Flow-Mediated Vasodilation was Found to be an Independent Predictor of Changes in the Carotid Plaque Status During a 5-Year Follow-Up
— A Prospective Investigation of the Vasculature in the Uppsala Seniors (PIVUS) Study

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Aim: It has previously been shown that flow-mediated vasodilation is a predictor of the progression of the intima-media thickness (IMT). In the present study, the degree of endothelium-dependent vasodilation in both resistance and conduit arteries was evaluated as a predictor of the IMT and plaque progression.

Methods: In the population-based Prospective Study of the Vasculature in Uppsala Seniors (PIVUS) trial (1,016 subjects all 70 years of age), the invasive forearm technique using acetylcholine administered in the brachial artery (resistance artery, EDV) and the brachial artery ultrasound technique with measurement of flow-mediated dilatation (conduit artery, FMD) were evaluated. The IMT and number of carotid arteries with plaques (0, 1 or 2) were recorded using ultrasound at the baseline investigation and the follow-up visit conducted five years later.

Results: A total of 760 subjects had valid measurements of the IMT and carotid artery plaques at both the investigations conducted at 70 and 75 years of age. Neither the FMD nor EDV significantly predicted the change in IMT over five years. However, the FMD, but not EDV, was associated with the change in carotid plaque burden during the follow-up period, independent of classical risk factors, such as gender, waist circumference, fasting blood glucose, systolic and diastolic blood pressure, HDL- and LDL-cholesterol, serum triglycerides, BMI and smoking (OR 0.81 for a 1 SD change in FMD, 95%CI 0.68 to 0.95, \( p = 0.010 \)).

Conclusions: The FMD was found to be a predictor of changes in the carotid plaque status, but not IMT, during the 5-year follow-up period, independent of classical cardiovascular risk factors.

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Key words: Endothelium, Vasodilation, Atherosclerosis, Intima-media thickness, Plaque

Introduction

A defect in the endothelial function is regarded to be the primary event in the atherosclerotic process. In humans, the endothelial function is most often evaluated as the vasodilatory response to stimuli that increase the production of nitric oxide. Two common techniques used in this field are the measurement of the forearm blood flow response to the brachial artery infusion of acetylcholine\(^1,2\) and the measurement of flow-mediated vasodilation in the brachial artery during hyperaemia\(^3\). The former technique evaluates endothelial vasoreactivity primarily in resistance vessels, while the latter technique assesses vasodilation in a conduit artery. Both of these techniques have been shown to predict future cardiovascular (CV) events\(^4-6\).

Several studies have indicated an association between endothelium-dependent vasodilation and
atherosclerosis in cross-sectional investigations\(^7\text{-}^{15}\), although this association has not been found in all studies\(^6\). Recently, the first study evaluating whether the measurement of endothelium-dependent vasodilation can be used to predict changes in the carotid artery intima-media thickness (IMT) over a 6.2-year period showed the FMD to be predictive of IMT progression\(^17\).

In order to study the predictive power of endothelium-dependent vasodilation with respect to the development of atherosclerosis, we used data obtained from the population-based Prospective Study of the Vasculature in Uppsala Seniors (PIVUS) trial\(^18\) in which we measured both the FMD and acetylcholine-mediated vasodilation in almost 1,000 subjects. Since we recently showed that acetylcholine-mediated vasodilation, but not FMD, is a powerful predictor of major adverse cardiovascular events (MACE) during a 5-year follow-up period\(^6\), the primary hypothesis evaluated in the present study was whether acetylcholine-mediated vasodilation, but not FMD, is a predictor of the development of atherosclerosis over the same follow-up period. Atherosclerosis was evaluated both as the IMT and the number of carotid arteries with plaque on ultrasound.

**Methods**

**Subjects**

Eligible patients included all subjects 70 years of age living in the community of Uppsala, Sweden. The subjects were chosen from the register of community living and invited in a randomized order. The subjects received an invitation by letter within two months of their 70th birthday. Of the 2,025 subjects invited, 1,016 participated, for a participation rate of 50.1%. The baseline investigation was started in April 2001.

The study was approved by the Ethics Committee of the University of Uppsala, and all participants provided their informed consent.

**Baseline Investigation**

The participants were asked to complete a questionnaire regarding their medical history, smoking habits and regular medications.

All subjects were investigated in the morning after an overnight fast. No medication use or smoking was allowed after midnight. After obtaining recordings of height, weight and abdominal and hip circumference, an arterial cannula was inserted into the brachial artery for blood sampling and later regional infusions of vasodilators.

Blood pressure was measured using a calibrated mercury sphygmomanometer in the non-cannulated arm to the nearest mmHg after at least 30 minutes of rest, and the average of three recordings was used. The levels of lipids and fasting blood glucose were measured using standard laboratory techniques. The basic CV risk factor characteristics are presented in Table 1.

Approximately 10% of the cohort reported a history of coronary heart disease, 4% reported stroke and 9% reported diabetes mellitus. Almost half of the cohort reported using a cardiovascular medication (45%), with antihypertensive medications being the most prevalent (32%). Fifteen percent of the subjects reported using a statin, while the use of insulin and oral antiglycemic drugs was reported in 2% and 6% of the subjects, respectively (see reference 18 for details).

**Re-Examination at Age 75**

The cohort was invited to undergo re-examinations one month after their 75th birthday; 827 (81%) individuals attended. The time between the examinations was 5.13 (SD 0.10) years. The re-examinations were completed in September 2009. Fifty-two subjects died before the re-examinations.

**Invasive Forearm Technique**

The forearm blood flow (FBF) was measured

### Table 1. Major cardiovascular risk factors at 70 years of age in the subjects who attended both the examinations conducted at 70 and 75 years of age

<table>
<thead>
<tr>
<th></th>
<th>Age 70</th>
</tr>
</thead>
<tbody>
<tr>
<td>Females (%)</td>
<td>46</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>169 (9.3)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>75 (13)</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>89 (11)</td>
</tr>
<tr>
<td>BMI (kg/m(^2))</td>
<td>26.2 (3.7)</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>148 (23)</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>78 (10)</td>
</tr>
<tr>
<td>LDL-cholesterol (mmol/L)</td>
<td>3.4 (0.8)</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/L)</td>
<td>1.6 (0.4)</td>
</tr>
<tr>
<td>Serum triglycerides (mmol/L)</td>
<td>1.1 (0.4)</td>
</tr>
<tr>
<td>Fasting blood glucose (mmol/L)</td>
<td>5.0 (0.9)</td>
</tr>
<tr>
<td>Current smoking (%)</td>
<td>9.0</td>
</tr>
<tr>
<td>EDV (%)</td>
<td>541 (303)</td>
</tr>
<tr>
<td>EIDV (%)</td>
<td>385 (220)</td>
</tr>
<tr>
<td>FMD (%)</td>
<td>4.9 (3.6)</td>
</tr>
</tbody>
</table>

The mean and SD are given.

SBP = systolic blood pressure. DBP = diastolic blood pressure. BMI = Body mass index. EDV = endothelium-dependent vasodilation (invasive forearm technique). EIDV = endothelium-independent vasodilation (invasive forearm technique). FMD = flow-mediated dilatation.
using venous occlusion plethysmography (Elektro-
medicin, Kullavik, Sweden). A mercury in-silastic
strain-gauge was placed on the upper third of the fore-
arm, which rested comfortably slightly above the level
of the heart. The strain-gauge was connected to a cali-
brated plethysmograph. Venous occlusion was achieved
by applying a blood pressure cuff proximal to the
elbow inflated to 50 mmHg using a rapid cuff
inflator. The evaluations of FBF were made by calculat-
ing the mean of at least five consecutive recordings.

An arterial cannula was placed in the brachial
artery. No more than one attempt to insert the can-
nula in each arm was allowed. The resting FBF was
measured 30 minutes after cannula insertion. After
evaluating the resting FBF, local intra-arterial drug
infusions were administered for five minutes per dose,
with a 20-minute wash-out period between the drugs.
The infused doses were 25 and 50 ug/minute of ace-
tylocholine (Clin-Alpha, Switzerland) to evaluate the
EDV and 5 and 10 ug/minute of SNP (Nitropress,
Abbot, UK) to evaluate the EIDV. The doses of these
drugs were chosen to result in FBFs on the steep part
of the dose-response curve without inducing systemic
effects. The drugs were given in a random order at a
maximal rate of 1 mL/min.

In the present study, only data for the highest
doses of acetylcholine and SNP were used. The EDV
was defined as the FBF observed during the infusion
of 50 ug/min of acetylcholine minus the resting FBF
divided by the resting FBF. The EIDV was defined as
the FBF observed during the infusion of 10 ug/min of
SNP minus the resting FBF divided by the resting
FBF.

We previously demonstrated the reproducibility
(coefficient of variation, CV) of EDV and EIDV to be
8-10%[19]. The EDV technique was not used in sub-
jects receiving regular medication with warfarin due to
expected problems with bleeding (n = 32). In 106 sub-
jects, cannulation of the brachial artery failed or some
other technical error occurred, leaving 87% of the
subjects with a valid test.

**Brachial Artery Ultrasound Technique**

The brachial artery was assessed using external
B-mode ultrasound imaging (Acuson XP128 with a
10-MHz linear transducer, Acuson Mountain View,
California, USA). The common carotid artery (CCA),
bulb and internal carotid artery (ICA) were visualized,
and the occurrence of plaque was recorded on both
sides. A plaque was considered to present if the IMT
was locally thickened by more than 50% compared to
the surrounding IMT, in accordance with the defini-
tion used by the group at the Wallenberg laboratory in
Gothenburg[21]. We believe that this relative definition
of a plaque is better than using a cutoff value of 1.0 or
1.2 mm for the IMT, since a fixed cutoff value will
underestimate the prevalence of plaque in small sub-
jects with a normally thin IMT. The number of
carotid arteries with plaques (0, 1 or 2 plaques) were
recorded. The presence of plaque was investigated at
70 and 75 years of age in a blinded fashion, so that
the reader of the images obtained at age 75 did not
know the plaque status at age 70. When the two read-
ers evaluated the same images, the presence of plaque
was correctly scored by both readers in > 95% of the
cases. This was also the case when the same image was
analyzed by the same observer twice with one day sep-
arating the readings.

The images were digitized and imported into the
AMS (Artery Measurement Software) automated soft-
ware program[22] for a dedicated analysis of the IMT.

**Carotid Artery Ultrasound Evaluations**

The carotid artery was assessed using external
B-mode ultrasound imaging (Acuson XP128 with a
10-MHz linear transducer, Acuson Mountain View,
California, USA). The common carotid artery (CCA),
bulb and internal carotid artery (ICA) were visualized,
and the occurrence of plaque was recorded on both
sides. A plaque was considered to present if the IMT
was locally thickened by more than 50% compared to
the surrounding IMT, in accordance with the defini-
tion used by the group at the Wallenberg laboratory in
Gothenburg[21]. We believe that this relative definition
of a plaque is better than using a cutoff value of 1.0 or
1.2 mm for the IMT, since a fixed cutoff value will
underestimate the prevalence of plaque in small sub-
jects with a normally thin IMT. The number of
carotid arteries with plaques (0, 1 or 2 plaques) were
recorded. The presence of plaque was investigated at
70 and 75 years of age in a blinded fashion, so that
the reader of the images obtained at age 75 did not
know the plaque status at age 70. When the two read-
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arating the readings.

The images were digitized and imported into the
AMS (Artery Measurement Software) automated soft-
ware program[22] for a dedicated analysis of the IMT.
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FMD of 25% of 1SD between the subjects who exhibited progression in the plaque status and those who did not. The statistical software package STATA 11 (Stata Corporation, College Station, USA) was used for the calculations.

Results

Changes in IMT

Among the 739 subjects with valid measurements of the IMT at both 70 and 75 years of age, the mean IMT was $0.88 \pm 0.16$ (SD) mm at baseline.

During the 5-year follow-up period, the mean increase in IMT was $0.057 \pm 0.12$ mm, corresponding to a yearly change of 0.011 mmHg. Expressed as the percentage change from the baseline value, the 5-year change was $+7.7 \pm 14\%$.

Baseline EDV and FMD vs Changes in IMT

When correlations between the vasodilatory variables at baseline and changes in the IMT were assessed, neither the EDV nor FMD or EIDV predicted the change in IMT, irrespective of whether the change in IMT was calculated in absolute numbers ($p=0.70$ for EDV and $p=0.58$ for FMD) or as the percentage change from baseline ($p=0.94$ for EDV and $p=0.46$ for FMD). All models were adjusted for gender.

The baseline diameter of the brachial artery was not related to the change in IMT ($p=0.97$).

Changes in the Plaque Status

Of the 760 subjects with valid measurements of carotid artery plaques at both the investigations conducted at 70 and 75 years of age, 307 (40%) exhibited progression in the plaque status and those who did not. The statistical software package STATA 11 (Stata Corporation, College Station, USA) was used for the calculations.

The IMT was evaluated in the far wall in the CCA 1-2 cm proximal to the bulb. A maximal 10-mm segment with good image quality was chosen for the IMT analysis of the carotid artery. The program automatically identifies the borders of the intima-media thickness of the far wall and the inner diameter of the vessel and calculates the intima-media thickness and diameter based on multiple discrete measurements obtained through the 10-mm long segment. The automated analysis was manually corrected if not found to be appropriate upon visual inspection. The given value for the carotid artery IMT is the mean value of both sides.

The mean length of the evaluated intima-media segments was 9.0 (SD 2.1) mm when subjects with a segment recording of less than 5 mm were excluded. The measurements of the intima-media thickness were repeated in 30 random subjects, giving a coefficient of variation of the carotid artery IMT of 7.2%.

The same technician used the same equipment for carotid plaque evaluation at both examinations.

Statistics

An ordinal logistic regression analysis was used to identify relationships between changes in the plaque status from age 70 to 75 during the 5-year follow-up and the EDV and FMD, adjusting for either sex or multiple risk factors. Since it has been shown that subjects with bilateral carotid plaques are at higher risk of future CV disorders than subjects with unilateral plaques\(^{21}\), we used a graded response to assess the presence of carotid plaque (0, 1 or 2 carotid arteries with plaques) rather than a binary outcome (plaque or no plaque) in order to use the variation in carotid plaque prevalence in a more powerful fashion.

*P*-values of $<0.05$ in two-sided tests were considered to be statistically significant. With a sample size of 760 individuals, this study has the power to detect a linear relationship between the baseline FMD and changes in the IMT with an $R^2$ of 0.005 at the $p<0.05$ level. This study also has a 90% power to detect a difference in the mean values of EDV and FMD of 25% of 1SD between the subjects who exhibited progression in the plaque status and those who did not.

Table 2. The number of carotid arteries with plaque was determined at both 70 and 75 years of age in 760 subjects

<table>
<thead>
<tr>
<th>Number of arteries with plaques</th>
<th>0 at age 75</th>
<th>1 at age 75</th>
<th>2 at age 75</th>
<th>Row sum</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 at age 70</td>
<td>99</td>
<td>89</td>
<td>82</td>
<td>270</td>
</tr>
<tr>
<td>1 at age 70</td>
<td>43</td>
<td>95</td>
<td>136</td>
<td>274</td>
</tr>
<tr>
<td>2 at age 70</td>
<td>11</td>
<td>20</td>
<td>185</td>
<td>216</td>
</tr>
<tr>
<td>Column sum</td>
<td>153</td>
<td>204</td>
<td>403</td>
<td>Total sum 760</td>
</tr>
</tbody>
</table>

The table shows the number of subjects according to the plaque status at both investigations. A significant trend ($p<0.0001$) towards a higher number of carotid arteries with plaque was seen over the 5-year follow-up period.
Baseline EDV and FMD vs Changes in the Plaque Status

By subtracting the number of arteries with plaque at age 70 from the number of arteries with plaque at age 75, we obtained a measurement of the change in the plaque status during the five years of follow-up. Using the change in the plaque status as the dependent variable in ordinal logistic regression models, it was found that the FMD (OR 0.86 for a 1 SD change in FMD, 95%CI 0.74 to 0.98, \( p = 0.027 \)), but neither the EDV (OR 1.01 for a 1 SD change in EDV, 95%CI 0.87 to 1.17, \( p = 0.84 \)) nor EIDV, predicted the change in the plaque status after adjusting for gender adjustment.

As shown in Fig. 1, the relationship between the FMD (SD-transformed scale) and the log odds for the change in the plaque status was fairly linear, which was supported by the finding that the squared term for FMD was not significant when included in the model \( (p = 0.92) \).

No interactions were observed between the FMD and gender regarding the predictive power for the change in the plaque status \( (p = 0.91) \); therefore, all other analyses regarding the relationship between the FMD and the change in the plaque status were adjusted for gender and not stratified by gender.

The relationship between the baseline FMD and the change in the plaque status during the 5-year follow-up period remained significant \( (p = 0.010) \) following adjustment for the traditional risk factors of gender, blood pressure, fasting glucose, HDL and LDL-cholesterol, serum triglycerides, BMI, waist circumference and smoking (see Table 3 for details). Further adjustments for the use of antihypertensive drugs, statins, insulin or oral antidiabetic medications only marginally changed the relationship between the FMD and the number of arteries with plaque (now \( p = 0.018 \) for FMD).

To ensure that the relationship between the FMD and the change in the plaque status was not due to the influence of the subjects who showed a reduction in the number of arteries with plaque during the follow-up period, these 72 subjects were excluded from the analysis. The baseline FMD, however, was still found to predict the change in the plaque status during the follow-up period (OR 0.82 for a 1 SD change, 95%CI 0.70 to 0.96, \( p = 0.014 \)).

When we excluded the 185 subjects with bilateral plaques at both investigations (thereby by this approach only could be evaluated properly regarding plaque status regression but not progression), a similar picture emerged, as described above in the total sample (OR 0.83 for a 1 SD change, 95%CI 0.71 to 0.97, \( p = 0.022 \) for the association between the FMD and the change in the plaque status).

The baseline diameter of the brachial artery was significantly related to the change in the plaque status during the 5-year follow-up when adjusted for gender only (OR 1.41, 95%CI 1.07-1.85, \( p = 0.014 \)). However, following adjustment for the FMD and CV risk factors, the baseline diameter of the brachial artery was no longer significantly related to the number of arteries with plaque \( (p = 0.27) \).

**Discussion**

The present study showed that the FMD, but not EDV, predicted changes in the carotid artery plaque status during a 5-year follow-up period, independent of traditional CV risk factors.

**Comparison with the Literature**

Only one previous study has evaluated endothelium-dependent vasodilation as a predictor of the vascular status \( 17 \). That study used the FMD and found that this marker of vasoreactivity predicted the progression of IMT during a follow-up of 6.2 years.

The present investigation extends the research on the relationship between the endothelial function and future atherosclerosis by using the acetylcholine-mediated vasodilation (EDV) in addition to the FMD and the change in the plaque status in addition to the IMT. Despite the fact that the progression of IMT was very similar in the two studies, the present investigation did not find that the FMD predicts the IMT, rather that it predicts only the change in the plaque status.

**Fig. 1.** Relationship between the flow-mediated vasodilation (FMD) given on an SD-scale and the log odds of the change in the carotid artery plaque status using a generalized additive model \( (p = 0.010) \).
status.

In a recently published meta-analysis of 21 different studies that evaluated the progression of IMT over time, the mean change in the common carotid artery IMT ranged from 0.001 mm/year to 0.30 mm/year\textsuperscript{23}. Therefore, it is clear that the rate of progression varies significantly in different samples. Age, gender distribution and whether the study was conducted in a population-based sample or in subjects with diseases known to induce atherosclerosis, such as diabetes, hypercholesterolemia, etc., certainly play a role in the rate of progression of the IMT.

**IMT vs Plaque**

Both the IMT and the occurrence of plaque have been shown to predict CV events. However, while the IMT is primarily measured in the far wall of the common carotid artery, as in the present study, no uniform definition of a carotid artery plaque measured on ultrasound exists. In the present study, we used the definition of a plaque as an area of local thickening of the IMT of $>50\%$, in accordance with other investigators\textsuperscript{21}. This definition appears to be more valid than the use of a fixed cutoff value for the IMT, such as 1.2 or 1.5 mm, as employed in other studies, since the definition takes into account the fact that different subjects have different "normal" IMT values. In particular, in studies performed in both sexes, using a fixed cutoff value would clearly underestimate the prevalence of plaque in women.

The FMD predicted the change in the plaque status over five years; however, it did not predict the change in IMT. Although the IMT of the common carotid artery and the prevalence of plaque are related, these two vascular parameters partly measure different characteristics. Plaque formation is most commonly found in areas of bifurcation and is a disease of the intima. Nitric oxide is most likely to play a major role in the development of overt atherosclerotic plaque. The IMT of the common carotid artery is, on the other hand, a measurement of both the intima and media and is at this location primarily governed by blood pressure and its effect on the media layer. Since the FMD is an indirect measurement of nitric oxide release, it is not strange that a relationship is observed between the FMD and plaque progression rather than IMT progression.

In the present study, the number of carotid plaques was calculated as a measurement of the plaque status. Other studies have used the plaque area\textsuperscript{24-27}. The latter is likely a better measurement of plaque progression in longitudinal studies; however, we have found it difficult to standardize the transducer angle over time in order to obtain reliable plaque area measurements. In concordance with the fact that the number of arteries with plaque increased in the present 5-year follow-up period, it has recently been shown that the plaque area steeply increases with age in both sexes\textsuperscript{27}.

We choose to count the number of carotid arteries in which we could find plaque. Using such an approach, both plaque progression and regression could be quantified in all subjects, not simply those with plaque at baseline. It was then found that 40% of the subjects exhibited an increased number of carotid arteries with plaque during the 5-year follow-up period, while 10% showed a decline in the number of carotid arteries with plaque. The main disadvantage of such a technique is that plaque progression cannot be properly evaluated in subjects with bilateral plaques at both examinations. It was however demonstrated that this was not a major problem in the present study since the association with the FMD was only marginally altered and remained significant when subjects with bilateral plaques at both investigations were excluded from the analysis.
The sample size and the fact that many subjects showed no changes in their plaque status gave the study good power to detect whether the EDV or FMD predicted the change in the IMT or the number of atherosclerotic plaques. Regarding the change in the IMT, the sample size was adequate to demonstrate a significant linear relationship, with an R^2^ of 0.005, meaning that a relationship with EDV or FMD would explain less than 0.5% of the variation in the change in the IMT. Therefore, the study size has the ability to demonstrate very small but significant physiological relationships with the change in the IMT, if such relationships exist.

**EDV vs FMD**

We recently demonstrated that the EDV, but not FMD, predicts future CV events in the PIVUS study. In the present study, we show that the FMD, but not EDV, is predictive of changes in the plaque status. This finding is contrary to our hypothesis and appears to be counterintuitive at first sight. However, atherosclerosis is a disease of the intima in conduit arteries and the FMD is a measurement of the endothelial function in a conduit artery. The EDV primarily measures vasoreactivity in smaller vessels and may therefore provide different, but also important, information from the FMD. It should be pointed out that the EDV and FMD were not found to be related in either the PIVUS study or other populations we have investigated. Therefore, the EDV and FMD are both important measurements in the study of atherosclerosis and its health consequences.

Both flow-mediated dilation and the vascular response to acetylcholine have previously been shown to be predictors of cardiovascular events; however, in the present study, only the FMD was found to be related to plaque progression. There are two likely explanations for this contradiction. First, atherosclerosis is a disease of the conduit arteries. The FMD measures vasodilation in conduit arteries, while the EDV primarily measures vasoreactivity in resistance arteries. Therefore, the anatomical locations of the two measurements differ. The FMD and progression of atherosclerosis are both measured in conduit arteries and are therefore more likely to be related. Second, most atherosclerosis-related CV events are due to thrombus formation at the site of vulnerable atherosclerotic plaque. It may well be that the EDV is related to that chain of events rather than plaque progression itself; therefore, no relationships were found between plaque progression and the EDV.

**Limitations**

The present findings are limited to Caucasians 70 years of age. Caution should therefore be used when drawing any conclusions for other ethnic and age groups.

The PIVUS study had a moderate participation rate. However, an analysis of non-participants showed the present sample to be fairly representative of the total population regarding most cardiovascular disorders and drug intake.

**Conclusion**

The FMD, but not EDV, was found to be a predictor of changes in the carotid plaque status during the 5-year follow-up period, independent of classical cardiovascular risk factors.

**Conflicts of Interest**

None.

**References**


