population, and in those with plaques divided by the echogenecity accordingly to the Gray-Weale classification into four groups. p=0.0092 for trend amongst those with plaque.

4. Discussion

In accordance with previous investigations, we could show that a low shear stress was associated with a thick IMT and plaque occurrence. Furthermore, the present study showed a low shear stress also to be related to an echolucent intima-media complex, as well as echolucent plaques, suggesting that shear stress does not only influence the extent of atherosclerosis, but also influence vascular wall and plaque tissue composition.

4.1 Shear stress vs plaque occurrence and IMT

The present study could confirm previous investigations showing that a low shear stress in the common carotid artery is related to both a thick IMT and to the prevalence of overt plaques [1,7-9,12-15,26,27]. Since the vast majority of the plaques in the present study are not flow obstructing, it is unlikely that plaques being distally to the site of the blood flow velocity measurement would influence the determination of shear stress. It is more likely that the effects of a low shear stress on the vascular wall, such as a prolonged contact time for atherogenic lipids, platelets and leukocytes, or a reduced stimulation of nitric oxide production by vascular wall mechano-receptors would influence the progression of atherosclerosis [3-6,22,24]. Findings that shear stress is lower in the carotid artery with plaques in those patients with plaques in only one of the carotid arteries [9], as well as in the carotid artery responsible for a unilateral ischemic stroke [2], support that assumption.

4.2 Shear stress vs echogenicity

The new finding in the present study is that a low shear stress also is associated with an echolucent IM-GSM and also echolucent plaques. These relationships were seen also after adjustment for the major coronary risk factors, by means of the Framingham risk score, and for IMT. Thus, shear stress seems to influence not only the extent of atherosclerosis, but also the composition of the vascular wall and plaques.

The histological correlate to an echolucent intima-media complex has not been evaluated, but studies in plaques have shown echolucent plaques to be rich in lipids and to contain less collagen and calcium [10,11]. We have recently shown the echogenecity of the intima-media complex and overt plaque to be closely correlated [17] and that an echolucent intima-media complex to be related to an atherogenic lipid profile [18], suggesting that IM-GSM might be used as a surrogate measure of lipid content in the vascular wall. This speculation has however to be confirmed in histological studies.

4.3 Calculated vs. measured whole blood viscosity

In the present study, whole blood viscosity was only directly measured in approximately half of the sample for practical reasons. However, we performed a regression model with variables being correlated with measured whole blood viscosity, and were able from the regression model to calculate whole blood viscosity in the rest of the sample. When compared to the measured whole blood viscosity, this calculated whole blood viscosity showed a correlation coefficient of 0.85, indicating a valid calculation (Fig. 1). Furthermore, when we compared the results obtained in the subjects in whom the whole blood viscosity was directly measured with the corresponding calculated values, similar relationships between shear stress and atherosclerosis were found (data not shown). This suggests this calculation of whole blood viscosity to be useful in order to be able to obtain information on shear stress in the total sample.

4.4 Shear stress vs. shear rate

From a physiological standpoint it is more likely that shear stress would influence the vascular wall rather than the shear rate. However, since we only have measured whole blood viscosity in half of the sample, we also investigated how shear rate was related to atherosclerosis since this variable does not include whole blood viscosity, and therefore could be properly evaluated in the total sample.
We found that shear rate also was related to different measures of atherosclerosis in a similar way as shear stress, although the relationships tended to be less close and in some instances not significant after adjustment. Thus, despite that whole blood viscosity was calculated, shear stress seems to be preferred over shear rate in this setting.

4.5 Limitation of the study

The present sample is limited to Caucasians aged 70. Caution should therefore be made to draw conclusions to other ethnic and age groups.
The present study had a moderate participation rate. Therefore, we carried out an evaluation of cardiovascular disorders and medications in 100 consecutive subjects who denied participation [19]. The prevalences of cardiovascular drug intake, history of myocardial infarction, coronary revascularization, antihypertensive medication, statin use and insulin treatment were similar to those in the investigated sample, while the prevalences of diabetes, congestive heart failure and stroke tended to be higher among the non-participants.

4.6 Conclusion

A low shear stress in the common carotid artery was associated with both a thick IMT and an echolucent intima-media complex. A similar picture was seen when overt plaques were evaluated, suggesting that shear stress is of importance for both the extent and composition of atherosclerosis.

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