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The influence of lipoproteins on whole-blood viscosity at multiple shear rates☆

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Abstract

Whole-blood viscosity appears to be an independent predictor of stroke, carotid intima-media thickening, and carotid atherosclerosis. The purpose of this study was to examine for relationships between whole-blood viscosity and blood lipids in young healthy subjects over a range of shear rates. Twenty-seven healthy men and women aged 10 to 25 years having a range of low-density lipoprotein (LDL) cholesterol values 88 to 258 mg/dL and body mass index *z* scores −1.18 to 2.64 SDs were studied. Whole-blood viscosity at shear rates from 1 to 1000 per second was measured using an automated capillary viscometer. Blood lipids were measured using standard techniques. Triglyceride-rich lipoproteins were isolated by ultracentrifugation at density of <1.020 g/mL, and a high ratio of cholesterol to triglyceride was used as an indicator of lipoprotein remnants. Whole-blood viscosity at shear rates of 100 to 1000 per second showed significant negative correlations with apolipoprotein A-1, but not with high-density lipoprotein cholesterol. Whole-blood viscosity at a shear rate of 1000 per second correlated with LDL cholesterol and inversely with LDL size. On stepwise multivariate analysis, apolipoprotein A-1 accounted for 14.7% of the variation in whole-blood viscosity at a shear rate of 150 per second. This study points to the importance of high-density lipoprotein particle number on whole-blood viscosity at physiological shear rates. The physiological significance of the relationships between whole-blood viscosity and LDL cholesterol and LDL particle size at a very high shear rate remains to be determined.

1. Introduction

Whole-blood viscosity is a predictor of stroke, carotid intima-media thickening, and carotid atherosclerosis, and these relationships are independent of traditional risk factors [1-3]. There are also strong relationships between blood viscosity and blood lipids [4-9]. However, in most studies, whole-blood viscosity was measured at a few nonspecific shear rates, and these data do not reflect the complete rheological characteristics of the study subjects [4-9].

Blood is a unique fluid in that it exhibits non-Newtonian characteristics, and its viscosity is dependent on shear rate [10-12]. Major determinants of whole-blood viscosity are hemotocrit, plasma viscosity, and red cell aggregation and red cell deformation under conditions of low and high shear [11,12].

The focus of this study was to examine relationships between whole-blood viscosity and blood lipids. To determine blood viscosity over a wide range of shear rates, whole-blood viscosity was measured using the Rheolog viscometer (Rheologics Inc, Exton, Pa), a capillary viscometer that simulates physiological blood flow conditions pertaining in vivo. Furthermore, in contrast to prior studies, this study was performed in children and young adults.

2. Methods

There were 27 male and female subjects who were healthy, aged 10 to 25 years, and without medical problems. None of the subjects were smokers, and none were on medication. Some of the subjects had attended the Pediatric

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Lipid Clinic at the Children's Hospital of Wisconsin because of hyperlipidemia or a family history of coronary disease, whereas others had responded to advertisements inviting their participation without respect to blood lipids or family history of coronary disease. Because of this recruitment strategy, participants exhibited a broad range of blood lipids. The study protocol was approved by the institutional review board of the Children's Hospital of Wisconsin.

Subjects attended the Clinical Research Center on the day of the study in the fasting state after a 12-hour fast. After signing informed consent, subjects underwent a brief physical examination, including height, weight, and blood pressure, and blood was drawn into EDTA tubes for blood lipids and whole-blood viscosity. Menstruating women were tested between days 7 and 14 of their menstrual cycle. The physical characteristics and laboratory values of the subjects are shown in Table 1.

Immediately after venipuncture, whole-blood viscosity was measured on the Rheolog viscometer (Rheologics Inc). In this instrument, blood is maintained at body temperature during the entire measurement. The EDTA-preserved blood is injected into the U-shaped disposable capillary assembly to fill one of the vertical tubes. Blood then flows from one arm of the instrument to the other through a precisely calibrated capillary, as illustrated in Fig. 1. Because of the decreasing pressure differential, blood flows at a gradually lower and lower speed, thereby allowing determination of whole-blood viscosity over a range of shear rates (1-1000 per second). The speed of blood flow is determined by continuous scanning of the height of the blood in the vertical tubes. The measured change in height in the columns over time translates into velocity, and shear rate is calculated on the assumption of laminar flow. The full range of blood viscosities is calculated from algorithms [13,14]. Results obtained with this instrument compare well with that obtained by other methodologies (data under review with the Federal Drug Administration).

Blood lipids were determined at the Emory Lipid Research Laboratory, which is a participant in the Centers for Disease Control and Prevention/National Heart, Lung, and Blood Institute Lipid Standardization Program. All analyses were performed on freshly isolated fasting EDTA plasma using a Beckman CX7 chemistry autoanalyzer. Total triglyceride and cholesterol were determined by enzymatic methods (Beckman Coulter Diagnostics, Fullerton, Calif). Direct high-density lipoprotein (HDL) and direct low-density lipoprotein (LDL) cholesterol were obtained using homogeneous assays (Equal Diagnostics, Exton, Pa). Apolipoprotein A-I, apolipoprotein B, and lipoprotein(a) were determined using immunoturbidometric reagents

Table 1
Characteristics and laboratory values of the 27 subjects

	Mean (range)
Age (y)	18.7 (11.0-25.2)
BP systolic (mm Hg)	112 (89-136)
BP diastolic (mm Hg)	65 (49-76)
BMI *z* score	0.48 (−1.18 to 2.64)
LDL cholesterol (mg/dL)	138 (66-258)
LDL size (nm)	26.2 (25.3-27.1)
HDL cholesterol (mg/dL)	49.2 (29.9-67.9)
Triglyceride (mg/dL)	81.7 (33-223)
Lipoprotein(a) (mg/dL)	58.8 (2.8-205.6)
Apolipoprotein A (mg/dL)	135.3 (81-198)
Apolipoprotein B (mg/dL)	101.3 (60-151)
TRL–TC/TG ratio	0.45 (0.24- 0.78)

BP indicates blood pressure; BMI, body mass index; TRL, triglyceride-rich lipoprotein; TC/TG, total cholesterol/triglyceride.

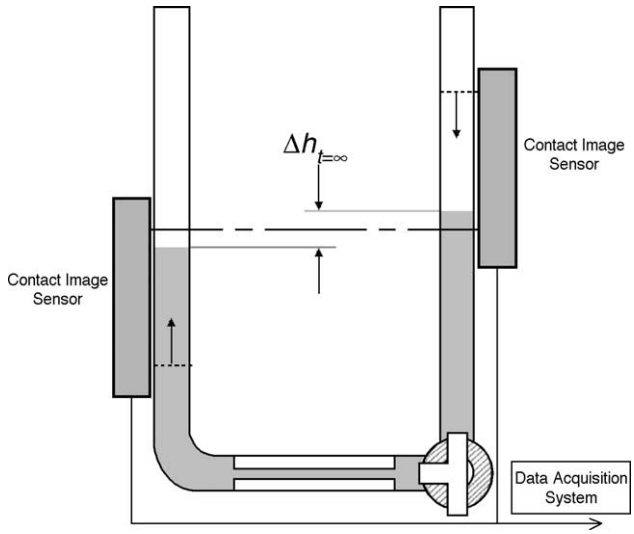

Fig. 1. Illustration of the capillary viscometer. Under gravity, the change in height of the blood in the 2 columns creates a continuously reducing pressure differential. This changing flow velocity creates different shear rates throughout the analysis; Δh at time infinity is the height difference at the end of the test because of yield stress and surface tension.

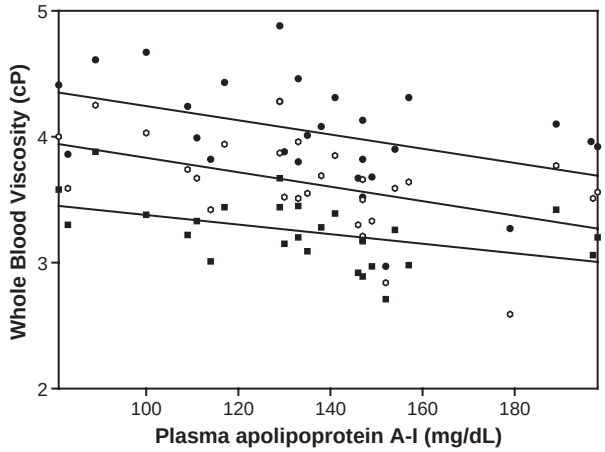

Fig. 2. Correlations between plasma apolipoprotein A and whole-blood viscosity at shear rates of 1000, 300, and 150 per second for the 27 subjects. Filled circles (●) indicate correlations at 150 per second ($r = -0.417$, $P = .027$); open circles (○) indicate correlations at 300 per second ($r = -0.480$, $P = .01$), and filled squares (■) indicate correlations at 1000 per second ($r = -0.450$, $P = .019$). The top, middle, and bottom lines are regression lines for whole-blood viscosity at shear rates 150, 300, and 1000 per second, respectively.

Table 2
Correlations between whole-blood viscosity and apolipoprotein A, direct LDL cholesterol, HDL cholesterol, and LDL size for the 27 subjects

Blood viscosity (per second)	Apolipoprotein A	LDL cholesterol	HDL cholesterol	LDL size
1000	−0.450 (0.019)	0.443 (0.021)	−0.257	−0.429 (0.029)
300	−0.480 (0.01)	0.228	−0.205	−0.234
150	−0.417 (0.027)	0.114	−0.190	−0.236
100	−0.36	0.036	−0.176	−0.226
50	−0.307	0.041	−0.135	−0.218
10	−0.005	0.014	−0.072	−0.163
5	0.082	0.005	−0.052	−0.131
2	0.145	−0.003	−0.034	−0.109
1	0.171	−0.007	−0.026	−0.101

R values are shown with significant *P* values in parentheses.

(DiaSorin, Stillwater, Minn). LDL particle diameter was determined using nondenaturing polyacrylamide gradient gel electrophoresis as previously described [15]. Triglyceride-rich lipoproteins were isolated by ultracentrifugation at density of <1.020 g/mL to include very low-density lipoprotein and intermediate-density lipoprotein. The supernatant was quantitatively recovered in volumetric tubes, and its content of triglyceride and cholesterol was determined by enzymatic methods as described above.

Correlations and multiple regression analysis were determined using the Minitab statistical package (State College, Pa). Additional terms in the regression model were tested hierarchically to examine what they added.

3. Results

At shear rates of 150, 300, and 1000 per second, whole-blood viscosity showed significant inverse correlations with apolipoprotein A-1 ($r = -0.417$, $P = .027$; $r = -0.480$, $P = .01$; and $r = -0.450$, $P = .019$, respectively), but not with HDL cholesterol (Fig. 2 and Table 2). Whole-blood viscosity

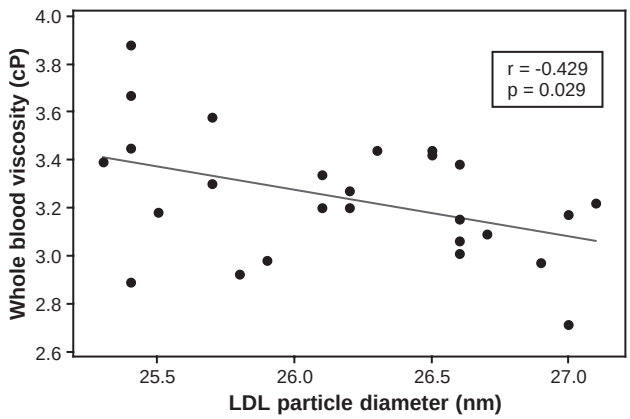

Fig. 3. Correlation between LDL particle diameter and whole-blood viscosity at a shear rate of 1000 per second for the 27 subjects. LDL particle diameter was determined using nondenaturing polyacrylamide gradient gel electrophoresis [15].

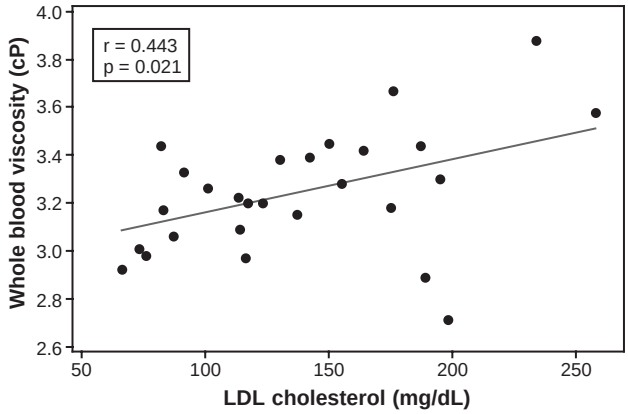

Fig. 4. Correlation between whole-blood viscosity and LDL cholesterol at a shear rate of 1000 per second for the 27 subjects. Direct LDL cholesterol was measured using a homogeneous assay (Equal Diagnostics).

at a shear rate of 1000 per second correlated with LDL cholesterol ($r = 0.443$, $P = .021$) and inversely with LDL size ($r = -0.429$, $P = .029$) (Figs. 3 and 4, and Table 2). No correlations were evident between whole-blood viscosity and the other lipoprotein variables, noted in Table 1, measured over the range of shear rates.

On a stepwise regression analysis of the determinants of whole-blood viscosity at a shear rate of 1000 per second that included apolipoprotein A-1, HDL cholesterol, LDL size, LDL cholesterol, age, and body mass index *z* score, the only independent determinants of whole-blood viscosity were LDL size and plasma apolipoprotein A-1, which accounted for 23.5% ($P = .029$) and 14.1% ($P = .039$) of the variation, respectively. With the same variables, apolipoprotein A-1 accounted for 14.7% of the variation in whole-blood viscosity at 150 per second ($P = .036$).

4. Discussion

This study is unique in examining relationships between blood lipids and whole-blood viscosity at multiple shear rates. This was made possible by use of the Rheolog capillary viscometer. A significant inverse relationship was demonstrated between whole-blood viscosity and apolipoprotein A-1, although not HDL cholesterol, at shear rates of between 150 and 1000 per second. These data suggest that, at these particular shear rates, whole-blood viscosity is influenced by the number of HDL particles in the blood.

The importance of whole-blood viscosity lies in the fact that it appears to be an independent predictor of stroke, carotid intima-media thickening, and carotid atherosclerosis [1-3]. The Edinburgh Artery Study of 1592 men and women aged 55 to 74 years found that, after correcting for conventional coronary risk factors, whole-blood viscosity and hematocrit were significant independent variables for stroke, but not total cardiovascular events [1]. In this study, whole-blood viscosity was measured at high shear (over 300 per second) using a Coulter-Harkness viscometer and was then corrected to a standard hematocrit of 45%. The

Edinburgh Artery Study also found that whole-blood viscosity and hematocrit were significantly related to common carotid intima-media thickening in men, and this relationship was maintained in a multivariate model that included total cholesterol, systolic blood pressure, and pack years [3]. However, these relationships did not hold for carotid intima-media thickening in women. Carallo et al [2] found that men with carotid atherosclerosis had significantly greater values for whole-blood viscosity and hematocrit compared with controls. In this study, blood viscosity was measured at shear rates of 450 and 225 per second using a cone-plate viscometer.

Blood lipids show a strong relationship to whole-blood viscosity. However, comparisons between this and previous studies should be done with caution, because no previous study has examined whole-blood viscosity and lipid relationships over a wide range of shear rates and, in particular, at the low shear rates that the Rheolog viscometer is capable of producing.

Using a capillary viscometer, Sloop and Garber [4] showed significant correlations between blood viscosity and LDL cholesterol, total cholesterol, and HDL cholesterol at calculated shear rates of between 75 and 132 per second in healthy nonfasting adult volunteers. Crowley et al [5] demonstrated significant relationships between whole-blood viscosity measured with a disposable porous bed viscometer and total cholesterol, triglyceride, LDL cholesterol, and HDL cholesterol in 982 normal volunteers. On stepwise regression analysis, the only independent influence from lipoproteins on blood viscosity was from LDL cholesterol. Using a coaxial cylinder microviscometer, Rosenson et al [6] showed an inverse correlation between normalized whole-blood viscosity and HDL cholesterol at shear rates of 100 and 20 per second in 126 healthy adults. Blood viscosity was normalized to a hematocrit of 45% by a regression equation. In a separate study, Rosenson et al [7] showed that triglyceride, HDL cholesterol, fibrinogen, total serum protein, and hematocrit were significant predictors of uncorrected whole-blood viscosity at a shear rate of 100 per second in healthy adult subjects referred for cardiovascular risk assessment. Because of the inverse relationship often noted between triglyceride and HDL cholesterol, Stamos and Rosenson [8] evaluated 70 normal normocholesterolemic and normotriglyceridemic adults (fasting cholesterol <5.2 mmol/L [200 mg/dL] and triglyceride <2.3 mmol/L [200 mg/dL]) and demonstrated an inverse correlation between whole-blood viscosity and HDL cholesterol at 20 and 100 per second using a coaxial cylinder microviscometer. In the West of Scotland Coronary Prevention Study which looked at more than 6000 middle-aged men, plasma viscosity was measured in a Coulter semiautomated capillary viscometer, and a high-shear whole-blood viscosity was calculated from an equation that incorporated the hematocrit value [9]. Plasma viscosity and whole-blood viscosity were related to prevalent cardiovascular disease, triglyceride, HDL cholesterol, cholesterol, very low-density lipoprotein, and LDL levels. In none of these previous studies was apolipoprotein A measured.

The data of Goldsmith and Turitto [12] suggest that, at physiological blood flow, peripheral shear rates range from 50 to 700 per second in large arteries, and from 250 to 2000 per second in the smallest arterioles and capillaries. The data from this study on whole-blood viscosity are therefore well within physiological shear rates. Interest in the past has focused on the influence of HDL to prevent red cell aggregation by competing with LDL-induced red cell aggregation [16,17]. It would seem logical to assume that this effect is mediated via the outer coat of the HDL particle which is composed of apolipoprotein A rather than the amount of cholesterol in HDL. The influence of LDL size at high shear has not previously been reported, and the importance of this observation could relate to LDL-induced red cell aggregation. These changes in whole-blood viscosity may in turn influence blood flow [18-20].

References

[1] Lee AJ, Mowbray PI, Lowe GD, Rumley A, Fowkes FG, Allan PL. Blood viscosity and elevated carotid intima-media thickness in men and women: the Edinburgh Artery Study. Circulation 1998;97:1467-73.

[2] Carallo C, Pujia A, Irace C, De Franceschi MS, Motti C, Gnasso A. Whole blood viscosity and haematocrit are associated with internal carotid atherosclerosis in men. Coron Artery Dis 1998;9:113-7.

[3] Lowe GD, Lee AJ, Rumley A, Price JF, Fowkes FG. Blood viscosity and risk of cardiovascular events: the Edinburgh Artery Study. Br J Haematol 1997;96:168-73.

[4] Sloop GD, Garber DW. The effects of low-density lipoprotein and high-density lipoprotein on blood viscosity correlate with their association with risk of atherosclerosis in humans. Clin Sci (Lond) 1997;92:473-9.

[5] Crowley JP, Metzger J, Assaf A, Carleton RC, Merrill E, Valeri CR. Low density lipopoprotein cholesterol and whole blood viscosity. Ann Clin Lab Sci 1994;24:533-41.

[6] Rosenson RS, McCormick A, Uretz EF. Distribution of blood viscosity values and biochemical correlates in healthy adults. Clin Chem 1996;42:1189-95.

[7] Rosenson RS, Shott S, Tangney CC. Hypertriglyceridemia is associated with an elevated blood viscosity. Atherosclerosis 2002; 161:433-9.

[8] Stamos TD, Rosenson RS. Low high density lipoprotein levels are associated with an elevated blood viscosity. Atherosclerosis 1999; 146:161-5.

[9] Lowe G, Rumley A, Norrie J, Ford I, Shepherd J, Cobbe S, et al. Blood rheology, cardiovascular risk factors, and cardiovascular disease: the West of Scotland Coronary Prevention Study. Thromb Haemost 2000;84:553-8.

[10] Koenig W, Ernst E. The possible role of hemorheology in atherothrombogenesis. Atherosclerosis 1992;94:93-107.

[11] Dutta A, Tarbell JM. Influence of non-Newtonian behavior of blood on flow in an elastic artery model. J Biomech Eng 1996;118:111-9.

[12] Goldsmith HL, Turitto VT. Rheological aspects of thrombosis and haemostatis: basic principles and applications. ICTH-Report—Subcommittee on Rheology of the International Committee on Thrombosis and Haemostatis. Thromb Haemost 1986;55(3):415-35.

[13] Kim S, Cho YI, Hogenauer WN, Kensey KR. A method of isolating surface tension and yield stress effects in a U-shaped scanning capillary-tube viscometer using a Casson model. J Non-Newton Fluid Mech 2002;103:205-19.

[14] Kim S, Cho YI, Jeon AH, Hogenauer B, Kensey KR. A new method for blood viscosity measurement. J Non-Newton Fluid Mech 2000;94:47-56.

[15] Innis-Whitehoues W, Brown WV, Le NA. Protocol for the separation of a segmental linear polyacrylamide gradient gel: simultaneous determination of Lp[a], LDL, and HDL particle sizes. J Lipid Res 1997;38(12):2603-14.

[16] Ruhenstroth-Bauer G, Mossmer G, Ottl J, Koenig-Erich S, Heinemann G. Highly significant negative correlations between erythrocyte aggregation value and serum concentration of high density lipoprotein cholesterol in a sample from a normal population and in patients with coronary disease. Eur J Clin Invest 1987;17:275-9.

[17] Rosenson RS, Lowe GDO. Effect of lipids and lipoproteins on thrombosis and rheology. Atherosclerosis 1998;140:271-80.

[18] Thomas DJ, Marshall J, Ross Russell RW, Wetherley-Mein G, Du Boulay GH, Pearson TC, et al. Cerebral blood-flow in polycythemia. Lancet 1977;2:161-3.

[19] Rim S-J, Leong-Poi H, Lindner JR, Wei K, Fisher NG, Kaul S. Decrease in coronary blood flow reserve during hyperlipidemia is secondary to an increase in blood viscosity. Circulation 2001;104:2704-9.

[20] Rubba P, Iannuzzi A, Postiglione A, Scarpato N, Montefusco S, Gnasso A, et al. Hemodynamic changes in the peripheral circulation after repeat low density lipoprotein apheresis in familial hypercholesterolemia. Circulation 1990;81:610-6.