Decrease in Coronary Blood Flow Reserve During Hyperlipidemia Is Secondary to an Increase in Blood Viscosity

Se-Joong Rim, MD; Howard Leong-Poi, MD; Jonathan R. Lindner, MD; Kevin Wei, MD; Nicholas G. Fisher, MD; Sanjiv Kaul, MD

Background—During maximal hyperemia, capillaries provide the greatest resistance to flow. A major determinant of capillary resistance is viscosity. We, therefore, hypothesized that abnormal coronary blood flow (CBF) reserve observed during hyperlipidemia is secondary to increased blood viscosity and not abnormal coronary vasomotion.

Methods and Results—Maximal hyperemia was induced in 9 dogs using adenosine. Serum triglyceride levels were increased by incremental doses of Intralipid. A good correlation was noted between serum triglyceride levels and blood viscosity ($r = 0.82$). Neither total coronary blood volume nor myocardial blood volume changed with increasing serum triglyceride levels, indicating lack of vasomotion. Myocardial vascular resistance (MVR) increased with increasing triglyceride levels ($r = 0.84$), while hyperemic myocardial blood flow (MBF) decreased ($r = -0.64$). The decrease in hyperemic MBF was associated with a decrease in blood velocity ($r = -0.56$). These findings were confirmed with direct intravital microscopic observations in the mice cremaster muscle.

Conclusions—Increasing lipid levels in a fully dilated normal coronary bed causes no change in large or small vessel dimensions. Instead, the increase in blood viscosity causes capillary resistance to rise, which attenuates hyperemic CBF. Therefore, the abnormal CBF reserve associated with hyperlipidemia is due to increase blood viscosity and not abnormal vascular function. (Circulation. 2001;104:2704-2709.)

Key Words: risk factors ■ lipids ■ microcirculation

Abnormal coronary blood flow (CBF) reserve has been associated with hyperlipidemia even in the absence of structural changes in coronary arteries.\(^1\)–\(^4\) It is generally thought that this phenomenon is secondary to endothelial dysfunction that affects the ability of coronary vessels to dilate.\(^5\)–\(^6\) At rest, maximal resistance to CBF is offered by the coronary arterioles with only a small portion offered by the capillaries and venules.\(^7\) During hyperemia, however, coronary arterial and venular resistances decrease significantly, but capillary resistance remains unaffected. Consequently, capillaries offer the most resistance to CBF during hyperemia and thus determine the maximal possible increase in hyperemic CBF.\(^8\)

According to Hagen-Poiseille’s equation, resistance to flow within a tube depends on the dimensions of the tube and the viscosity of the fluid in it. In large vessels, resistance is determined mainly by the dimensions of the vessels with viscosity playing a negligible role. In vessels with $<30 \ \mu m$ diameter, however, an abrupt increase in viscosity is seen with an inverse relation between vessel size and effective viscosity.\(^9\)

Increased whole blood and plasma viscosity are observed in patients with hyperlipoproteinemia.\(^10\)–\(^12\) A major factor affecting capillary resistance is blood viscosity. Because capillaries play a dominant role in limiting maximal hyperemic CBF, we hypothesized that abnormal CBF reserve observed during hyperlipidemia in the absence of coronary stenosis is secondary to increased blood viscosity rather than abnormalities in coronary vessel function.

Methods

Animal Preparation

The study was approved by the Animal Research Committee at the University of Virginia and conformed to The American Heart Association Guidelines for the Use of Animals in Research. Nine open-chest, anesthetized, adult mongrel dogs were used for the study. Catheters (7F) were placed in both femoral veins for administration of fluids, drugs, and microbubbles and in both femoral arteries for withdrawal of blood samples. Catheters were also placed in the ascending aorta as well as the right and left atria to measure pressures. The catheter in the left atrium was also used for injection of radiolabeled microspheres.

The proximal portion of the left anterior descending coronary artery (LAD) was dissected free from surrounding tissue. An
ultrasonic time-of-flight flow probe (series SB; Transonics) was placed around it and connected to a flowmeter (model T206, Transonics). Two 20-gauge catheters were introduced into separate distal branches of the LAD for intracoronary infusion of adenosine and intracoronary injection of microbubbles, respectively. Heparin sodium was administered intravenously as a bolus (2000 to 3000 IU), followed by intermittent administration of 1000 IU during the rest of the experiment. Myocardial vascular resistance (MVR) of the LAD bed was calculated by dividing the coronary driving pressure (the difference between the mean aortic and right atrial pressure) by LAD flow.

Myocardial Contrast Echocardiography

An HDI-5000 system (Philips-ATL) was used for myocardial contrast echocardiography (MCE). High mechanical index (1.0) intermittent harmonic imaging was used with ultrasound transmitted at 1.67 MHz. The ultrasound transducer was fixed in position, and a saline bath served as an acoustic interface between the transducer and the heart. Imaging was performed at the mid-papillary muscle short-axis level of the left ventricle. Image depth, focus, and gains were optimized at the beginning of each experiment and were held constant during the study. All data were recorded digitally. Backscatter was measured in acoustic intensity (AI) units from digitally stored images. AI is measured prior to log compression and post-processing, allowing a larger linear range between backscatter and its measurement.

Total LAD coronary blood volume (CBV), which is the volume of blood in the entire LAD system (including arteries, arterioles, capillaries, venules, and veins) was measured at each stage to determine if any vasomotion had occurred.13 For this purpose, 1 mL of a diluted solution (5%) of Albunex ( Molecular Biosystems) was injected directly into the LAD over 1 second with a power injector (Pulsar, Medrad). Images were acquired from 4 to 5 frames before contrast appearance until its disappearance from the myocardium. A large region-of-interest was drawn over the myocardium in the LAD bed and AI was derived from all end-systolic aligned images in the injection sequence. A y-variance function, \( y = A e^{-t} \), was fitted to the background-subtracted time-intensity plots, where \( A \) is a scaling factor, \( t \) is time, and \( \alpha \) is proportional to the mean transit rate of the tracer. Total LAD CBV was calculated by dividing LAD flow at each stage by the mean microbubble transit rate at that stage.13

Myocardial blood volume (MBV), which is a component of CBV and consists of blood only within the myocardium (90% of which is in capillaries), was also measured, as was mean myocardial microbubble velocity. We have previously shown that the microvascular rheology of microbubbles is similar to that of red blood cells (RBCs). A suspension of 1 mL of perfluorocarbon-filled polymer microspheres (PBC 127, Point Biomedical) in 49 mL of 5% Dextrose water was infused at a constant rate (100 mL/min) via the femoral venous catheter. Imaging was initiated after steady-state was reached (~2 minutes). The relation between microbubble concentration in the LV cavity and AI was linear for the doses of microbubbles used.

End-systolic triggered imaging was performed using pulsing intervals (PI) of 200 ms to 20 cardiac cycles to allow for progressively greater microbubble replenishment of the ultrasound beam. At least 7 frames were recorded at each PI. A large region-of-interest was drawn over the LAD territory (defined by direct LAD injection of Albunex) for measurement of regional AI. At each experiment, the same region-of-interest was used for all stages. The AI within frames for each PI was averaged and digitally subtracted from similarly averaged baseline frames. PI versus AI plots were then generated and fitted to the exponential function: \( y = A(1 - e^{-t}) \), where \( y \) is the AI at a PI of \( t \), \( A \) is the plateau AI which represents MBV, and \( \beta \) represents mean microbubble velocity in tissue.15 The product of \( A \) and \( \beta \) reflects MCE-derived MBF. MBV was divided by AI in the LV cavity at each stage to determine MBV fraction. We have previously demonstrated a close linear relation between MCE-and radiolabeled microsphere-derived MBF (\( r = 0.92 \)).15 Our interobserver and intraobserver variability for the MCE-derived parameters are good (\( r = 0.88 \) and \( r = 0.91 \) for \( A \); \( r = 0.91 \) and \( r = 0.92 \) for \( \beta \)).16

Experimental Protocol

After baseline measurements were performed, maximal LAD hyperemia was induced with a subselective infusion of 10 \( \mu \)g \( \cdot \) kg\(^{-1} \) \( \cdot \) min\(^{-1} \) of adenosine directly into the distal LAD. After ensuring that systemic hemodynamics and CBF had stabilized for 10 minutes, the infusion was maintained for the duration of the study. Baseline hemodynamics were acquired and MCE was performed. Blood was also sampled for serum triglyceride levels and whole blood viscosity. The latter was measured using a rotational viscometer (EW-98936-00, Cole-Parmer) at 37°C at a shear rate of 7.34 s\(^{-1} \). Radiolabeled microspheres were injected at each stage to determine MBF.13 All measurements were repeated 20 minutes after each intravenous administration of Intralipid (range 50 to 450 mL). Because free fatty acids cause arteriolar vasodilation from cytosolic calcium sequestration,18 2.0 mmol/L of calcium chloride was injected intravenously prior to each administration of Intralipid. At the conclusion of the study, the dog was euthanized, and the heart was excised for radiolabeled microsphere-derived MBF analysis.

Intravital Microscopy

To confirm the findings of the MCE studies, changes in RBC velocity in response to hyperlipidemia were assessed by intravital microscopy in 5 wild-type C57BL/6 anesthetized mice. The left carotid artery was cannulated for arterial pressure measurement and a catheter was placed in jugular vein for administration of microbubbles and Intralipid. The cremaster muscle was exteriorized through a scrotal incision and superfused with isothermic bicarbonate-buffered saline. Microscopic observations of the muscle were made using an intravital microscope (Axioskop 2 FS, Carl Zeiss) with a saline immersion objective (×40/0.8 N.A.). Video recordings were made using a high-resolution camera interfaced with video time display unit and connected to a S-VHS recorder.

At baseline, recordings of reference arterioles and venules (20 to 40 \( \mu \)m) were made. Centerline RBC velocities were measured in these vessels using a dual-slit photodiode (CircuSoft), and converted to mean velocity by multiplying by 0.625. Approximately 60 \( \mu \)L of a solution containing fluorescein isothiocyanate (FITC)-dextran (1 mg/mL; Sigma) was administered to outline the individual capillaries. A surrogate of RBCs—500 nm red fluorescent polymer microspheres—was injected with an excitation peak of 542 nm (R500, Duke Scientific) was administered to study velocity in capillaries. Ten optical fields were recorded using fluorescent epi-illumination with both 460 to 490 nm and 530 to 560 nm excitation filters. All measurements were repeated during topical administration of adenosine (10 \( \mu \)mol/L final concentration in the superfuse) before and 5 minutes after intravenous injection of Intralipid (3 \( \mu \)L/g).

Arteriolar and venular diameters were measured off-line and blood flow was calculated. Capillary blood velocity was determined using freeze-frame advancing of fluorescent epi-illumination recordings to track individual polymer microspheres over a distance of 30 to 150 \( \mu \)m. The total distance traveled was divided by the elapsed time to derive the mean velocity. The percent of capillaries perfused in each optical field was determined by the presence or absence of RBC flux observed during fluorescent epi-illumination of FITC-dextran.

Statistical Methods

All data are expressed as mean±SD. Comparisons between 2 stages were made using Student’s \( t \) test whereas those between multiple stages were made using one-way ANOVA. Correlations were performed using least squares fit linear regression analysis. Differences were considered significant at \( P < 0.05 \) (two-sided).

Results

Canine Experiments

A total of 33 stages were performed in the 9 dogs. Incremental doses of Intralipid resulted in progressive increases in serum triglyceride levels without any change in hemodynamics (Table 1). Ionized calcium levels did not change despite
intravenous injections of calcium chloride before each dose of Intralipid. A close correlation was noted between serum triglyceride levels and blood viscosity for the 16 stages where the latter was measured (Figure 1).

Increasing doses of Intralipid had no effect on either total CBV or MBV fraction of the LAD bed during hyperemia (Figure 2). The lack of change in CBV indicates that the total net dimensions of all coronary vessels did not change over a wide range of serum triglyceride levels, whereas the constant MBV fraction indicates that there was no capillary recruitment or derecruitment with Intralipid. Despite no changes in CBV or MBV fraction, MVR of the LAD bed increased during hyperemia with increasing doses of triglycerides and a concomitant decrease in hyperemic MBF was noted. Figure 3 illustrates a linear relation between serum triglyceride levels and MVR and an inverse relation between serum triglyceride levels and hyperemic MBF.

Because MBV fraction did not change during hyperlipidemia, the decrease in hyperemic MBF resulted from a reduction in mean RBC velocity. Figure 4 illustrates PI versus AI plots obtained during hyperemia at 3 different levels of serum triglycerides from one dog. It is apparent that while MBV did not change over a several-fold increase in serum triglyceride levels, mean microbubble velocity decreased in proportion to the decrease in hyperemic MBF. Figure 5 depicts the inverse relation between mean microbubble velocity during hyperemia versus serum triglyceride levels.

Intravital Microscopy
Topical adenosine and intravenous lipid administration were well tolerated and did not alter mean arteriolar blood pressure in the mice. Adenosine produced marked arteriolar vasodilation, a mild increase in mean arteriolar blood velocity, and a 3.5-fold increase in mean arterial blood flow (Table 2). Although Intralipid administration did not affect arteriolar diameter in the presence of topical adenosine, it decreased both arteriolar blood velocity and flow. A similar, albeit attenuated, response was seen in venules.

Mean capillary blood flow velocity increased in response to adenosine by approximately 3.5-fold (Figure 6), while the proportion of capillaries with RBC flux remained unchanged (85% to 90%). Therefore, augmentation of capillary perfusion in response to adenosine was mediated by increases in capillary velocity without any concomitant capillary recruitment. Intralipid markedly attenuated the adenosine-mediated increase in capillary blood velocity without any effect on capillary dimension or the proportion of capillaries with RBC flux.

Discussion
The new findings of our study are that increasing lipid levels in a fully dilated normal coronary bed causes no change in large or small vessel dimensions. Instead, the increase in blood viscosity causes capillary resistance to rise, which attenuates hyperemic CBF. Therefore, the abnormal CBF reserve associated with hyperlipidemia is due to increased blood viscosity and not abnormal vascular function.

Under normal resting conditions, the mean myocardial capillary pressure is about one-third the mean aortic pressure. The resting coronary arteriole tone serves to reduce the pressure from the aortic to the capillary level,7 although about 25% of the MVR is offered by the capillary network itself. When a coronary vasodilator is used to produce maximal

![Figure 1. Relation between serum triglyceride levels (x-axis) and whole blood viscosity (y-axis) in 16 stages where the latter was measured. See text for details.](image1)

![Figure 2. Effect of serum triglyceride levels (x-axis) on CBV (left y-axis, shown as filled circles) as well as MBV fraction (right y-axis, shown as triangles) in the presence of maximal hyperemia. All values are normalized to baseline hyperemic values in each dog. See text for details.](image2)

<table>
<thead>
<tr>
<th>Variable Measured</th>
<th>Baseline (n=9)</th>
<th>50 mL (n=9)</th>
<th>150 mL (n=7)</th>
<th>300 mL (n=5)</th>
<th>450 mL (n=3)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum triglyceride level, mg/dL</td>
<td>17±7</td>
<td>118±82</td>
<td>411±257</td>
<td>990±549</td>
<td>1533±1122</td>
<td>&lt;0.001</td>
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<td>Ionized calcium, mg/dL</td>
<td>4.2±4.0</td>
<td>4.2±0.5</td>
<td>3.9±0.8</td>
<td>3.8±0.6</td>
<td>3.8±0.8</td>
<td>0.50</td>
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<tr>
<td>Aortic pressure, mm Hg</td>
<td>108±13</td>
<td>104±11</td>
<td>109±11</td>
<td>107±14</td>
<td>103±19</td>
<td>0.922</td>
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<tr>
<td>Right atrial pressure, mm Hg</td>
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<td>6±3</td>
<td>6±2</td>
<td>5±3</td>
<td>6±2</td>
<td>0.933</td>
</tr>
<tr>
<td>Left atrial pressure, mm Hg</td>
<td>12±4</td>
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<td>14±2</td>
<td>13±1</td>
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<tr>
<td>Heart rate, beats/min</td>
<td>127±28</td>
<td>123±24</td>
<td>119±16</td>
<td>116±17</td>
<td>123±21</td>
<td>0.921</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SD.
vasodilation, total MVR decreases by about 67%, with the arteriolar resistance decreasing by approximately 85%. Consequently, arterioles contribute only 25% to the total MVR during hyperemia. Because capillaries lack smooth muscle, their resistance is not affected by adenosine, and thus, they contribute the most (about 75%) to total MVR during maximal hyperemia. The Hagen-Poiseuille’s equation states that flow (Q) in a tube can be calculated as follows: 

\[ Q = \frac{8 \cdot \pi \cdot r^4 \cdot \Delta P}{8 \cdot l \cdot \eta} \]

where \( l \) and \( r \) are the length and radius of the tube, respectively, and \( \eta \) is viscosity of the fluid flowing through the tube. By analogy to Ohm’s law, the total resistance to flow (R) is given by \( \Delta P/Q \) or \( Q=\Delta P/R \). By combining the two equations we get \( R = \frac{(8 \cdot l \cdot \eta)}{(\pi \cdot r^4)} \). That is, total resistance equals the product of vascular resistance and viscosity. In large vessels (>30 \( \mu \)m in diameter), vascular resistance is the major determinant of R, with viscosity playing a minor role. In vessels <30 \( \mu \)m in diameter, however, viscosity assumes a greater role, with relative effective viscosity increasing 6 to 7 fold at the level of the capillaries. Because the effect of vascular resistance and viscosity are multiplicative, small changes in viscosity produce a large difference in R. Furthermore, it has also been demonstrated that unlike glass tubes, R in the same sized capillaries is almost 2-fold higher for the same blood hematocrit probably from the interaction between the irregular vessel lining and blood components. A number of studies have shown an increase in blood viscosity with hyperlipoproteinemia. A strong positive correlation between increased blood viscosity and coronary artery disease (CAD) has also been reported. Several studies have shown abnormal CBF reserve in patients with CAD risk factors in the absence of CAD on angiography. Furthermore, it has been shown that the use of lipid lowering drugs (especially statins) can normalize abnormal CBF reserve without affecting coronary artery morphology. Thus, it is possible that in hyperlipoproteinemia, reduced CBF reserve may lead to repeated episodes of exercise-induced ischemia, which may ultimately have a detrimental effect on microvascular and myocyte integrity.

Other than hyperviscosity itself, increased lipid levels in blood can also affect RBCs directly. Because they have a lipid membrane, it is possible that an altered lipid milieu may affect the rigidity of RBC membranes and, thus, their deformability. Because RBC deformability is another major determinant of R in capillaries, lipid-lowering drugs have been found to favorably affect RBC rheology within the capillaries via this mechanism. The transient reduction in the vasodilatory capacity of peripheral or coronary arteries in response to a fatty meal has been construed as a defect in endothelial function. Two points need to be emphasized in this regard. First, large vessel dilatation is usually induced by changes in microvascular flow and not vice versa; that is, adenosine has no direct effect on large vessels and any change in their dimension is due to an increase in flow mediated at a more distal level. Second, because the capillaries are the bottleneck to flow during hyperemia, no amount of direct large vessel dilatation will increase flow to tissue if capillary R is increased. Therefore, the increased capillary R caused by increased blood viscosity can explain the reduction in hyperemic flow to tissue as well as the lack of flow-mediated vasodilation in larger vessels.

In our study, the coronary bed was fully vasodilated before serum lipid levels were increased. No net change in CBV (volume of blood in the entire LAD circulation, including the LAD itself, its branches, arterioles, capillaries, and venules) was found. Furthermore, no change in MBV fraction of the LAD bed (90% of which is composed of blood in capillaries) was seen. Therefore, even high levels of lipids had no net effect on large or small vessel dimensions in the presence of ischemia.
maximal hyperemia. The reduction in hyperemic flow was explained solely on the basis of reduced RBC velocity, which likely resulted from increased whole blood viscosity and/or reduced RBC deformability in the presence of hyperlipidemia. These findings were confirmed by direct observation of the hamster cremaster muscle microcirculation.

Critique of our Methods

Microbeads behave like RBCs in the microcirculation. They are also pure intravascular tracers, with their concentration in tissue reflecting MBV. To account for any changes in blood microbubble concentration that may have developed despite using a constant infusion, background-subtracted AI in the myocardium was normalized to that within the LV cavity. We also used a dose of microbeads that lies within the linear relation between AI and microbubble concentration.

Our purpose was not to ascertain the effect of hypertriglyceridemia per se on CBF reserve, but to study the effect of blood viscosity. Intralipid was a convenient means of changing blood viscosity. Achieving a wide range of blood viscosity levels would not have been as easy with hyperglycemia or hypercholesterolemia.

Our results do not imply that endothelial dysfunction does not occur with hyperlipidemia. All we have shown is that the abnormal CFR during hyperlipidemia is not primarily due to abnormalities in large vessel vasomotion, but rather to increased blood viscosity that is an important determinant of tissue blood flow. Intralipid may simply be concurrent epiphenomena, or even conceivably result, in part, from increased blood viscosity.

Finally, we used an acute model that may not be entirely relevant in chronic CAD. It, however, allowed us to study physiological changes directly in the heart. Despite possible adaptations to chronic hyperlipidemia that might occur in chronic CAD, the biophysical principles that govern tissue flow will still hold, and thus, blood viscosity will surely play an important role in the attenuation of CBF reserve. It may be for this reason that statins and other lipid lowering drugs can have a marked beneficial effect in patients with CAD without producing a major change in coronary artery morphology.

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References


![Figure 6. Velocity of 500 nm fluorescent particles at baseline, during topical adenosine, and after administration of Intralipid during topical adenosine. See text for details.](image-url)


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