Myocardial Uptake of $^{99m}$Tc-N-NOET and $^{201}$Tl During Dobutamine Infusion

Comparison With Adenosine Stress

Dennis A. Calnon, MD; Mirta Ruiz, MD; Gérald Vanzetto, MD, PhD; Denny D. Watson, PhD; George A. Beller, MD; David K. Glover, ME

**Background**—The myocardial uptake of $^{99m}$Tc-sestamibi is attenuated by dobutamine stress, resulting in underestimation of ischemia. N-Ethyl-N-ethoxy-dithiocarbamato-$^{99m}$Tc ($^{99m}$Tc-N-NOET) is a new $^{99m}$Tc-labeled perfusion agent that is highly extracted by the myocardium by a mechanism different from that defined for $^{99m}$Tc-sestamibi. We therefore hypothesized that $^{99m}$Tc-N-NOET uptake would not be attenuated by dobutamine and that $^{99m}$Tc-N-NOET uptake would be comparable to $^{201}$Tl uptake during dobutamine stress.

**Methods and Results**—In 28 open-chest dogs, after placement of a stenosis in the left anterior descending coronary artery that reduced flow reserve by $\geq$50%, adenosine (300 $\mu$g · kg$^{-1}$· min$^{-1}$; $n$=15) or dobutamine (2.5 to 30 $\mu$g · kg$^{-1}$· min$^{-1}$; $n$=13) was infused. During adenosine stress, the stenotic-to-normal activity ratio for $^{99m}$Tc-N-NOET was 0.55±0.05. The stenotic-to-normal flow ratio was 0.33±0.04 at the time of $^{99m}$Tc-N-NOET injection. During dobutamine stress, the stenotic-to-normal $^{99m}$Tc-N-NOET activity ratio was 0.63±0.04, comparable to the $^{201}$Tl activity ratio of 0.59±0.04. The stenotic-to-normal flow ratio was 0.47±0.04 at the time of $^{99m}$Tc-N-NOET and $^{201}$Tl injection. The relationship between $^{99m}$Tc-N-NOET uptake and blood flow was comparable for adenosine and dobutamine stress, with no evidence of attenuation of $^{99m}$Tc-N-NOET extraction by dobutamine.

**Conclusions**—In the presence of coronary stenoses that reduced regional flow reserve, the myocardial uptake of $^{99m}$Tc-N-NOET and $^{201}$Tl are closely proportional to blood flow during both adenosine and dobutamine stress, suggesting that the adverse effect of dobutamine on $^{99m}$Tc-sestamibi uptake is a tracer-specific phenomenon rather than a generalized effect. The clinical implication of this finding is that $^{99m}$Tc-N-NOET might be preferable to $^{99m}$Tc-sestamibi when used with dobutamine stress for detection of coronary stenoses. *(Circulation. 1999;100:1653-1659.)*

**Key Words:** imaging ▪ radioisotopes ▪ inotropic agents ▪ adenosine

For detection of coronary artery disease, the success of pharmacological stress myocardial perfusion imaging relies on 2 fundamental principles: (1) the pharmacological stressor must produce a blood flow disparity between myocardial regions supplied by normal and stenotic arteries, and (2) the radionuclide tracer must be distributed in the myocardium in proportion to blood flow. Therefore, selection of an optimal perfusion imaging protocol requires a thorough understanding of the unique properties of the available pharmacological stressors and radionuclide tracers and their potential for interactions with one another.

Until recently, it was believed that the myocardial uptake of a perfusion tracer was determined entirely by the intrinsic properties of the tracer and the myocardial blood flow distribution present at the time of tracer injection. However, we and others have shown that the myocardial uptake of $^{99m}$Tc methoxyisobutyl isonitrile ($^{99m}$Tc-sestamibi) is “stressor dependent,” with less favorable myocardial sestamibi uptake during dobutamine stress than during adenosine stress (uptake plateaus at flows of 1.0 mL·min$^{-1}$·g$^{-1}$ versus 2.0 to 2.5 mL·min$^{-1}$·g$^{-1}$, respectively). This observation has fueled speculation that other tracers might be preferable to $^{99m}$Tc-sestamibi for dobutamine stress myocardial perfusion imaging.

Among the available agents, $^{201}$Tl would be a rational choice as a first-line tracer for dobutamine stress perfusion imaging. However, $^{201}$Tl has well-known limitations as a perfusion imaging agent, including a long physical half-life (which limits the injectable dose) and a low photon energy, which results in less optimal image quality compared with that achieved with technetium-labeled tracers. N-Ethyl-N-ethoxy-dithiocarbamato-$^{99m}$Tc ($^{99m}$Tc-N-NOET) is a recently developed, technetium-labeled neutral lipophilic myocardial perfusion imaging agent that has favorable properties.

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From the Experimental Cardiology Laboratory, Cardiovascular Division, Department of Medicine, University of Virginia Health Sciences Center, Charlottesville. Dr Calnon is now at MidOhio Cardiology Consultants, Columbus, Ohio.

Reprint requests to David K. Glover, ME, Cardiovascular Division, Department of Medicine, Box 158, University of Virginia Health Sciences Center, Charlottesville, VA 22908. E-mail dglover@virginia.edu

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1653
myocardial uptake properties during dipyridamole-induced hyperemia and, like 201Tl, redistributes in the myocardium over time. Therefore, 99mTc-N-NOET would appear to be well suited for pharmacological stress perfusion imaging, because 99mTc-N-NOET combines the favorable myocardial kinetic properties of 201Tl with the favorable radiophysical properties of Tc-labeled tracers.

However, we hypothesized that the myocardial uptake of 201Tl and 99mTc-N-NOET might also be attenuated by dobutamine stress, reflecting a "generalized" (rather than a "tracer-specific") effect of dobutamine on tracer uptake. Therefore, the objectives of the present study were (1) to define the initial myocardial uptake of 99mTc-N-NOET and 201Tl during dobutamine stress in the presence of coronary artery stenoses and (2) by comparison to uptake during adenosine stress, to determine whether the dobutamine-induced attenuation of myocardial 99mTc-sestamibi uptake represents a tracer-specific phenomenon or a generalized effect.

### Methods

#### Surgical Preparation

Twenty-eight fasted adult mongrel dogs (mean weight, 27.4 kg) were anesthetized with sodium pentobarbital (30 mg/kg IV), tracheally intubated, and mechanically ventilated with room air (Harvard Apparatus) with a positive end-expiratory pressure of 4 cm H₂O. Arterial blood gases were monitored (model 158, Ciba-Corning) and maintained in the normal physiological range. The left femoral vein was cannulated with an 8F catheter for the administration of fluids, 99mTc-N-NOET, 201Tl, and sodium pentobarbital. Both femoral arteries were cannulated with 8F catheters and used for microsphere reference blood withdrawal. An additional 7F catheter was placed in the right femoral artery for arterial pressure monitoring. A 7F Millar high-fidelity pressure catheter was inserted into the left ventricle through an 8F sheath in the left carotid artery. The left external jugular vein was cannulated with an 8F catheter for administration of adenosine or dobutamine.

A left lateral thoracotomy was performed at the level of the fifth intercostal space, and the heart was suspended in a pericardial cradle. A flare-tipped catheter was inserted into the left atrium for pressure measurement and for the injection of radiolabeled microspheres. A snare ligature was placed loosely on a proximal portion of the left anterior descending coronary artery (LAD). Ultrasonic flow probes (T201, Transonic Systems, Inc) were placed on a more distal portion of the LAD and on the left circumflex coronary artery (LCx). Throughout each protocol, the ECG, arterial and left atrial pressures, LAD and LCx flows, and left ventricular pressure and its first time derivative (dP/dt) were monitored continuously and recorded on an 8-channel strip-chart recorder (model 7458A, Hewlett-Packard).

All experiments were performed with the approval of the University of Virginia Animal Research Committee and were in compliance with the position of the American Heart Association on the use of research animals.

#### Experimental Protocols

**Group 1: Myocardial Uptake of NOET During Adenosine Stress**

In 15 dogs, after instrumentation, microspheres were injected to determine baseline myocardial blood flow (Figure 1). The LAD was then occluded for 10 seconds, and the peak flow that followed was recorded as the normal reactive hyperemic response. The snare ligature was then adjusted to create an LAD stenosis that reduced the normal reactive hyperemic response by 50% without reducing resting flow. Microspheres were injected 15 minutes later to determine myocardial flow in the presence of the stenosis. Adenosine (300 μg · kg⁻¹ · min⁻¹ IV) was then infused for 5 minutes, with continuous monitoring of blood flow in the normal LCx to indicate the point of maximal adenosine-induced hyperemia. 99mTc-N-NOET (8 mCi, 296 MBq) and microspheres were injected simultaneously during maximal adenosine-induced hyperemia, and the adenosine infusion was terminated 1 minute later. The animals were euthanized with an overdose of sodium pentobarbital and potassium chloride 5 minutes after 99mTc-N-NOET injection, and regional myocardial blood flow and 99mTc-N-NOET activity were measured by gamma-well counting and ex vivo gamma-camera imaging.

**Group 2: Myocardial Uptake of 99mTc-N-NOET and 201Tl During Dobutamine Stress**

In 13 dogs, after baseline measurements were made and the LAD stenosis was created, dobutamine was infused intravenously in 3-minute dose increments of 2.5, 5, 10, 20, and 30 μg · kg⁻¹ · min⁻¹ (Graseby Medical infusion pump, model 3400). 99mTc-N-NOET (8 mCi, 296 MBq), 201Tl (0.75 mCi, 27.8 MBq), and microspheres were simultaneously injected at the peak 30-μg · kg⁻¹ · min⁻¹ dobutamine dose, and the dobutamine infusion was terminated 2 minutes later. Animals were euthanized 5 minutes after tracer injection (Figure 1).

#### Image Acquisition and Quantification of the Stenotic-to-Normal Count Ratio

Our ex vivo imaging protocol has been described previously. The hearts were divided into 4 concentric slices from apex to base, and these slices were placed directly on the collimator of a standard nuclear medicine gamma camera (Technicare 420, Ohio Nuclear). 99mTc-N-NOET images were acquired with an all-purpose, low-to-medium-energy collimator with a 20% window centered around the 99mTc photopeak and recorded with a 128×128 matrix for 4 minutes. In the dobutamine group (group 2), after at least 24 hours had passed (to allow for 99mTc decay), 201Tl images were acquired with a 25% window centered around the 201Tl photopeak. Image quantification was performed on a nuclear medicine computer (Sophia Medical Systems). No background subtraction, thresholding, or filtering was applied to the images. A region of interest (ROI) was drawn on the anteroseptal wall to represent the stenotic zone, and a second ROI was drawn on the normally perfused posterolateral wall. The stenotic-to-normal count ratio was calculated by dividing the counts per pixel in the stenotic ROI by the counts per pixel in the normal ROI. The stenotic-zone ROI was limited to an area of ~20% of the LV in the central stenotic zone. The normal-zone ROI was limited to ~20% of the LV in the area with maximal myocardial counts.

#### Determination of Regional Myocardial Blood Flow and Sestamibi Activity

The microsphere technique used in our laboratory has been described previously. To measure regional tracer activity and microsphere-determined blood flow, each of 4 left ventricular slices was divided...
into 6 transmural sections, which were then subdivided into epicardial, midwall, and endocardial segments. The resulting 72 myocardial tissue samples were counted in a gamma-well scintillation counter (MINAXI 5550, Packard Instruments) with standard window settings. The tissue counts were corrected for background, decay, and isotope spillover, and regional myocardial blood flow was calculated with computer software (PCGERDA, Scientific Computing Solutions, LLC). Blood flow and tracer activities for each of the 24 transmural sections were calculated as the weighted average of the 3 corresponding epicardial, midwall, and endocardial segments. The 5 transmural sections with the lowest flows at the time of tracer injection were defined as the stenotic region, and the 5 transmural sections with the highest flows were defined as the normal region. Stenotic-to-normal ratios for blood flow and tracer activities were calculated by dividing the average flow or tracer activity in the stenotic region by the average values in the normal region.

Statistical Analysis
All statistical computations were made with SYSTAT software (SYSTAT, Inc.). The results are expressed as the mean±SEM. Differences between means within a group were assessed by a repeated-measures ANOVA or by a paired t test as appropriate. Comparisons between groups were made with 1-way ANOVA and Tukey’s post hoc testing.

Results

Hemodynamics
As expected, mean heart rate (Table 1) increased only modestly during adenosine stress (121±5 to 134±5 bpm, P<0.05) but increased markedly during dobutamine stress (127±4 to 208±7 bpm, P<0.001). Similarly, peak positive left ventricular dP/dt (an index of global left ventricular contractility) increased only slightly during adenosine (1729±74 to 2140±105 mm Hg/s, P<0.001) and increased more during dobutamine (1950±84 to 6619±446 mm Hg/s, P<0.001).

Regional Myocardial Blood Flow
Myocardial blood flow was unchanged by placement of the LAD stenoses (Table 2). In the normal zone, endocardial, midwall, epicardial, and transmural flow increased significantly during adenosine and dobutamine infusion (P<0.001). Adenosine (300 μg · kg⁻¹ · min⁻¹) increased transmural flow in the normal zone by a factor of 4 to 5 times baseline resting flow, to a mean peak flow of 4.52±0.17 mL · min⁻¹ · g⁻¹. Dobutamine (30 μg · kg⁻¹ · min⁻¹) increased transmural flow in the normal zone by a factor of 2.5 to 3 times resting flow, to a mean peak flow of 2.50±0.28 mL · min⁻¹ · g⁻¹. These stressor-induced hyperemic flows were identical to those reported previously in these canine models. By design, flow reserve was significantly reduced in the stenotic zone, with a mean peak transmural flow of just 1.49±0.21 mL · min⁻¹ · g⁻¹ during adenosine stress and 1.18±0.17 mL · min⁻¹ · g⁻¹ during dobutamine stress, similar to the stenotic-zone flows reported previously in these models.

Stenotic-to-Normal Ratios for Myocardial Blood Flow and ⁹⁹mTc-N-NOET and ²⁰¹Tl Activity

Adenosine Stress
The left panel in Figure 2 depicts the mean stenotic-to-normal ratios for transmural myocardial flow at the time of ⁹⁹mTc-N-NOET injection during adenosine stress, with corresponding ⁹⁹mTc-N-NOET activity ratios by gamma-well counting and ex vivo imaging. A stenotic-to-normal image count ratio <0.75 is typically associated with visually detectable perfusion defects on clinical imaging. Although ⁹⁹mTc-N-NOET count ratios by gamma-well counting (0.55±0.05) and ex vivo imaging (0.58±0.04) underestimated the adenosine-induced blood flow disparity at the time of NOET injection (flow ratio=0.33±0.04; P<0.001), the NOET ratios easily exceeded the 0.75 threshold, suggesting that perfusion defects would be easily detectable on adenosine ⁹⁹mTc-N-NOET perfusion imaging. For comparison, the right panel in Figure 2 depicts the corresponding ²⁰¹Tl ratios during adenosine stress from previous studies in our laboratory using the same...
canine model. Note that $^{99m}$Tc-N-NOET uptake during adenosine stress in the present study is similar to that reported previously for $^{201}$Tl.

Dobutamine Stress

The left panel in Figure 3 depicts the mean stenotic-to-normal ratios for transmural myocardial flow at the time of tracer injections during dobutamine stress, with corresponding $^{201}$Tl and $^{99m}$Tc-N-NOET activity ratios by gamma-well counting and ex vivo imaging. Although the $^{201}$Tl and $^{99m}$Tc-N-NOET activity ratios by gamma-well counting (0.59 ± 0.04 and 0.63 ± 0.04, respectively) and ex vivo imaging (0.60 ± 0.05 and 0.57 ± 0.03, respectively) underestimated the dobutamine-induced blood flow disparity at the time of tracer injection (flow ratio = 0.47 ± 0.04; $P<0.001$), the ratios are again <0.75, implying that perfusion defects would be detectable on dobutamine perfusion imaging. The $^{201}$Tl and $^{99m}$Tc-N-NOET activity ratios observed during dobutamine stress in the present study are markedly different from the previously reported stenotic-to-normal activity ratio for $^{99m}$Tc-sestamibi (Figure 3, right panel). Despite an identical dobutamine-induced flow ratio of 0.47 ± 0.03 at the time of sestamibi injection, the $^{99m}$Tc-sestamibi activity ratios were much less favorable (0.82 ± 0.02 and 0.81 ± 0.03 by gamma-well and ex vivo imaging, respectively) and did not achieve the 0.75 detection threshold. Thus, $^{99m}$Tc-N-NOET uptake was not attenuated by dobutamine infusion as was previously observed with $^{99m}$Tc-sestamibi.

Relationship Between Myocardial Blood Flow and $^{201}$Tl and $^{99m}$Tc-N-NOET Uptake

Figure 4 is a scatterplot of normalized myocardial $^{99m}$Tc-N-NOET activity versus flow (normalized to 1 mL / min / g) at the time of $^{99m}$Tc-N-NOET injection during adenosine stress, plotted together with the mathematically derived curve relating flow and $^{201}$Tl and $^{99m}$Tc-sestamibi activity during adenosine stress in the same canine models. The curve fits are based on the solute transport model of Gosselin and Stibitz. Note that the myocardial uptake of $^{99m}$Tc-N-NOET

![Figure 2](http://circ.ahajournals.org/)

Figure 2. Mean stenotic-to-normal ratios for myocardial flow and $^{99m}$Tc-N-NOET activity during adenosine stress. For comparison, results from our previous studies of adenosine $^{201}$Tl are shown on right. Although both tracers slightly underestimated adenosine-induced blood flow disparity at time of tracer injection, stenotic-to-normal count ratios for both $^{99m}$Tc-N-NOET and $^{201}$Tl were <0.75, suggesting that perfusion defects would be readily detectable on perfusion imaging. * $P<0.001$ vs flow ratio.
and $^{201}$Tl is proportional to myocardial blood flow over a wide range of adenosine-induced hyperemic flows.

Figure 5 is a scatterplot of normalized myocardial $^{201}$Tl and $^{99m}$Tc-N-NOET activity versus flow (normalized to $1\text{ mL} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$) at the time of tracer injection during dobutamine stress, plotted together with the mathematically derived curve relating flow and $^{99m}$Tc-sestamibi activity during dobutamine stress in the same canine models. The data points represent 429 individual myocardial tissue samples from the present study. First, note that the myocardial uptake of $^{201}$Tl and $^{99m}$Tc-N-NOET during dobutamine stress (Figure 5) is nearly identical to that during adenosine stress (Figure 4), suggesting the absence of a stressor-dependent effect of dobutamine on the myocardial uptake of these tracers. In addition, note that the myocardial uptake of $^{201}$Tl and $^{99m}$Tc-N-NOET during dobutamine stress (Figure 5) is much more favorable than that of $^{99m}$Tc-sestamibi, with a plateau in $^{201}$Tl and $^{99m}$Tc-N-NOET uptake only at very high flow rates ($>4\text{ mL} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$), compared with the earlier plateau in $^{99m}$Tc-sestamibi uptake at flow rates of only $1\text{ mL} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$. During dobutamine stress, for any given level of hyperemic flow, there is relatively greater myocardial uptake of $^{201}$Tl and $^{99m}$Tc-N-NOET than uptake of $^{99m}$Tc-sestamibi.

**Discussion**

The major finding of this study was that in these canine models of coronary stenoses, the myocardial uptake of $^{201}$Tl and $^{99m}$Tc-N-NOET was favorable during both adenosine and dobutamine stress, implying the absence of a stressor-dependent effect of dobutamine on the myocardial uptake of these tracers. These findings suggest that the dobutamine-induced attenuation of myocardial $^{99m}$Tc-sestamibi uptake is a tracer-specific phenomenon rather than a generalized effect.
Effects of Adenosine and Dobutamine Stress on Myocardial Blood Flow

For purposes of myocardial perfusion imaging, the capacity to increase blood flow in normally perfused myocardium is the most important attribute of a pharmacological stressor, because flow in the stenotic zone is determined largely by the severity of the coronary stenosis. In the present study, adenosine increased blood flow in normal myocardium by a factor of 5 times baseline flow, and dobutamine increased blood flow by a factor of 2.5 to 3 times resting flow. The degree of hyperemia produced by both stressors is adequate for myocardial perfusion imaging, provided that the tracer selected is taken up by the myocardium in proportion to blood flow over the range of flows produced by the stressor.

Myocardial Uptake of $^{99m}$Tc-N-NOET and $^{201}$Tl During Adenosine and Dobutamine Stress

The myocardial uptake of all diffusable tracers is dependent on both myocardial blood flow and myocardial extraction of the tracer. The myocardial uptake of $^{99m}$Tc-sestamibi, a lipophilic cationic molecule, is thought to occur through electrical charge-driven diffusion across sarcolemmal membranes, with cellular retention in mitochondrial membranes due to the negative transmembrane potential. Dobutamine stress produces a less favorable relationship between myocardial blood flow and sestamibi uptake than that produced by adenosine stress in the same canine models, suggesting the presence of a stressor-specific adverse effect of dobutamine on myocardial sestamibi uptake. This attenuation of uptake yields poor perfusion defect contrast on dobutamine $^{99m}$Tc-sestamibi images. A possible explanation for this phenomenon is that the myocardial uptake of sestamibi is diminished by dobutamine-induced calcium influx, with blunting of the negative mitochondrial membrane driving potential due to mitochondrial calcium sequestration. Although the effect of dobutamine stress on the myocardial uptake of $^{99m}$Tc-tetrofosmin is unknown, an attenuation of $^{99m}$Tc-tetrofosmin uptake by dobutamine would be expected on the basis of the similar myocardial binding mechanisms of $^{99m}$Tc-tetrofosmin and $^{99m}$Tc-sestamibi.

In contrast, $^{99m}$Tc-N-NOET uptake during dobutamine stress in the present study yielded perfusion defects that more closely reflected the myocardial blood flow at the time of $^{99m}$Tc-N-NOET injection. This lack of attenuation of $^{99m}$Tc-N-NOET uptake with dobutamine stress is explained by a different mechanism of myocardial extraction of this neutral, lipophilic compound. Although the mechanism of myocardial uptake of $^{99m}$Tc-N-NOET remains incompletely defined, studies to date have suggested that $^{99m}$Tc-N-NOET binds to the hydrophilic cardiomyocyte cell membrane, with no significant accumulation in the cytosolic or mitochondrial compartments. The cellular binding of $^{99m}$Tc-N-NOET appears to be linked to the L-type calcium channel, and it is independent of cellular ATP content. After initial myocardial uptake, $^{99m}$Tc-N-NOET redistribution occurs via bidirectional exchange between red blood cells and the myocardium. Early clinical data suggest that exercise stress $^{99m}$Tc-N-NOET single photon emission CT perfusion imaging provides diagnostic information comparable to that by $^{201}$Tl, with evidence of myocardial redistribution evident on delayed $^{99m}$Tc-N-NOET images. To date, there are no clinical studies addressing the detection of coronary stenoses with dobutamine $^{99m}$Tc-N-NOET imaging.

The biokinetics of $^{201}$Tl in humans has been defined, and the clinical accuracy of dobutamine stress $^{201}$Tl perfusion imaging has been well established. The reported sensitivity for detection of CAD has ranged from 86% to 97%. The results of the present study suggest that $^{201}$Tl might be superior to $^{99m}$Tc-sestamibi for the detection of coronary stenoses during dobutamine stress, although the more favorable myocardial uptake properties of $^{201}$Tl must be weighed against the relatively unfavorable radiophysical imaging properties of $^{201}$Tl compared with $^{99m}$Tc-labeled agents.

Clinical Implications

The experimental data provided in the present study suggest that $^{99m}$Tc-N-NOET should be as accurate as $^{201}$Tl and superior to $^{99m}$Tc-sestamibi for the detection of coronary artery disease with dobutamine stress perfusion imaging. A clinical comparison is warranted.

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