SUMMARY One hundred neurologically asymptomatic male subjects (aged 50–69 years), randomly selected through population registers, were screened for atherosclerotic lesions of the cervical carotid arteries, using a high resolution multi-gate pulsed Doppler system. In 93 subjects serum was assayed for total and HDL cholesterol. Besides, cuff arterial blood pressure measurements were made. Twenty-three of these subjects (Group III) were classified as abnormal according to the Doppler investigation (degree of narrowing < 50% in 78% of the cases). Seven of these 23 subjects also had a history of ischemic heart disease or intermittent claudication. Of the 70 subjects with a normal Doppler examination 16 had a history of ischemic heart disease and/or intermittent claudication (Group II). The remaining 54 subjects served as controls (Group I). The frequency of asymptomatic atherosclerotic lesions of the cervical carotid arteries in the population under investigation was 23%. The HDL/total cholesterol ratio was lower and the frequency of high blood pressure higher in the Groups II and III than in Group I. The findings in this study support the idea that a low serum HDL/total cholesterol ratio and high blood pressure have a high degree of association with atherosclerotic lesions of the cervical carotid arteries. This association is already apparent at an early stage of the disease, i.e. in asymptomatic subjects with a slight to moderate degree of carotid artery narrowing.

STROKE Vol 16, No 1, January-February 1985

Serum HDL/Total Cholesterol Ratio and Blood Pressure in Asymptomatic Atherosclerotic Lesions of the Cervical Carotid Arteries in Men

TINY VAN MERODE, PAUL HICK, ARNOLD P.G. HOEKS, AND ROBERT S. RENEMAN

RISK FACTORS for carotid artery lesions have been investigated extensively in the past decades. Especially high blood pressure, serum lipoprotein abnormalities and diabetes were found to be related to the risk of cerebrovascular disorders like stroke and transient ischemic attacks.1-11 These investigations, however, were performed on patients suffering from these disorders, which means that information has been obtained about risk factors at a progressed stage of the disease. Besides, in most of these investigations the control subjects were assumed to be healthy, despite the fact that asymptomatic carotid artery lesions may be present in the age groups studied.12-14

In the present investigation arterial blood pressure as well as serum total cholesterol and high density lipoprotein (HDL) cholesterol levels were assessed in asymptomatic subjects with atherosclerotic lesions of the cervical carotid arteries and in healthy subjects without cervical carotid artery lesions. The subjects were randomly selected through the population registers of small, average and large urban and rural towns, and screened for cervical carotid artery lesions using a high resolution multi-gate pulsed Doppler system.15-17 Arterial lesions were diagnosed, using the criteria of spectral broadening as developed for pulsed Doppler systems18-19 and adapted to the characteristics of the multi-gate device. The study was limited to men above the age of 50 years because cervical carotid artery lesions are likely to occur infrequently below this age.20 Men were selected since differences in HDL-cholesterol levels between patients and control subjects are often more pronounced in males than in females.4,6 The serum HDL cholesterol level and the HDL/total cholesterol ratio were used as indicators for dyslipoproteinemia because a low HDL-cholesterol level is considered to be an independent risk factor for
Materials and Methods

The study was performed on 100 male volunteers from the Maastricht area, varying in age between 50 and 69 years. Their names and addresses were randomly selected from the population registers of small, average and large urban and rural towns. Two hundred and fifty subjects were contacted once to obtain 100 volunteers. All respondents were included in the study. From all volunteers informed consent was obtained before they entered the study. A history was taken on symptoms related to atherosclerosis. They all had a physical examination, including cuff blood pressure measurements in the supine position. In case of hypertensive blood pressure readings (see below), the measurements were repeated 2–3 times and the lowest value was taken as the subject’s reading.

The cervical carotid arteries were screened for disease, using a high resolution multi-gate pulsed Doppler system25–27 with the following characteristics: emission frequency 6.1 MHz, pulse repetition frequency 18 KHz, emission duration 1.0 μs, sample interval 0.5 mm and number of gates 64. The sample volume of this system as measured in vitro (23) is 1.2 mm³ at a range of 15 mm and 1.7 mm³ at a range of 20 mm (20). The Doppler examinations were performed with the volunteers in the supine position with the head in the mid-position. Doppler spectra were recorded midstream at various sites in the common, internal and external carotid arteries. The site of sampling was localized through a velocity image of the bifurcation. All spectra were stored on a 4-channel tape recorder (Rackal; 0–10 KHz-3dB) and analyzed off-line with a Nicolet spectrum analyzer (100 frequency bins, each 100 Hz), providing amplitude as a function of frequency. For display the amplitude of the spectrum was scaled with respect to the peak amplitude. The spectra were classified according to the criteria of spectral broadening as developed for pulsed Doppler systems18–19 and adapted to the characteristics of our multi-gate device (table 1). Since doubt has been raised whether B classifications always represent cervical carotid artery lesions, even when small sample volumes are used,20 only B classifications associated with disturbed flow patterns as indicated by other criteria, were considered to be representative of vessel disease. These criteria include asymmetry of the velocity profile and/or disturbed instantaneous velocity waveforms along the ultrasound beam, locally or along the whole vessel diameter. Velocity profiles at discrete time intervals during the cardiac cycle and instantaneous velocity waveforms at various sites along the ultrasound beam can be recorded on-line with our multi-gate pulsed Doppler system.24, 25 In prospective studies the pulsed Doppler/spectrum analysis method to detect atherosclerotic lesions of the cervical carotid arteries has been compared with biplane arteriography.18, 19 These studies revealed that cervical carotid artery lesions associated with a

### Table 1: Grading of Artery Narrowing from the Degree of Spectral Broadening according to Langlois et al19 and Breslau22 and Adapted to the Characteristics of our Multi-gate Pulsed Doppler System

<table>
<thead>
<tr>
<th>Classification</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>Normal</td>
<td>(A) no spectral broadening; max. frequency &lt;6 kHz*</td>
</tr>
<tr>
<td>&lt;15% stenosis†</td>
<td>(B) spectral broadening in deceleration phase of systole; max. frequency &lt;6 kHz</td>
</tr>
<tr>
<td>16–49% stenosis</td>
<td>(C) spectral broadening throughout systole; max. frequency &lt;6 kHz</td>
</tr>
<tr>
<td>50–99% stenosis</td>
<td>(D) spectral broadening throughout systole/ increased frequencies in diastole; max. frequency &gt;6 kHz</td>
</tr>
<tr>
<td>Total occlusion</td>
<td>(E) No signal</td>
</tr>
</tbody>
</table>

*The maximum systolic peak frequency for our system was considered to be 6 kHz, because of the higher emission frequency and the slightly steeper angle between vessel and probe in our multi-gate device than in the ATL Duplex scanner as used by Langlois et al and Breslau.

†Diameter reduction.

degree of artery narrowing of 16–49% (C classification) and 50–99% (D classification) can be diagnosed accurately, provided that the sample volume of the Doppler system is small, as is the case in the present investigation. In our hands these lesions can be diagnosed with a sensitivity of 89%, a specificity of 83% and a diagnostic accuracy of 87% as compared to arteriography (unpublished results of a prospective study).

Venous blood was taken from each person in a non-fasting condition for the estimation of total cholesterol and HDL cholesterol in whole serum. The concentration of total cholesterol in serum was measured after direct addition of Liebermann-Burchard reagent and the results were calibrated with human serum pools with known cholesterol concentrations as described by Katan and co-investigators.26 Reproducibility for blind control sera provided by the Centers for Disease Control, Atlanta USA, was within 0.9% and the accuracy was within 1.6% of the true (target) values. The concentration of cholesterol in HDL was measured after the precipitation of low and very low density lipoproteins by heparin-MnCl2.27 Reproducibility for blind control sera as obtained in the Center for Disease Control Survey of HDL-measurements was within ± 2.2% and this, with regard to the overall survey mean, was an average of 0–1%. Blood samples were taken scattered over the day. This is allowed because previous studies have shown that there are no detectable differences in the concentrations of total and HDL cholesterol with time of the day and the time interval since the last meal.21, 22 Differences between the mean values of the variables in the various groups were evaluated for statistical significance (p < 0.05) in an one-way analysis of variance as implemented in a standard computer program (BMDP statistical software 1981, p. 106).

Results

In 5 of the 100 volunteers the serum lipid concentrations could not be assessed for technical reasons. Two subjects were excluded from the study because of insulin-dependent diabetes mellitus.
Twenty-three of the remaining 93 subjects had an abnormal Doppler investigation (30% in the 6th age decade and 18% in the 7th age decade, a non-significant difference; Pearson χ² test). The arteries involved and the degree of narrowing are given in Table 2. In 21 cases lesions were found in the internal carotid artery, while the external carotid artery was involved in 2 cases. In 5 subjects multi-vessel disease was observed. All volunteers were asymptomatic for carotid artery disease, which means a negative history of stroke, transient ischemic attacks or amaurosis fugax. The Doppler examination was normal in the subjects that were excluded from the serum lipid estimation (see above). Hence, the frequency of asymptomatic carotid artery disease in the population under investigation was 23%. Of the 70 subjects with normal Doppler findings, 16 had a positive history of angina pectoris, myocardial infarction and/or intermittent claudication. Based upon these findings three groups were formed. One group of normal subjects, consisted of 54 volunteers with normal Doppler findings and no history of atherosclerotic disease (Group I). A second group consisted of 16 volunteers with normal Doppler findings, but with a positive history of atherosclerotic disease (Group II). The third group consisted of the 23 subjects with abnormal Doppler findings (Group III). Seven of these subjects had a positive history of angina pectoris or intermittent claudication. Beta blocking agents were taken by one subject in Group I (Lopresor (metoprolol)), 9 subjects in group II (6 Inderal (propranolol), 2 Trasicor (oxprenolol), 1 Aptine (alprenolol)) and 2 subjects in Group III (Trasicor). Two subjects in each group took thiazides (Esidrex).

Twenty-five of the volunteers were hypertensive according to the criteria of the W.H.O. (blood pressure > 140/90). The frequency of hypertension was significantly higher in the Groups II (4%) and III (35%) than in Group I (19%). No significant difference could be detected between the frequency in Group II and Group III. Diastolic blood pressure was significantly higher in Group II (87.6 ± 9.9 mmHg; x ± sd) and Group III (86.0 ± 10.6 mmHg) than in Group I (81.5 ± 8.7 mmHg). The diastolic blood pressure readings in the Groups II and III were not significantly different. No significant differences could be detected between the systolic blood pressure readings in the three groups. In all hypertensive subjects in Group I blood pressure was lower than 160/95 and hence their hypertensive status could be considered borderline. Three subjects in Group II and 3 subjects in Group III were definitely hypertensive (blood pressure > 160/95). There was no significant difference in the frequency of hypertension between the 6th and 7th age decade in the Groups I and III, but a higher frequency of hypertension in the 6th age decade in Group II (Pearson χ² test). The groups were comparable as far as age and heart rate are concerned.

The serum total and HDL cholesterol concentrations as well as the HDL/total cholesterol ratio in the various groups are shown in Table 3. The total cholesterol level was significantly higher in Group III than in Group I, while the HDL cholesterol level was significantly lower in Group II than in Group I. The HDL/total cholesterol ratio was significantly lower in the Groups II and III than in Group I. No significant differences could be detected between the Groups II and III. The serum total and HDL cholesterol concentrations, and the HDL/total cholesterol ratio were similar for hypertensive and non-hypertensive subjects (Table 4).

Discussion

In the present study serum total cholesterol was found to be higher and the serum HDL/total cholesterol ratio lower in subjects with asymptomatic cervical carotid artery lesions than in age matched subjects devoid of obvious atherosclerosis as evidenced by a normal Doppler examination and a negative history of symptoms related to atherosclerosis. In the former subjects the frequency of hypertension and the diastolic blood pressure level were higher than in the control subjects. These findings support the idea that at least in men a

<table>
<thead>
<tr>
<th>TABLE 2 Arteries Involved and the Degrees of Artery Narrowing in the 23 Subjects with Abnormal Doppler Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Artery</td>
</tr>
<tr>
<td>-----------------------------------------------</td>
</tr>
<tr>
<td>Internal carotid artery (n = 21)</td>
</tr>
<tr>
<td>External carotid artery (n = 2)</td>
</tr>
</tbody>
</table>

*Significantly higher than in Group 1.
†Significantly lower than in Group 1.

<table>
<thead>
<tr>
<th>TABLE 3 Serum Total and HDL Cholesterol Concentrations (mmol/l) and HDL/Total Cholesterol Ratio in Normal Subjects (Group I), Subjects with a Positive History of Atherosclerosis, but without Detectable Cervical Carotid Artery Lesions (Group II) and Subjects with Asymptomatic Cervical Carotid Artery Lesions (Group III) (x ± sd)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
</tr>
<tr>
<td>-----------------------------------------------</td>
</tr>
<tr>
<td>Group I (n = 54)</td>
</tr>
<tr>
<td>Group II (n = 16)</td>
</tr>
<tr>
<td>Group III (n = 23)</td>
</tr>
</tbody>
</table>

*Significantly higher than in Group 1.
†Significantly lower than in Group 1.

<table>
<thead>
<tr>
<th>TABLE 4 Serum Total and HDL Cholesterol Concentrations (mmol/l) and HDL/Total Cholesterol Ratio in Normotensive and Hypertensive Subjects (x ± sd)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normotensive (n = 68)</td>
</tr>
<tr>
<td>-----------------------------------------------</td>
</tr>
<tr>
<td>Total cholesterol</td>
</tr>
<tr>
<td>HDL cholesterol</td>
</tr>
<tr>
<td>HDL/total cholesterol ratio</td>
</tr>
</tbody>
</table>
low serum HDL/total cholesterol ratio\(^4\text{--}^8\) and high blood pressure\(^9\text{--}^{11}\) can be considered as risk factors for atherosclerotic lesions of the cervical carotid arteries, especially since these aberrations are already observed at an early stage of the disease. In the present investigation, after all, all subjects were asymptomatic for cerebrovascular disease, while 78% of the lesions were associated with less than 50% narrowing of the artery (table 2). That these risk factors are related to atherosclerosis is supported by the finding that the serum lipid disturbances, the frequency of high blood pressure and the diastolic blood pressure level were similar in the group with asymptomatic cervical carotid artery lesions (Group III) and in the group of subjects with a history of ischemic heart disease and/or intermittent claudication (Group II), but with a normal Doppler examination. Furthermore, 30% of the subjects in Group III also had a history of ischemic heart disease or intermittent claudication. These findings also imply that the risk factors under investigation are indiscriminate as far as the localization of atherosclerosis is concerned. A major advantage of the use of the serum HDL/total cholesterol ratio as risk factor in epidemiologic studies over other lipids is that overnight fasting is not required to estimate this ratio accurately.\(^5\text{--}^9\) Several subjects, especially in Group II, were taking beta-blocking agents or thiazides. Whether this has influenced the results as obtained in the present investigation is not known because the effect of these compounds on serum lipids is still a matter of debate.\(^10\text{--}^{12}\)

In the present investigation the Doppler spectral classifications A and classifications B with normal velocity profiles and instantaneous velocity waveforms were grouped and assumed to be representative of normal arteries, despite the fact that B classifications per se have been considered to be associated with very minor lesions. In a recent study, however, it was indicated that B classifications per se do not necessarily reflect cervical carotid artery disease, especially in the internal carotid artery\(^20\) where the flow conditions are rather complicated, even under normal circumstances.\(^33\) On the other hand, the inclusion of subjects with very minor carotid artery lesions in the group of normals cannot be excluded when A and some B classifications are combined.

The frequency of asymptomatic cervical carotid artery lesions of 23% as found in this study compares favorably with the results obtained by Martin and co-investigators\(^13\) in post-mortem studies, but is significantly higher than the incidence of 5.9% found by Hennerici and his colleagues\(^12\) in neurologically asymptomatic at-risk patients. The latter investigators, however, only considered patients with a degree of carotid artery narrowing of more than 50%. The incidence of lesions in this category was 5% in our study, a value in close agreement with the one described by Hennerici.

In summary the findings of the present study support the idea that a low serum HDL/total cholesterol ratio and high blood pressure have a high degree of association with atherosclerotic lesions of the cervical carotid arteries. This association is already apparent at an early stage of the disease.

Acknowledgments

The authors are greatly indebted to Astrid Dingjan for her help in collecting and processing the blood samples, and to Mr. Z. Kraswijk and Dr. C.E. West (Agriculture University, Wageningen) for the estimation of the serum total and HDL cholesterol concentrations. The help of Jos Heemskerk and Mariet de Groot in preparing the manuscript is kindly acknowledged.

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Modification of Cerebral Ischemia With Fluosol


SUMMARY Fluosol-DA (Perfluorochemical Blood Substitute) was investigated in a previous study and found to provide some protection from ischemia and possible usefulness in limiting the size of infarction. In the present study, larger doses over longer periods of acute focal cerebral ischemia were used. Twenty-four cats had transorbital ligation of the middle cerebral artery (MCA). The 12 experimental animals were given Fluosol-DA and might be useful in limiting the size of infarction to the brains of the cats treated, it may have helped to slow the development of the pathological changes.

Fluosol-DA did provide some protection from ischemia and might be useful in limiting the size of infarction. The present report is based on further investigation of the effectiveness of Fluosol-DA, using larger doses over longer periods of acute focal cerebral ischemia.

Material and Methods

Implantation of Occlusive Device (Tourniquet)

Twenty-four cats were anesthetized with intraperitoneal injection of ketamine-HCl (30 mg/kg). Intubation was done for the security of airway and the head was immobilized in an operating apparatus. As described in our previous study, the microtourniquet was surgically placed in position, around the proximal portion of the left MCA. The tourniquet was not tightened at this time.

During the recovery period of four or five days following the operation, the cats were kept under observation and showed no apparent neurological deficit. Following this period, each animal was anesthetized

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Received June 2, 1983; revision #2 accepted June 15, 1984.

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T van Merode, P Hick, P G Hoeks and R S Reneman

Stroke. 1985;16:34-38
doi: 10.1161/01.STR.16.1.34

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