A Kinetic Model of the Circulatory Regulation of Tissue Plasminogen Activator During Exercise, Epinephrine Infusion, and Endurance Training

By Wayne L. Chandler, Wayne C. Levy, Richard C. Veith, and John R. Stratton

A computer simulation of the circulatory system was used to kinetically model secretion, inhibition, and clearance of tissue plasminogen activator (t-PA) during three different processes that increase active t-PA levels: epinephrine infusion, exercise, and endurance training. Infusion of epinephrine stimulated an increase in t-PA secretion that was proportional to the plasma epinephrine concentration. In addition, epinephrine infusion increased hepatic blood flow and t-PA clearance, thus slowing the increase of plasma t-PA levels. During exercise, t-PA levels increased due both to increased t-PA secretion and to decreased clearance secondary to reduced hepatic blood flow. The increase in t-PA secretion during exercise was directly proportional to the epinephrine concentration in blood with the same ratio of t-PA secretion to epinephrine as found during epinephrine infusion. During epinephrine infusion, suggesting that increased plasma epinephrine during exercise was the primary stimulus for t-PA secretion. Lastly, the simulation predicted that 6 months of endurance training produced a decrease in resting plasminogen activator inhibitor type 1 (PAI-1) secretion, resulting in less t-PA inhibition and an overall increase in active t-PA after training. Accurate analysis of the regulation of active t-PA levels in blood required simultaneous modeling of t-PA and PAI-1 secretion, hepatic clearance, and inhibition of t-PA by PAI-1.

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Materials.
Human glu-plasminogen, goat antihuman–t-PA IgG and its peroxidase conjugate, and one-chain melanoma-derived t-PA (512,000 IU/mg) were obtained from American Diagnostica Inc (Greenwich, CT), Human fibrinogen, cyanogen bromide (CNBr), and Triton-X-100 were obtained from Sigma Chemical Co (St Louis, MO). CNBr-cleaved human fibrinogen fragments were prepared as previously described. Chromogenic substrate D-valyl-phenyl alanly-lysyl-p-nitro-anilide (S-2390) was obtained from Kabi Vitrum Inc (Franklin, OH). Microtiter plates precoated with goat antihuman t-PA and monoclonal antihuman PAI-1 peroxidase conjugate were obtained from Biopool Inc (Norcross, GA). All other materials not described below were reagent or laboratory requirements included a normal hematocrit, fasting blood glucose, total cholesterol, resting and exercise electrocardiogram including stress redistribution tomographic thallium imaging in subjects over the age of 60 years, and M-mode and two-dimensional echocardiogram.

Exercise and infusion protocol. Epinephrine infusion and exercise studies were performed as previously described on nine healthy males (average age, 27 ± 3 years). All studies were performed in the

From the Department of Laboratory Medicine, the Division of Cardiology, Department of Medicine, the Geriatric Research, Education and Clinical Center, and the Department of Psychiatry and Behavioral Science, University of Washington and Seattle VA Medical Center, Seattle.

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Address reprint requests to Wayne L. Chandler, MD, Department of Laboratory Medicine, SB-10, University of Washington, Seattle, WA 98195.

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late morning and early afternoon. Two intravenous lines were placed in the right arm. Epinephrine was infused into the antecubital vein while blood samples were obtained from a hand or wrist vein. The hand was warmed before obtaining the venous sample as previously described. For the epinephrine infusion data, baseline samples were taken after 30 minutes of supine rest just before beginning the infusion. Samples were then obtained after infusion of 10, 25, and 50 ng/kg/min of epinephrine for 10 minutes each. Again, for the exercise data, baseline samples were taken after another 30 minutes of supine rest period just before starting the exercise. Samples were taken at the end of each 3-minute supine bicycle exercise stage, which began at 33 W and increased by 33-W steps until exhaustion.

**Table 1. Resting Plasma Volume Assumptions in the Circulatory Model**

<table>
<thead>
<tr>
<th>Location</th>
<th>% Total Plasma Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiopulmonary circulation</td>
<td></td>
</tr>
<tr>
<td>Thoracic vena cava</td>
<td>6.0</td>
</tr>
<tr>
<td>Right heart</td>
<td>3.6</td>
</tr>
<tr>
<td>Pulmonary arteries</td>
<td>2.6</td>
</tr>
<tr>
<td>Pulmonary capillaries</td>
<td>2.2</td>
</tr>
<tr>
<td>Pulmonary veins</td>
<td>4.0</td>
</tr>
<tr>
<td>Left heart</td>
<td>3.8</td>
</tr>
<tr>
<td>Thoracic aorta</td>
<td>3.0</td>
</tr>
<tr>
<td>Splanchnic circulation</td>
<td></td>
</tr>
<tr>
<td>Splanchnic arteries</td>
<td>2.0</td>
</tr>
<tr>
<td>Splanchnic capillaries</td>
<td>1.5</td>
</tr>
<tr>
<td>Portal and splanchnic veins</td>
<td>12.5</td>
</tr>
<tr>
<td>Hepatic sinusoids</td>
<td>2.0</td>
</tr>
<tr>
<td>Hepatic veins</td>
<td>2.0</td>
</tr>
<tr>
<td>Systemic circulation</td>
<td></td>
</tr>
<tr>
<td>Inferior aorta and arteries</td>
<td>9.2</td>
</tr>
<tr>
<td>Systemic capillaries</td>
<td>4.3</td>
</tr>
<tr>
<td>Systemic veins</td>
<td>41.5</td>
</tr>
</tbody>
</table>

**Fig 1.** Schematic of human circulatory model. Open boxes indicate large arteries or veins. Shaded boxes indicate capillary beds or hepatic sinusoids in which t-PA and PAI-1 secretion occurs. The area of each box is proportional to the plasma volume at rest in that segment. TPF, total plasma flow in mL/s; SPF, splanchnic plasma flow; SysPF, systemic plasma flow. Note that the systemic circulation in the model consists of all the noncardiopulmonary, nonsplanchnic circulatory beds, ie, cerebral, renal, extremity, etc.

**Endurance training protocol.** To study the effects of endurance training on fibrinolytic variables, 12 healthy elderly male subjects participated in 6 months of vigorous supervised training as previously described. In brief, elderly subjects aged 60 to 82 years exercised four to five times per week beginning at 50% to 60% of heart rate reserve and increasing to 80% to 85% by 4 months. After 6 months of exercise training, the subjects showed a significant, 21% increase in their maximum oxygen uptake, indicating that a substantial training effect had occurred. Samples for fibrinolytic studies were obtained in the morning at rest at least 48 hours after the last period of exercise.

**Blood sampling and sample preparation.** Blood for fibrinolytic studies was anticoagulated by the addition of 1.8 mL whole blood to 0.2 mL of 130 mmol/L sodium citrate. To stabilize t-PA activity, 0.5 mL of citrate anticoagulated whole blood was mixed with 0.25 mL of 0.5 mol/L sodium acetate, pH 4.2, within 1 minute after the sample was drawn. All samples were centrifuged for 10 minutes at 1,000g at room temperature. Plasma and acidified plasma were then removed and frozen at −80°C until analyzed.

**Catecholamine analysis.** For catecholamine analysis, 2.5 mL of whole blood was added to a prechilled tube containing 50 μL of 0.15 mol/L NaCl, 90 g/L EGTA, and 60 g/L reduced glutathione. Catecholamine samples were centrifuged at 4°C for 15 minutes at 900g. The plasma was removed and recentrifuged as before, and the supernatant removed and stored in tightly sealed plastic tubes at −80°C.

**t-PA activity assay.** t-PA activity was measured in acidified plasma using an amidolytic method as previously described. Results were initially expressed in international units (IU) by comparison with the second international t-PA activity standard (86/670) from the National Institute for Biologic Standards and Control (NIBSC; London, UK). t-PA activity results were converted into molar concentrations of active t-PA using a specific molar activity.
of $4.48 \times 10^{12}$ IU/mol. Molar concentrations of all proteins were corrected for hemocconcentration based on changes in total protein levels in plasma. The interassay imprecision for active t-PA was 20.5 ± 1.4 (SD) pmol/L (CV = 7%).

**Active PAI-1 assay.** PAI-1 activity was measured using a back titration assay with active one-chain t-PA as previously described.\(^\text{17}\)

Active PAI-1 levels in activity units (AU/mL) were converted to molar concentrations using a specific molar activity of $4.48 \times 10^{13}$ IU/mol.\(^\text{3}\) The interassay imprecision of the active PAI-1 assay was 8 ± 5 (SD) pmol/L (CV = 10%).

**Total t-PA assay.** Total t-PA antigen was determined using an enzyme-linked immunosorbent assay (ELISA) as previously described.\(^\text{18-20}\) The interassay imprecision for total t-PA was 90 ± 9 (SD) pmol/L (CV = 10%).

**t-PA/PAI-1 complex assay.** t-PA/PAI-1 complex antigen was measured using an ELISA assay as previously described.\(^\text{14}\) The interassay imprecision for t-PA/PAI-1 complex was 164 ± 17 (SD) pmol/L (CV = 10%).

**Epinephrine assay.** Epinephrine was measured using a single isotope radioenzymatic assay as previously described.\(^\text{21}\)

**Circulatory model.** The effects of secretion, clearance, and inhibition reactions on the concentrations of active t-PA, active PAI-1, t-PA/PAI-1 complex, and total t-PA (active t-PA plus complex) were modeled throughout a simplified human circulatory system (Fig 1). The model circulatory system consisted of a cardiopulmonary circulation that split into splanchnic and systemic circulations. The systemic circulation consisted of all the noncardiopulmonary, nonsplanchnic circulatory beds, ie, cerebral, renal, extremity, etc.

**Blood volumes.** Estimates of total resting blood volume were based on weight, assuming a total blood volume of 5 L for a 70-kg male. Estimated resting blood volumes for different organs are shown in Table 1 expressed as a percentage of the total blood volume.\(^\text{22,23}\)

In this model of healthy subjects, we assumed there was no change in cardiopulmonary blood volume during exercise or epinephrine infusion.\(^\text{1,12,22}\) Low-dose epinephrine infusion was assumed to have no effect on splanchic blood volume.\(^\text{22}\) During exercise, splanchnic blood volume in the model remained unchanged at the resting value up to a heart rate of 100 beats/min, after which the splanchnic blood volume was given by:

$$\text{Exercise SBF} = \text{Resting SBF} - (0.35 \times \text{HR}[100]) + 1.35$$

where SBF is splanchnic blood flow.\(^\text{3,11}\) Systemic blood volume in the model increased by an amount equal to the decrease in splanchnic blood volume.

As fibrinolytic factors are in the plasma fraction and not in red blood cells, the model simulated secretion, inhibition, and clearance of fibrinolytic factors in the plasma only. Resting plasma volume was estimated from total blood volume and resting hematocrit.

**Blood flow rates.** During exercise, splanchnic blood flow is inversely proportional to oxygen uptake, cardiac output, and heart rate.\(^\text{11,12}\) In this simulation, the splanchnic circulation at rest received 25% of total resting blood flow, whereas the systemic circulation received the remaining 75%.\(^\text{11}\) During exercise, splanchnic blood flow in the model remained unchanged at the resting value up to a heart rate of 100 beats/min, after which the splanchnic flow decreased linearly to 43% of the resting value at a heart rate of 171 beats/min. Splanchnic flow based on heart rate was given by:

$$\text{Exercise SBF} = \text{Resting SBF} - (0.803 \times \text{HR}[100]) + 1.803$$

where SBF is splanchnic blood flow.\(^\text{3,11}\) Systemic blood flow in the model was equal to total blood flow minus splanchnic flow.

Infusion of epinephrine at the doses used in this study (10 to 50 ng/kg/min) results in an increase in splanchnic blood flow that is proportional to the change in cardiac output; the percentage of total blood flow to the splanchnic bed is essentially unchanged.\(^\text{9}\) In contrast, higher infusion rates (100 ng/kg/min) may increase both absolute and percentage splanchnic blood flow.\(^\text{10,26}\) During epinephrine infusion in this model, splanchnic blood flow was set to 25% of the total cardiac output.

**Fibrinolytic model.** The fibrinolytic system of the model contained only three proteins: active t-PA, active PAI-1, and t-PA/PAI-1 complex. The molar concentration of these proteins was regulated by three processes: t-PA and PAI-1 secretion, t-PA inhibition by PAI-1 forming t-PA/PAI-1 complex, and clearance of t-PA, PAI-1, and t-PA/PAI-1 complex by the liver. Total t-PA was equal to active t-PA plus complex t-PA.

**t-PA is secreted by endothelial cells.**\(^\text{27}\) The fibrinolytic model was simplified by assuming that: (1) t-PA was secreted only by vascular endothelial cells; (2) essentially all of the endothelium was located in three capillary beds (the pulmonary, splanchnic, and systemic capillary beds); and (3) all capillary endothelial beds secreted t-PA at the same rate.

PAI-1 is produced by human hepatocytes and human endothelium in culture.\(^\text{36-38}\) Juhan-Vague et al\(^\text{31}\) studied PAI-1, insulin, and acute-phase proteins and concluded that the majority of active PAI-1 was produced by the liver. Others have shown that human liver secretes PAI-1 into the blood.\(^\text{22}\) Most studies on venous occlusion concluded that the vascular endothelium of the upper and lower extremities does not contribute significantly to the basal level of active PAI-1 in the blood of healthy subjects,\(^\text{3,32,34}\) but this finding is
Clearance (L min⁻¹) = E × Q (L min⁻¹)  

where E is the hepatic extraction fraction and Q is the hepatic plasma flow rate.³⁹,⁴⁰ In humans, the hepatic extraction fraction for t-PA is approximately 55%, equivalent to a hepatic clearance half-life of about 4 minutes.⁶,³²,³⁸,⁴¹ Brommer et al.³² reported a slightly lower hepatic extraction fraction of 42% for total t-PA antigen. As t-PA/PAI-1 complex makes up the majority of total t-PA antigen in blood,³ this suggests that t-PA/PAI-1 complex is cleared at a slightly slower rate than active t-PA. In this model, we assumed hepatic extraction fractions of 55% for active t-PA and 42% for t-PA/PAI-1 complex. This clearance model was validated (see Results) by simulating the t-PA clearance study of de Boer et al.⁷

Estimation of hepatic PAI-1 clearance was more difficult. Active PAI-1 is secreted by the liver, cleared by the liver, and cleared by reaction with active t-PA forming t-PA/PAI-1 complex.³ In human, rabbit, and rat models, clearance of PAI-1 by the liver is much slower than clearance of either active t-PA or t-PA/PAI-1 complex.³³,⁴²,⁴³ Although precise data on the rate of active PAI-1 clearance by the liver were not available, the hepatic extraction fraction has been estimated to be on the order of 10%.³³,⁴² For this model it was assumed that E = 0.10 for active PAI-1.

Secretion, clearance, and inhibition reaction kinetics. t-PA is secreted by the vascular endothelium. More than 95% of all endothelium is located in the capillary beds.⁶,⁲² In large arteries and veins the ratio of blood volume to endothelial surface area is high.²²,²³ It was assumed that no secretion of t-PA or PAI-1 occurs in large vessels; the only changes in concentration were due to the reaction between t-PA and PAI-1. For example, in a large vessel:

\[
d_{[t-PA]} = -k_1 [t-PA][PAI-1] 
\]

where k₁ is the second-order rate constant for the reaction.

In the capillary beds of the lung, splanchnic, and systemic circulation, changes in concentration occur due both to t-PA secretion and to the inhibition reaction. Using t-PA as an example again:

\[
d_{[t-PA]} = -k_1 [t-PA][PAI-1] + S_{t-PA} 
\]

where S_{t-PA} is the t-PA secretion rate in pmol/L/s. In this report, t-PA and PAI-1 secretion rates are given as the rate averaged over the entire plasma volume; in the actual simulation, local capillary rates were used.

In the liver sinusoids, changes in concentration occur due to the secretion of active PAI-1 and the reaction between PAI-1 and t-PA, which is followed by clearance of the appropriate fraction of each factor as the plasma flowed into the hepatic veins.

Computer simulation. During the simulation, cardiac outputs in the model were set to the actual measured cardiac output for each subject. To begin a simulation, the resting concentrations for active t-PA, active PAI-1, and t-PA/PAI-1 complex were loaded into all segments of the model circulatory system. During each iteration, the change in concentration of each reactant due to secretion, inhibition, and clearance was determined for each segment of the model as described above. A fourth-order Runge-Kutta method was used to approximate the solution of these kinetic equations over time for each segment in the model circulatory system.³¹ Based on the measured cardiac output, the appropriate plasma volume then "flowed" out of one segment into the next segment downstream in the circulation while an equivalent amount "flowed" into the segment from upstream. When plasma flow was completed, the next iteration of secretion, inhibition, and clearance was started.

The time interval between iterations was set so that a maximum of 50% of the blood moved out of the smallest vascular segment (about 0.15 L in the pulmonary capillaries) into the next segment during each iteration. As maximum cardiac output among the nine subjects during exercise and epinephrine infusion was 0.65 L/s, an
and exercise (Table 2). To validate our clearance model, we compared measured versus simulated levels of active and total t-PA (plus complex) were unchanged during exercise and epinephrine infusion. The model predicted an average 0.01% change in the plasma t-PA levels if clearance or secretion were incorrectly held constant. For example, the simulation predicted a steady-state total t-PA level of 192 ng/mL (2,952 pmol/L) at rest and 373 ng/mL (5,732 pmol/L) during exercise. These predictions compared well with the total t-PA antigen levels of 193 ± 69 ng/mL at rest and 376 ± 117 ng/mL during exercise reported by de Boer et al. The close agreement between predicted and measured total t-PA levels indicated that the clearance model in the simulation accurately reflected changes in clearance seen during exercise in the study by de Boer et al.

Effect of epinephrine infusion and exercise on t-PA secretion and clearance. We simulated the infusion of epinephrine and exercise individually for each of the nine subjects in the study. Figure 2 shows an example of a complete cardiovascular and fibrinolytic data set for one subject. Note that t-PA levels increased linearly with epinephrine infusion but increased exponentially with exercise level. Also note that the predicted t-PA secretion rates closely follow the pattern of plasma epinephrine levels. Averaged data for the entire group at baseline, maximum epinephrine infusion, and maximum exercise are given in Table 3.

Epinephrine infusion resulted in an increase in plasma epinephrine, cardiac output, heart rate, and t-PA. The predicted t-PA secretion rate was directly proportional to the plasma epinephrine concentration in all subjects. Figure 3 shows the predicted effect of alterations in t-PA secretion versus alterations in t-PA clearance on plasma t-PA levels during epinephrine infusion. The model predicted an average threefold increase in t-PA secretion during epinephrine infusion and a twofold increase in hepatic blood flow and, thus, t-PA clearance. Overall, the change in plasma t-PA levels during epinephrine infusion was due both to increased secretion of t-PA as well as an increase in hepatic clearance, which moderated the effect of increased secretion. The dotted lines in Fig 3 shows the model’s predictions of plasma t-PA levels if clearance or secretion were incorrectly held constant. For example, the simulation predicted that total t-PA levels would have been 63% ± 15% higher during epinephrine infusion if hepatic clearance were not increased.

### Table 3. Average Measured and Predicted Variables During Epinephrine Infusion and Exercise

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Epinephrine (60 ng/kg/min)</th>
<th>Baseline</th>
<th>Maximum Exercise</th>
</tr>
</thead>
<tbody>
<tr>
<td>Measured cardiovascular variables</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Cardiac output (mL/s)</td>
<td>78 ± 15</td>
<td>155 ± 38</td>
<td>104 ± 31</td>
<td>311 ± 84</td>
</tr>
<tr>
<td>2. Heart rate (beats/min)</td>
<td>56 ± 4</td>
<td>69 ± 9</td>
<td>62 ± 7</td>
<td>171 ± 14</td>
</tr>
<tr>
<td>Predicted cardiovascular variables</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Hepatic blood flow (mL/s)</td>
<td>19 ± 4</td>
<td>38 ± 9</td>
<td>19 ± 4</td>
<td>8 ± 3</td>
</tr>
<tr>
<td>Measured fibrinolytic variables</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. Active t-PA (pmol/L)</td>
<td>15 ± 8</td>
<td>48 ± 30</td>
<td>15 ± 8</td>
<td>88 ± 99</td>
</tr>
<tr>
<td>5. Active PAI-1 (pmol/L)</td>
<td>196 ± 98</td>
<td>161 ± 95</td>
<td>154 ± 69</td>
<td>*</td>
</tr>
<tr>
<td>6. Total t-PA (pmol/L)</td>
<td>95 ± 25</td>
<td>143 ± 38</td>
<td>85 ± 37</td>
<td>147 ± 76</td>
</tr>
<tr>
<td>7. Epinephrine (ng/L)</td>
<td>68 ± 27</td>
<td>668 ± 224</td>
<td>91 ± 50</td>
<td>893 ± 819</td>
</tr>
<tr>
<td>Predicted fibrinolytic variables</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8. t-PA secretion (pmol/L/s)</td>
<td>0.13 ± 0.03</td>
<td>0.38 ± 0.13</td>
<td>0.12 ± 0.04</td>
<td>0.55 ± 0.59</td>
</tr>
<tr>
<td>9. PAI-1 secretion (pmol/L/s)</td>
<td>0.20 ± 0.06</td>
<td>0.20 ± 0.06</td>
<td>0.16 ± 0.07</td>
<td>0.16 ± 0.07</td>
</tr>
</tbody>
</table>

Results are presented as the mean ± SD, n = 9. * Measurement of PAI-1 activity at the maximum exercise level was not accurate due to high active t-PA levels.

### Statistics

The secretion rates for t-PA and PAI-1 were adjusted to produce the best weighted least-squares fit between the concentration of fibrinolytic factors in the systemic venous circulation of the model and the measured concentrations of these factors in peripheral venous blood samples. Estimates of interassay impression were used to weight the least-squares fit. When a best fit between measured and simulated values was achieved, the output of the model was an estimate of the rate of t-PA and PAI-1 secretion under each set of conditions. For the endurance training simulations we compared measured versus simulated active t-PA, active PAI-1, and t-PA/PAI-1 complex levels as previously described. Rapid changes in active t-PA levels, particularly during the exercise experiments, made accurate measurement of t-PA/PAI-1 complex and active PAI-1 levels difficult. As total PAI-1 levels (active PAI-1 plus complex) were unchanged during exercise and epinephrine infusion, we held PAI-1 secretion constant at the baseline level and compared measured versus simulated levels of active and total t-PA. Group distributions are expressed as the mean ± 1 standard deviation (SD) unless otherwise noted. Group comparisons were made using Student’s t test.

### RESULTS

Validation of the clearance model. de Boer et al. measured t-PA levels and liver blood flow at rest and during constant exercise while infusing t-PA at a constant rate. Using the model described above, we simulated their exercise clearance study based on their results for body weight, heart rate, hepatic clearance, and t-PA infusion rate during rest and exercise (Table 2). To validate our clearance model, we compared their measured level of total t-PA during rest and exercise versus our simulated level. The simulation predicted a steady-state total t-PA level of 192 ng/mL (2,952 pmol/L) at rest and 373 ng/mL (5,732 pmol/L) during exercise. These predictions compared well with the total t-PA antigen levels of 193 ± 69 ng/mL at rest and 376 ± 117 ng/mL during exercise reported by de Boer et al. The close agreement between predicted and measured total t-PA levels indicated that the clearance model in the simulation accurately reflected changes in clearance seen during exercise in the study by de Boer et al.

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During exercise (Fig 2), epinephrine levels, t-PA levels, and the t-PA secretion rate remained near baseline until an exercise level of 100 to 133 W was reached, after which all three factors increased exponentially until exhaustion. Again during exercise, the predicted t-PA secretion rate was directly proportional to the plasma epinephrine concentration.

Figure 4 shows the predicted effect of t-PA secretion versus t-PA clearance on plasma t-PA levels during exercise.
The average predicted t-PA secretion rate increased fivefold during exercise whereas t-PA clearance decreased 50%. Overall, the simulation predicted that approximately 80% of the increase in the total plasma t-PA concentration during graded exercise was due to an increase in t-PA secretion whereas 20% of the increase was due to a reduction in clearance of t-PA by the liver as a result of lower hepatic blood flow.

The simulation predicted that the PAI-1 secretion rate remained at the baseline level during both epinephrine infusion and exercise. During epinephrine infusion, the predicted PAI-1 secretion rate ranged among subjects from 0.09 to 0.27 pmol/L/s, with an average of 0.16 ± 0.07 pmol/L/s. During the exercise study, which occurred later in the day, the predicted PAI-1 secretion ranged from 0.05 to 0.27 pmol/L/s, with an average of 0.16 ± 0.07 pmol/L/s.

**Relationship between plasma epinephrine and t-PA secretion.** Figure 5 shows a comparison of predicted t-PA secretion rates versus plasma epinephrine concentrations during epinephrine infusion and exercise. To simplify Fig 5, only the regression lines are shown for each subject, not the individual data points. All points were used in determining the regression lines. The average response is indicated by the dark line. In all nine subjects, the predicted t-PA secretion rate increased linearly with the epinephrine concentration during both exercise and epinephrine infusion. The average slope of t-PA secretion rate (pmol/L/s) versus the epinephrine concentration (μg/L) during epinephrine infusion was not significantly different than the slope during exercise (0.504 ± 0.116 and 0.668 ± 0.124 [SEM] pmol/s/μg, respectively; paired t-test, P = .34).

**Effect of endurance training on resting t-PA and PAI-1 secretion.** Figure 6 shows the effect of 6 months of endurance training on the predicted t-PA and PAI-1 secretion rates. The simulation predicted that 10 of 12 subjects showed a decrease in PAI-1 secretion, whereas 7 of 12 showed a slight decrease in t-PA secretion. The remainder showed no change or increases in secretion after endurance training. On average, the PAI-1 secretion rate marginally decreased from a baseline of 0.202 ± 0.093 [SD] pmol/L/s to 0.135 ± 0.064 pmol/L/s after endurance training (P = .056, paired t-test). Average t-PA secretion was unchanged (0.158 ± 0.073 to 0.139 ± 0.062 pmol/L/s, P = .4).

**DISCUSSION**

Three mechanisms play a role in regulating the level of active t-PA in blood: secretion of t-PA, inhibition by PAI-1, and hepatic clearance. Prior studies evaluated each of these mechanisms separately, providing estimates of the average rate of t-PA secretion, the rate of reaction between t-PA and PAI-1, and the rate of t-PA clearance by the liver. We used these individual results to study how the different regulatory mechanisms interact when combined into a single kinetic model of the circulatory regulation of t-PA. Using this model, we studied three processes known to result in higher levels of active t-PA: graded epinephrine infusion, exercise to exhaustion, and 6 months of endurance training.

Epinephrine infusion at 50 ng/kg/min stimulated an average 193% increase in the predicted t-PA secretion rate compared with baseline. This rapid increase in secretion was opposed by an increase in the hepatic clearance of t-PA secondary to the epinephrine-induced increase in cardiac output and hepatic blood flow. The model would have predicted t-PA secretion rates approximately 39% lower during epinephrine infusion if changes in clearance had not been included in the simulation.

Clearance also played a role in determining plasma t-PA...
levels during exercise. The simulation predicted that increased epinephrine during exercise stimulated a fourfold increase in t-PA secretion at the point of exhaustion. However, in contrast to epinephrine infusion, exercise is known to reduce hepatic blood flow, which decreases t-PA clearance. The simulation predicted that 81% ± 14% of the increase in t-PA during exercise was due to increased t-PA secretion, whereas approximately 19% of the increase in t-PA was due to reduced clearance.

Inhibition of t-PA by PAI-1 was important in analyzing both epinephrine infusion and exercise. There were three-fold variations in the basal rate of PAI-1 secretion among different subjects, leading to substantial differences in the amount of secreted t-PA that was inhibited. Furthermore, the average basal rate of PAI-1 secretion was 20% lower during exercise as compared with epinephrine infusion. This reduction in PAI-1 secretion during exercise may be due in part to the circadian rhythm of PAI-1; on average, the exercise experiments were performed about 2 hours after the epinephrine infusion study.

Regulation of PAI-1 levels was also important during endurance training. The simulation predicted that endurance training reduced resting PAI-1 secretion approximately 33% in older males without changing t-PA secretion rates significantly. Before training, the PAI-1 secretion rate was predicted to be 28% higher than the t-PA secretion rate. After training, the PAI-1 secretion rate was 3% lower than the t-PA secretion rate. The reduction in PAI-1 secretion resulted in less t-PA being inhibited. The overall effect was an increase in the active t-PA level as a result of training.

The importance of including all regulatory mechanisms was seen in evaluating the effect of epinephrine levels on t-PA secretion rates. Epinephrine infusion rapidly stimulated the release of t-PA at a rate directly proportional to the concentration of epinephrine in plasma. Therefore, it was likely that other processes that increase epinephrine in the blood would also lead to an increase in t-PA secretion. To test this assumption we evaluated t-PA secretion and epinephrine levels during exercise to exhaustion. In a previous descriptive study we found that plasma t-PA levels increased linearly with the plasma epinephrine concentration during exercise but at twice the rate observed during epinephrine infusion. This finding suggested that factors other than epinephrine-induced t-PA secretion were increasing plasma t-PA levels during exercise. When changes in clearance and PAI-1 inhibition were taken into account in the model presented here, the predicted rate of t-PA secretion versus plasma epinephrine concentration during exercise was not significantly different than the secretion rate versus epinephrine level during epinephrine infusion. In other words, within the limits of this model, the increase in t-PA secretion during exercise was entirely due to stimulation by epinephrine. This finding was supported by recent studies showing that pretreatment with β-blockers reduced t-PA release during exercise, suggesting the response was β adrenergically mediated. The model showed that the more rapid increase in plasma t-PA levels during exercise versus epinephrine infusion was due to differences in t-PA clearance rates.

Although it is possible that factors other than epinephrine play a small role in stimulating t-PA secretion during exercise, our model suggests that these factors account for less than 10% of the total. Recent studies indicate that hypoxia-, acidosis-, or exercise-induced increases in norepinephrine, vasopressin, or lactate do not significantly stimulate t-PA secretion.

We conclude that all three mechanisms (secretion, inhibition, and clearance) must be taken into account in analyzing the in vivo regulation of active t-PA levels in blood. This conclusion is particularly true when rapid changes in fibrinolytic activity are occurring. In the basal state, the fibrinolytic system is a relatively stable steady-state system showing slow circadian changes principally due to variations in PAI-1 secretion. The fibrinolytic system is not always stable though. As shown in this study, it is capable of rapid increases in t-PA activity. Due to the dynamic nature of fibrinolytic regulation (rapid clearance and continuous inhibi-
tion), rapid increases in active t-PA do not last long; steady-state levels return within 20 to 30 minutes after the t-PA secretion stimulus is removed.

While useful, this kinetic model has several limitations. At the time of this study, there was no way to both block the reaction between t-PA and PAI-1 and still measure PAI-1 activity later, making it difficult to estimate in vivo active PAI-1 levels at the end of exercise and the epinephrine infusions when active t-PA levels in the sample were high. As there was no evidence of increased release of active PAI-1 during epinephrine infusion or exercise, we held PAI-1 secretion constant at the baseline rate, which was estimated from samples obtained before the rapid changes in t-PA. As total t-PA antigen levels could be measured accurately, the simulation tracked both active t-PA and total t-PA (active plus bound) during the epinephrine infusion and exercise studies.

To improve the accuracy of the model, further regional measurements of t-PA and PAI-1 are needed. To be of use in kinetic models, future studies should measure the absolute level t-PA and PAI-1 activity using specific assays. Results from global assays such as the euglobulin lysis time are a combination of the level of activators and inhibitors. Results expressed as lysis times cannot be used in kinetic models as they cannot be converted to molar concentrations of a specific factor.

Lastly, further work is needed on t-PA inhibitors. Little is known about the quantitative levels of t-PA inhibition by CI inhibitor. Studies in rabbits indicate that human rt-PA is cleared with an \( \alpha \)-phase half-life of about 1.5 minutes, whereas rPAI-1 has an \( \alpha \)-phase half-life of 8.8 minutes. In humans, rt-PA has an \( \alpha \)-phase half-life of 4 minutes. If the ratio of PAI-1 to t-PA half-life is similar in humans and rabbits, the estimated half-life for PAI-1 in humans would be about 23 minutes, close to the value used in this model based on the work of Brommer et al.

Despite these limitations, the model provides useful insights into the complex and dynamic regulation of fibrinolytic activity in humans.

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A kinetic model of the circulatory regulation of tissue plasminogen activator during exercise, epinephrine infusion, and endurance training

WL Chandler, WC Levy, RC Veith and JR Stratton