Effects of Increased Lipid Concentration and Hyperemic Blood Flow on the Intrinsic Myocardial Washout Kinetics of ^{99m}TcN-NOET

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Bis(*N*-ethoxy,*N*-ethyldithiocarbamato)nitrido technetium (V) (^{99m}Tc) (^{99m}TcN-NOET) is a myocardial perfusion imaging agent demonstrating significant redistribution and currently in phase III clinical trials. Previous studies have suggested that ^{99m}TcN-NOET is bound intravascularly. Therefore, we sought to determine whether modifications in the vascular compartment would provide further insights into the mechanisms of ^{99m}TcN-NOET myocardial washout and redistribution. **Methods:** ^{99m}TcN-NOET cardiac washout was studied ex vivo in 15 isolated perfused rat hearts after bolus injection (1.5 MBq) in the absence ($n = 6$) or presence of bovine serum albumin ([BSA] 0.03%) with ($n = 5$) or without ($n = 4$) bound lipids. The intrinsic myocardial washout of the tracer was also studied in vivo in 6 dogs after intracoronary bolus injection of the tracer (0.75 MBq) before and after hyperlipidemia induced by intravenous administration of 300 mL of 20% intralipids ($n = 3$) or hyperemia induced by intravenous infusion of the adenosine A_{2A} receptor agonist ATL-146e (0.3 μg/kg/min; $n = 6$). **Results:** On isolated hearts, there was no significant myocardial washout of ^{99m}TcN-NOET with Krebs–Henseleit buffer. Addition of BSA without bound lipids resulted in a significant cardiac washout of the tracer ($P < 0.001$ by repeated measures ANOVA). The presence of lipids bound to BSA further accelerated the washout rate of ^{99m}TcN-NOET (half-life [$t_{1/2}$], 431.5 ± 23.2 min vs. 242.9 ± 63.2 min; $P < 0.05$). In vivo in dogs, intralipid administration significantly increased the intrinsic washout rate of ^{99m}TcN-NOET ($t_{1/2}$, 108.0 ± 23.9 min vs. 51.8 ± 11.8 min; $P < 0.05$). In addition, vasodilatation with ATL-146e resulted in a 4.9-fold increase in coronary flow ($P < 0.05$ vs. baseline) and a significantly faster intrinsic ^{99m}TcN-NOET myocardial washout ($t_{1/2}$, 81.1 ± 12.1 min vs. 40.7 ± 7.3 min; $P < 0.05$). **Conclusion:** The myocardial washout kinetics of ^{99m}TcN-NOET are affected by a variety of intravascular factors, supporting the hypothesis that the tracer is most likely localized on the vascular endothelium. The potential impact of variations in circulating lipid levels among patients on clinical imaging with ^{99m}TcN-NOET requires further investigation.

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Bis(*N*-ethoxy,*N*-ethyldithiocarbamato)nitrido technetium (V) (^{99m}Tc) (^{99m}TcN-NOET) is a neutral and lipophilic myocardial perfusion imaging agent (*1*) currently in phase III clinical trials in the United States and Europe. The relationship between the myocardial uptake of ^{99m}TcN-NOET and regional blood flow as well as the redistribution kinetics of this tracer have been shown to correlate very closely with that of ^{201}Tl in experimental and clinical studies (*2–4*) despite the very different chemical properties of the 2 tracers.

Indeed, whereas the cationic and hydrophilic tracer ^{201}Tl is believed to be taken up by the myocardium through energy-dependent mechanisms similar to that of potassium (*5*), there is indirect evidence that the neutral and lipophilic characteristics of ^{99m}TcN-NOET are responsible for nonspecific binding of the tracer to the vascular endothelium (*6–8*). These divergent mechanisms are likely responsible for the different patterns of ^{201}Tl and ^{99m}TcN-NOET myocardial uptake observed in the setting of acute reperfused myocardial infarction in vivo (*9*) and lead to the conclusion that ^{99m}TcN-NOET is not a technetium analog to ^{201}Tl.

Although the mechanisms for ^{201}Tl myocardial kinetics and redistribution are now well understood (*10–12*), little is known about the determinants of ^{99m}TcN-NOET myocardial kinetics in vivo. Johnson et al. have suggested that bidirectional transfer of ^{99m}TcN-NOET between the myocardium and blood elements (*7*) may play a role in the mechanism for the observed ^{99m}TcN-NOET redistribution, in accordance with the hypothesis of ^{99m}TcN-NOET binding on the vascular endothelial surface. In this study, we sought to further test this hypothesis by determining whether other modifications in the intravascular compartment—that is, lipid levels and coronary blood flow—would alter the myocardial washout kinetics of ^{99m}TcN-NOET and would pro-

vide further insights into the redistribution mechanisms of this tracer.

MATERIALS AND METHODS

Isolated Rat Heart Experiments

Buffers. Bovine serum albumin ([BSA] Sigma fraction V; A2153) was used. This albumin contains physiologic levels of fatty acids (FAs) (*13*) and is called BSA throughout this article. In some experiments, FAs were extracted from the BSA to give FA-free BSA by shaking 1 g of BSA for three 1-h periods with 10 mL of methanol and removing the methanol-soluble fraction each time after centrifugation (10,000*g* × 10 min at room temperature) (*14*). FA-free BSA was then vacuum-dried to remove methanol and used as described below for addition to Krebs–Henseleit (KH) perfusing buffer.

KH perfusing buffer containing (mmol/L) 118.0 NaCl, 25.0 $NaHCO_3$, 4.75 KCl, 1.19 $MgSO_4$, 1.18 KH_2PO_4, 1.5 $CaCl_2$, and 11.1 glucose was used. BSA (0.6 g) or FA-free BSA (0.6 g) was dissolved in 800 mL of KH buffer, dialyzed overnight against 4 L of KH buffer without BSA using a semipermeable membrane with a 6,000–8,000 molecular weight cutoff (Spectra/Por), and further diluted with 1.2 L of KH containing no BSA on the day of the experiment. The KH containing BSA or FA-free BSA was then filtered using 1.2-μm nitrocellulose filters (Millipore) immediately before use.

Experimental Apparatus and Protocol. Fifteen male Sprague–Dawley rats (300–400 g) were anesthetized with 25 mg (0.5 mL) intraperitoneal sodium pentobarbital. Heparin (100 U; 0.1 mL) was injected intravenouly through the saphenous vein and the heart was quickly removed and immersed into ice-cold KH buffer. Hearts were perfused via the aortic route according to the method described by Langendorff (*15*). Perfusion flow was held constant throughout all experiments at 12 mL/min without recirculation by means of a peristaltic pump (Masterflex L/S; Cole-Parmer Instrument Co.), which also removed sinus drainage. The perfusate was heated to 37°C and saturated with 95% O_2 and 5% CO_2. A soft plastic balloon catheter connected to a pressure transducer was inserted into the left ventricle and filled with saline to reach an end-diastolic pressure of 2–5 mm Hg. Coronary perfusion pressure (CPP) was also measured throughout each experiment by a similar pressure transducer connected to the perfusion line. Hearts were atrially paced at 300 beats per minute (bpm). CPP and systolic and diastolic pressures (mm Hg), heart rate (bpm), and maximal first derivative of left ventricular pressure (dP/dt_{max} [mm Hg/s]) were monitored and recorded on a personal computer throughout the entire perfusion period using data acquisition software (Dataflow; Coyotebay).

Hearts were perfused with KH ($n = 6$), KH + FA-free BSA 0.03% ($n = 4$), or KH + BSA 0.03% ($n = 5$). All experiments involved a minimum 10-min baseline period during which the hemodynamics of the hearts were allowed to stabilize. ^{99m}TcN-NOET was injected in the form of a 1.5-MBq bolus in 0.3 mL over a 10-s period through a side port located at the top of the perfusion cannula, and its myocardial activity was monitored for 60 min. At the end of the protocol, the hearts were removed from the cannula, dried of surface moisture, and then weighed. Next, the whole hearts were counted in a dose calibrator to measure final ^{99m}Tc activity.

Canine Experiments

Surgical Preparation. Six fasted, adult mongrel dogs (24.9 ± 1.5 kg; range, 17.3–31.8 kg) were anesthetized with sodium pentobarbital (30 mg/kg intravenously), tracheally intubated, and mechanically ventilated. Open-chest surgery and instrumentation were performed as described (*16*). A 22-gauge intracoronary catheter was inserted into the main left anterior descending coronary artery through a small side branch.

Influence of Increased Lipid Concentration on ^{99m}TcN-NOET Myocardial Kinetics. After baseline stabilization, 0.75 MBq ^{99m}TcN-NOET were injected into the intracoronary catheter, and the catheter was immediately removed. Twenty-five minutes later, hyperlipidemia was induced by a rapid intravenous infusion of 300 mL of 20% intralipids (Baxter Healthcare Corp.). Myocardial ^{99m}TcN-NOET activity was monitored continuously throughout both the normo- and hyperlipidemic phases. Arterial blood sampling was performed beginning 5 min before intralipid injection and at 5, 10, 20, and 30 min afterward. Serum triglyceride levels were assessed at the University of Virginia clinical laboratory.

Influence of Coronary Flow on ^{99m}TcN-NOET Myocardial Kinetics. After baseline stabilization, 0.75 MBq ^{99m}TcN-NOET were injected into the intracoronary catheter under resting flow conditions. Immediately after the injection, the catheter was removed. After 30 min of resting flow, an intravenous infusion of the adenosine A_{2A} receptor agonist ATL-146e (0.3 μg/kg/min) (*17*) was begun and continued for an additional 30 min. Myocardial washout of ^{99m}TcN-NOET was monitored for the entire 60-min period using the external NaI probe.

Preparation of ^{99m}TcN-NOET

^{99m}TcN-NOET was prepared as described (*2*) using kits provided by CIS Bio International, Schering SA. The preparation included the solubilizer dimethyl-β-cyclodextrin.

Tracer Kinetic Monitoring

In both isolated rat hearts and canine myocardium, ^{99m}TcN-NOET myocardial activity was monitored at 10-s intervals after injection using a lead-collimated NaI scintillation detector interfaced with a multichannel analyzer.

Data Analysis and Statistics

Results are presented as mean ± SEM. ^{99m}TcN-NOET initial myocardial activity was expressed as a percentage of the injected dose per gram of wet tissue (%ID/g). The individual tracer activity curves were normalized by expressing the serial counts as a percentage of the initial activity. Computations were performed with SYSTAT software (SPSS, Inc). Comparisons were performed using 1-way ANOVA and repeated measures ANOVA. When appropriate, post hoc testing was performed using Bonferroni tests. P values < 0.05 were considered significant.

RESULTS

Lipid Extraction from BSA

The amount of lipids extracted from the BSA after a 1-h incubation period with methanol decreased progressively from the first to the third period of incubation (10.2, 2.6, and 1.0 mg lipids per gram of BSA, respectively), indicating successful separation of FAs from BSA.

TABLE 1
Hemodynamic Parameters: Isolated Rat Heart Experiments

	Time after ^{99m}TcN-NOET injection (min)					
	Baseline	0	15	30	45	60
LVESP (mm Hg)						
Control (no BSA)	100.0 ± 4.6	103.1 ± 4.2	104.3 ± 4.8	102.1 ± 5.0	96.0 ± 3.3	97.6 ± 3.2
FA-free BSA	99.3 ± 7.4	93.5 ± 3.4	94.8 ± 4.0	98.2 ± 2.3	91.9 ± 1.4	94.8 ± 3.3
BSA	107.9 ± 5.8	104.5 ± 6.2	107.0 ± 6.6	99.1 ± 6.1	99.0 ± 4.2	100.2 ± 3.9
LVEDP (mm Hg)						
Control (no BSA)	4.5 ± 0.2	4.3 ± 0.3	3.5 ± 0.3	4.0 ± 0.3	3.2 ± 0.3	3.1 ± 0.3
FA-free BSA	3.8 ± 0.2	4.0 ± 0.4	4.8 ± 0.6	5.2 ± 0.5	5.2 ± 0.2*	5.0 ± 0.5†
BSA	5.3 ± 0.3	4.3 ± 0.2	4.5 ± 0.2	4.2 ± 0.4	4.3 ± 0.5	5.0 ± 0.5†
CPP (mm Hg)						
Control (no BSA)	35.0 ± 1.9	33.6 ± 1.4	35.2 ± 1.6	36.3 ± 2.1	35.3 ± 1.8	37.3 ± 1.6
FA-free BSA	32.4 ± 2.0	33.2 ± 2.7	33.3 ± 1.8	35.1 ± 1.9	35.9 ± 1.6	36.3 ± 1.7
BSA	37.2 ± 2.4	34.5 ± 2.6	37.2 ± 2.5	36.0 ± 2.5	36.3 ± 1.7	38.0 ± 2.0
dP/dt_{max} (mm Hg/s)						
Control (no BSA)	4,507 ± 403	4,506 ± 462	4,480 ± 402	4,660 ± 352	4,484 ± 205	4,676 ± 483
FA-free BSA	4,209 ± 384	3,667 ± 560	3,612 ± 214	4,164 ± 367	4,207 ± 101	3,882 ± 236
BSA	4,547 ± 557	4,526 ± 303	4,900 ± 538	4,542 ± 334	4,567 ± 604	4,288 ± 348

*$P < 0.05$ vs. baseline.
†$P < 0.05$ vs. control (no BSA).
LVESP = left ventricular end-systolic pressure; LVEDP = left ventricular end-diastolic pressure; CPP = coronary perfusion pressure; dP/dt = peak positive first derivative of left ventricular pressure with respect to time.

Isolated Rat Hearts Experiments

Hemodynamic Parameters. Results are presented in Table 1. Left ventricular end-systolic pressure (LVESP), CPP, and dP/dt_{max} remained constant for the entire duration of the experiments, and there was no difference in any of these parameters between the experimental groups throughout the protocol. Sixty minutes after ^{99m}TcN-NOET injection, left ventricular end-diastolic pressure (LVEDP) was slightly but significantly higher in the groups perfused with BSA compared with the control (no BSA) group. Wet weights of hearts perfused with KH buffer in the absence or presence of BSA were not statistically different when measured at the end of the experiments (1.55 ± 0.06 vs. 1.51 ± 0.05 g, respectively; P = not significant).

^{99m}TcN-NOET Myocardial Uptake and Washout Kinetics. Initial ^{99m}TcN-NOET myocardial uptake (%ID/g) is shown in Figure 1 for the 3 groups of hearts perfused with KH (no BSA), KH + BSA, or KH + FA-free BSA buffer. The presence of BSA in the KH buffer resulted in a significant 18% decrease in the initial ^{99m}TcN-NOET myocardial uptake ($P < 0.05$ vs. control). Note that the reduction in myocardial uptake of ^{99m}TcN-NOET with BSA compared with control (no BSA) hearts was not dependent on the degree of BSA lipid loading. Figure 2A compares the myocardial ^{99m}TcN-NOET washout kinetics of control hearts perfused with BSA-free KH buffer with hearts perfused with KH buffer containing either BSA or FA-free BSA. In the control hearts, there was no significant myocardial washout of ^{99m}TcN-NOET over time ($P = 0.148$ by repeated measures ANOVA), and myocardial ^{99m}TcN-NOET activity 60 min after injection represented 98.7% ± 1.6% of the initial activity. Note that the addition of BSA to the KH buffer resulted in a significant increase in ^{99m}TcN-NOET myocardial washout over 60 min ($P < 0.001$ by repeated measures ANOVA for both conditions). Myocardial end activity of the tracer represented 90.6% ± 1.1% initial with FA-free BSA (P = not significant vs. control) and 79.6% ± 3.9% initial with BSA ($P < 0.001$ vs. control; $P < 0.05$ vs. FA-free BSA). Moreover, as shown in Figure 2B, the presence of lipids on the BSA resulted in a significantly faster myocardial washout of the tracer as reflected by the shorter

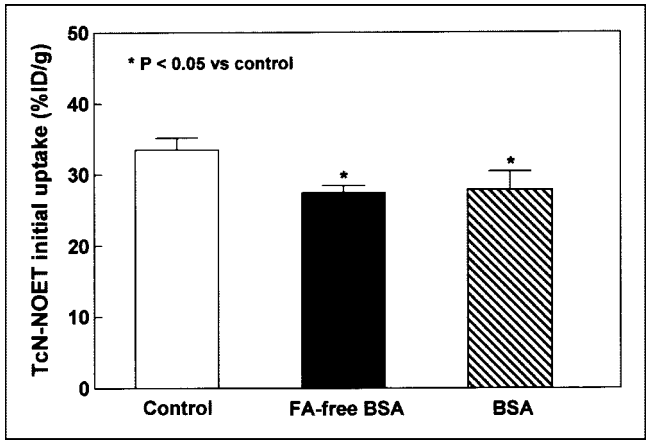

FIGURE 1. Initial ^{99m}TcN-NOET myocardial uptake after bolus injection in isolated rat hearts perfused with KH buffer (control; $n = 6$), KH with FA-free BSA 0.03% ($n = 5$), or BSA 0.03% ($n = 4$). Myocardial ^{99m}TcN-NOET uptake was reduced in presence of BSA and reduction was not dependent on degree of lipid loading.

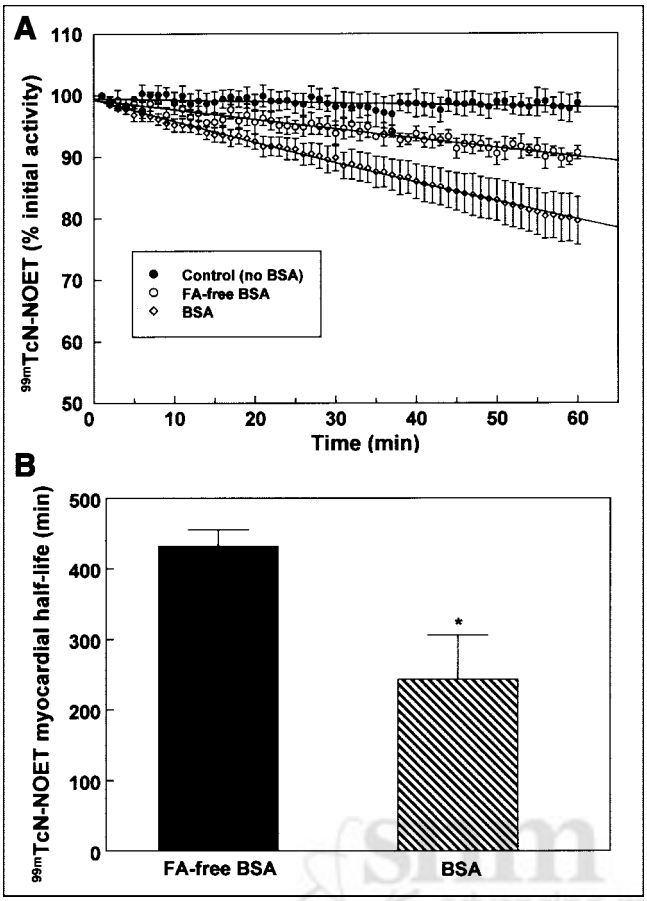

FIGURE 2. (A) Time course of ^{99m}TcN-NOET myocardial activity after bolus injection in isolated perfused rat hearts. Hearts were perfused with KH buffer (control; $n = 6$), KH with FA-free BSA 0.03% ($n = 5$), or BSA 0.03% ($n = 4$). (B) $t_{1/2}$ of ^{99m}TcN-NOET myocardial washout from isolated perfused rat hearts. $t_{1/2}$ values were determined by applying exponential fit ($y = a_0 \times \exp[-x/a_1]$) to individual ^{99m}TcN-NOET myocardial washout curves in presence of FA-free BSA or BSA. Data are mean ± SEM of 5 and 4 experiments, respectively. *$P < 0.05$ vs. FA-free BSA.

half-life ($t_{1/2}$) with the BSA than with the FA-free BSA groups (242.9 ± 63.2 min vs. 431.5 ± 23.3 min, respectively, $P < 0.05$).

Canine Experiments

Influence of Increased Lipid Concentration on ^{99m}TcN-NOET Intrinsic Myocardial Washout Kinetics. Results are presented in Figures 3 and 4. Before intralipid injection, the $t_{1/2}$ of ^{99m}TcN-NOET myocardial washout was 108.0 ± 23.9 min (baseline serum triglyceride concentration = 15.5 ± 3.5 mg/dL). Immediately after intralipid injection (serum triglyceride concentration = 3,520 ± 168 mg/dL), there was rapid loss of ^{99m}TcN-NOET from the myocardium. Once blood lipid levels had stabilized (serum triglyceride concentration = 2,708 ± 133 mg/dL, 2,511 ± 20 mg/dL, and 2,302 ± 91 mg/dL at 5, 15, and 30 min after injection, respectively), the intrinsic myocardial washout of ^{99m}TcN-NOET remained markedly accelerated during the hyperlipidemic state (Fig. 3), with a mean $t_{1/2}$ of 51.8 ± 11.8 min ($P < 0.05$ vs. control; Fig. 4). Ultrasonic coronary flow did not change between the normal and hyperlipidemic state (Fig. 4).

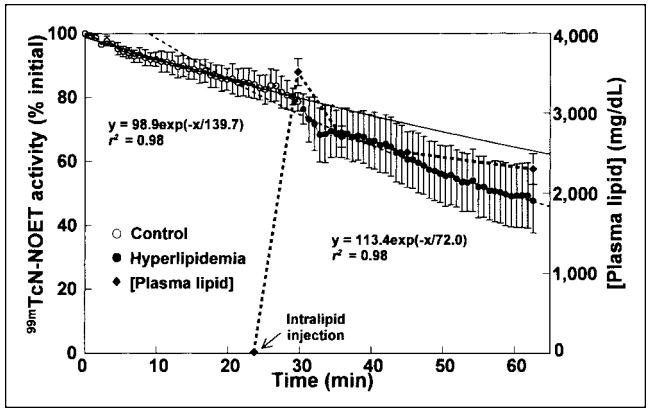

FIGURE 3. Time course of ^{99m}TcN-NOET intrinsic myocardial washout from canine myocardium before (control) and after intravenous administration of intralipids (hyperlipidemia). Data are mean ± SEM of 3 experiments.

Influence of Coronary Flow on ^{99m}TcN-NOET Intrinsic Myocardial Washout Kinetics. Results are depicted in Figures 5 and 6. Immediately after intracoronary injection, there was rapid washout of nonextracted ^{99m}TcN-NOET. Under normal flow conditions, the $t_{1/2}$ of ^{99m}TcN-NOET intrinsic myocardial washout was 81.1 ± 12.1 min. The intravenous infusion of the adenosine A_{2A} receptor agonist ATL-146e (0.3 μg/kg/min) resulted in a 4.9-fold increase in coronary flow ($P < 0.05$ vs. baseline). Under these hyperemic conditions, ^{99m}TcN-NOET intrinsic myocardial washout was markedly accelerated with a $t_{1/2}$ of 40.7 ± 7.3 min ($P < 0.05$ vs. control).

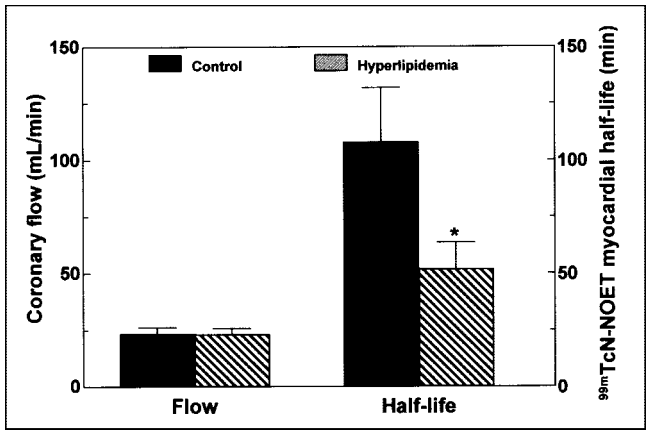

FIGURE 4. Ultrasonic coronary blood flow and $t_{1/2}$ of ^{99m}TcN-NOET intrinsic myocardial washout from canine myocardium before (control) and after intravenous administration of intralipids (hyperlipidemia). $t_{1/2}$ values were determined by applying exponential fit ($y = a_0 \times \exp[-x/a_1]$) to individual ^{99m}TcN-NOET intrinsic myocardial washout curves. Data are mean ± SEM of 3 experiments. *$P < 0.05$ vs. preintralipids administration.

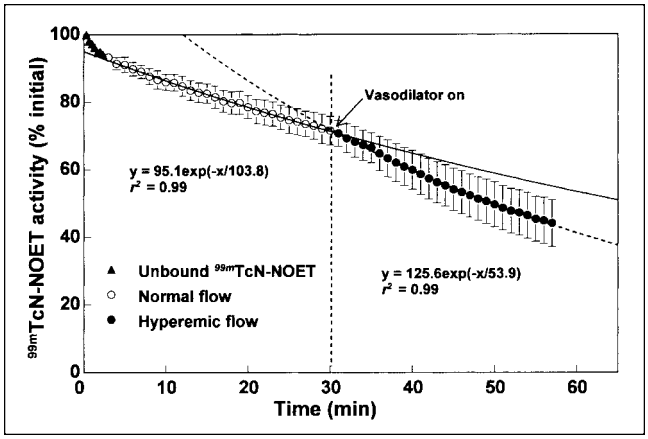

FIGURE 5. Time course of ^{99m}TcN-NOET intrinsic myocardial washout from canine myocardium after intracoronary bolus injection at rest (normal flow) and during vasodilator stress induced by adenosine A_{2A} receptor agonist ATL-146e (0.3 μg/kg/min) (hyperemic flow). Data are mean ± SEM of 6 experiments.

DISCUSSION

In this study, the effect of increased lipid concentration on ^{99m}TcN-NOET myocardial washout was evaluated ex vivo and in vivo. No ^{99m}TcN-NOET washout was observed ex vivo in the isolated rat heart model when the myocardium was perfused with crystalloid KH buffer. Such high retention has been described by Johnson et al. in a similar buffer-perfused, isolated rat heart model (*6,7*). These authors also performed successful attempts at accelerating ^{99m}TcN-NOET myocardial washout in this same model. Significant myocardial washout of the tracer was observed when the perfusing buffer was supplemented with a detergent such as Triton X-100 (*6*) or with red blood cells or red blood cells and albumin (*7*), leading the authors to propose that ^{99m}TcN-NOET was bound to the vascular endothelium in whole organ models.

In this study, we showed that albumin alone was responsible for a decrease in ^{99m}TcN-NOET initial myocardial uptake and for an increase in ^{99m}TcN-NOET myocardial washout rate, suggesting that the tracer binds nonspecifically to the protein. Albumin binds to and solubilizes FAs in vivo in blood plasma, acting as an FA supplier for the myocardium where albumin-FA complexes are dissociated (*18*). Because ^{99m}TcN-NOET is a lipophilic agent, we hypothesized that the effect of albumin on ^{99m}TcN-NOET kinetics would be dependent on the level of albumin lipid loading. Using methanol extraction, we found that a total of 13.8 mg of FAs could be extracted per gram of BSA. Considering that the main FAs bound to BSA are palmitic acid and stearic acid (*13*), we calculated that the approximate number of FAs per albumin molecule before methanol extraction was 3.4, a value that is in the normal range for BSA lipid-carrying capacity (*13*). The ex vivo data indicated that, at a constant albumin concentration, the myocardial washout of ^{99m}TcN-NOET was significantly accelerated when lipids were bound to the albumin.

We do not believe that the differences in myocardial washout rate of ^{99m}TcN-NOET that we observed in this study can be attributed to tissue edema. First, the fact that the heart wet weights were not statistically different in the absence or presence of albumin suggests that cellular edema was minimal or at least similar between the groups. Second, myocardial edema resulting in increased intramyocardial tissue pressure would be expected to increase over the perfusion period. If this had occurred, we should have observed a gradual slowing of ^{99m}TcN-NOET washout over time, particularly in the control (no BSA) group. However, as shown in Figure 2A, the ^{99m}TcN-NOET washout rate in this group was slow and constant over the 60-min washout period. Finally, in the 2 groups of hearts perfused with buffer containing BSA, where we would expect a small and similar degree of edema, we observed faster myocardial washout of ^{99m}TcN-NOET when the BSA contained lipids.

Nonspecific binding to vascular endothelial cell membranes has been suggested as the mechanism of ^{99m}TcN-NOET myocardial uptake (*6,7*). Due to the fact that ^{99m}TcN-NOET is a highly hydrophobic compound, our laboratory and others (*6–8*) have previously observed that the myocardial retention of the tracer on cellular membranes is extremely high when isolated hearts are perfused with a crystalloid buffer. The introduction of a protein such as albumin in the perfusion buffer may provide nonspecific binding sites for the tracer in the intravascular compartment and, therefore, promote ^{99m}TcN-NOET washout from the myocardium. The data from our study indicate that this mechanism is accentuated when albumin is loaded with hydrophobic FA molecules that may further modify the partition of ^{99m}TcN-NOET between vascular endothelial cells and the intravascular compartment. The fact that ^{99m}TcN-NOET initial myocardial uptake was similar in the presence of KH + FA-free BSA and KH + BSA indicates

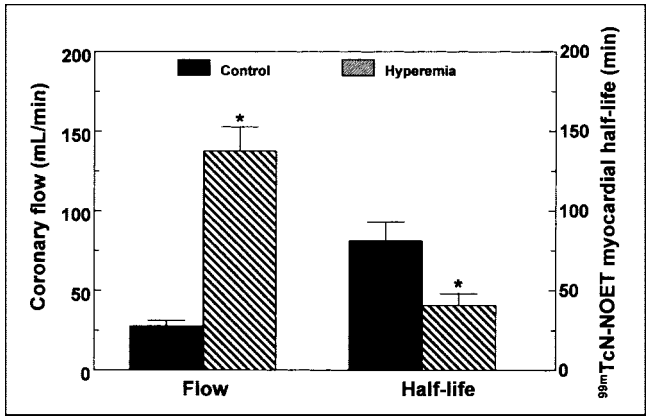

FIGURE 6. Ultrasonic coronary blood flow and $t_{1/2}$ of ^{99m}TcN-NOET intrinsic myocardial washout from canine myocardium at rest (control) and during vasodilator stress (hyperemia). $t_{1/2}$ values were determined by applying exponential fit ($y = a_0 \times \exp[-x/a_1]$) to individual ^{99m}TcN-NOET intrinsic myocardial washout curves. Data are mean ± SEM of 6 experiments. *$P <$ 0.05 vs. control.

that lipids may play a more important role in ^{99m}TcN-NOET removal from the myocardium than in its initial extraction.

Because circulating lipid levels may vary among patients (*19*), we evaluated the effect of an intravenous injection of intralipids on ^{99m}TcN-NOET myocardial washout in vivo from canine myocardium to determine whether the influence of FAs observed ex vivo on isolated hearts could still be evidenced in vivo. The dose of intralipids used in our study has been used by others with the same experimental model and has been shown to have little effect on hemodynamic parameters such as aortic pressure, left atrial pressure, and heart rate (*20*). The intravenous injection of intralipids significantly increased the myocardial washout rate of ^{99m}TcN-NOET in vivo without affecting ultrasonically measured epicardial coronary blood flow, indicating that the mechanism for ^{99m}TcN-NOET myocardial washout determined ex vivo occurred in vivo as well. Rim et al. (*20*) showed that increasing plasma triglyceride levels using intravenous injections of intralipids resulted in an increase in blood viscosity and a decrease in microcirculatory flow. Therefore, the faster intrinsic myocardial washout of ^{99m}TcN-NOET after intravenous administration of intralipids cannot be attributed to the effect of intralipids on regional myocardial blood flow because a decrease in regional flow would have resulted in a slower rather than faster ^{99m}TcN-NOET myocardial intrinsic washout rate (*21*).

The purpose of our study was to determine whether changes in a vascular compartment variable (i.e., circulating lipid levels) could affect ^{99m}TcN-NOET kinetics. Although the serum triglyceride level reached in this experimental study was an order of magnitude higher than that which is usually observed in hyperlipidemic patients (*22*), our results highlight the fact that ^{99m}TcN-NOET uptake or washout kinetics can be readily affected by changes in a variety of factors in the intravascular compartment and support the hypothesis that ^{99m}TcN-NOET is bound intravascularly. The potential impact of this finding on clinical imaging with ^{99m}TcN-NOET needs to be evaluated because variations in circulating lipid levels among patients (*19*) could result in variations in myocardial ^{99m}TcN-NOET uptake or washout kinetics.

A second potential determinant of ^{99m}TcN-NOET myocardial kinetics in vivo is coronary flow. Indeed, by using serial quantitative in vivo planar imaging, Petruzella et al. (*23*) showed that the mechanism for the apparent ^{99m}TcN-NOET redistribution likely involved differential washout of the tracer from normally perfused versus ischemic regions. However, one difficulty of using in vivo imaging after intravenous injection to study the mechanism of myocardial redistribution is that it is impossible to separate the myocardial activity from that of the underlying blood pool. By using an intracoronary injection of a tracer, one can assess the intrinsic myocardial washout of the tracer without the confounding effect of blood-pool activity. For example, Beller et al. (*10,24*) and Grunwald et al. (*12*) have shown that differential intrinsic myocardial washout rates between a normal and an ischemic zone were responsible for ^{201}Tl redistribution, together with delayed tracer reuptake in the previously ischemic region. In our study, we could not determine whether delayed reuptake of ^{99m}TcN-NOET is a factor in its mechanism of redistribution because the tracer was given via the intracoronary route so no circulating blood activity was available for myocardial reuptake. We did observe that the intrinsic myocardial washout rate of ^{99m}TcN-NOET was significantly increased after adenosine A_{2A} receptor-mediated vasodilatation, confirming that differential clearance of the tracer between normal and ischemic myocardium due to differences in perfusion flow clearly plays a major mechanistic role in the observed ^{99m}TcN-NOET redistribution. The fact that ^{99m}TcN-NOET shares a common mechanism of redistribution with ^{201}Tl (the dependence of the intrinsic myocardial washout of the tracer on perfusion flow) should not be interpreted as an indication that the information provided by the 2 tracers is identical. As mentioned above, ^{99m}TcN-NOET is likely bound to the vascular endothelium where its washout rate can be affected by a variety of intravascular factors, whereas ^{201}Tl is localized within the cytosol and is in constant active equilibrium with the extracellular tracer concentration (*5*).

Among the various ^{99m}Tc-labeled perfusion tracers, flow-dependent myocardial washout has only been observed with ^{99m}Tc-teboroxime, another neutral, lipophilic molecule (*25,26*). As observed with ^{99m}TcN-NOET, binding to blood components also increased the rate of ^{99m}Tc-teboroxime myocardial washout (*27*). Cationic, lipophilic ^{99m}Tc-labeled tracers, such as sestamibi, that are sequestered in the mitochondria of the myocyte do not exhibit flow-dependent washout (*28*) and show minimal redistribution (*29*).

CONCLUSION

Circulating lipid levels and coronary flow are important determinants of ^{99m}TcN-NOET myocardial kinetics in vivo. The dependence of ^{99m}TcN-NOET kinetics on changes occurring within the vascular compartment supports the hypothesis that the tracer is bound intravascularly. Further studies are needed to determine if variations in blood lipid concentration in the range seen in humans induce variations in ^{99m}TcN-NOET uptake and clearance kinetics in the setting of clinical imaging.

Another potential clinical implication of this study involves the finding that ^{99m}TcN-NOET intrinsic myocardial washout is flow dependent. With stress imaging, the duration of the hyperemic response is related to the choice of stress used—that is, adenosine stress is relatively short, whereas the elevation in coronary flow with exercise or dipyridamole stress may last considerably longer. It is unknown whether differences in the duration of hyperemia that could accelerate the washout of ^{99m}TcN-NOET from high-flow regions have an effect on the speed at which a defect resolves over time. Further experimental studies are needed to address the important question of whether the

choice of stress used has an effect on the redistribution kinetics of ^{99m}TcN-NOET that may impact on the timing of image acquisition.

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