Factors Influencing Serial Measurements of Cardiac Volumes by Count-Based Methods: Effects of Elevated Catecholamines, Position, and Exercise on Technetium-99m-Blood Radioactivity Concentration

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Most radionuclide methods for measuring cardiac volume require a determination of the blood radioactivity concentration. Thus, changes in blood radioactivity over time or during interventions might lead to spurious volume estimates unless blood radioactivity is serially measured. The effects of elevated epinephrine, posture and exercise on 99mTc-labeled blood radioactivity concentration were studied in 15 young (mean age = 28 yr) and 14 older (mean age = 68 yr) healthy males. An epinephrine infusion of 50 ng/kg/min resulted in a 4.1% ± 1.0% increase in 99mTc-blood radioactivity (p ≤ 0.001) compared to baseline. Sitting increased blood radioactivity concentration by 12.3% ± 3.0% (p < 0.0002) compared to the supine position and peak supine bicycle exercise caused an 11.0% ± 1.7% increase (p ≤ 0.0001) compared to supine rest. There was a significantly greater increase during peak supine exercise in the young compared to the older subjects (15.0% ± 2.3% versus 6.3% ± 2.0%, p ≤ 0.01). The mechanism of the increase in blood radioactivity concentration is uncertain, but presumably reflects the addition of hemococoncentrated red blood cells from the spleen and/or the loss of plasma volume. Failure to correct for the increased blood radioactivity concentration during exercise or pharmacological interventions will result in a significant error in serial measurements of cardiac volumes by methods requiring RBC radioactivity measurements.


Left ventricular volume changes during exercise or pharmacological interventions are frequently measured using radionuclide methods (1–5). Most radionuclide methods require a measure of the blood radioactivity concentration obtained from a blood sample as well as correction for attenuation and 99mTc decay (6,7). Most investigators obtain a single blood sample at rest (1) or peak intervention (2–5) to estimate cardiac volume changes, assuming that blood radioactivity does not change significantly during the intervention. However, several investigators have reported a 10%–15% increase in 99mTc counts/ml of blood (blood radioactivity concentration) with standing (8) or supine bicycle exercise (8,9). The postulated mechanism for the increase in blood radioactivity concentration is multifactorial and may include the mobilization of hemoconcentrated blood from the spleen (8,11) and/or a loss of plasma volume (12–14). Failure to draw separate blood samples at rest and at peak intervention to correct for the change in blood radioactivity concentration during the intervention may result in erroneous cardiac volume calculations.

Correction for the in-vivo decay of 99mTc between resting and intervention acquisitions is routinely performed using the 6-hr physical half-life of 99mTc. However, prior studies have suggested that the in-vivo effective half-life of 99mTc-labeled RBCs is significantly shorter than the physical half-life, which might constitute another source of measurement error (15–17).

The purpose of this study was to evaluate several factors that might alter blood radioactivity concentration and thus influence radionuclide measures of ventricular volumes. The factors we evaluated were increased epinephrine levels induced by an exogenous epinephrine infusion, position (supine versus sitting) and dynamic exercise. Since the cardiovascular and hormonal responses to these interventions might be altered with aging, both a young and an older group of healthy males were studied.

METHODS

The study population consisted of 15 young (mean age = 28 yr, range 24–32) and 14 older (mean age = 68 yr, range 60–82)
healthy, nonathletic male volunteers. Exclusion criteria included current smoking, hypertension, chronic medication use of any type, chronic disease, regular exercise, diabetes mellitus or morbid obesity. All subjects had unremarkable histories, physical examinations, electrocardiograms, complete blood counts, serum chemistries, including cholesterol, and normal M-mode and two-dimensional echocardiograms. Subjects meeting the initial screening criteria were then required to have a normal Bruce protocol maximal exercise test, which included postexercise and redistribution tomographic thallium imaging in the older subjects.

Protocol

On the morning of the study, subjects reported to the laboratory after a small breakfast and abstinence from caffeine. Intravenous catheters were placed in the right hand and the right antecubital fossa. RBCs were labeled with 1,435–1,500 MBq of \(^{99m}\)Tc using the modified in-vivo method of Callahan (18). The right hand was placed in a heating pad to provide arterialized venous samples for catecholamine measurements (19). Duplicate 1.0-ml blood samples were obtained after 20 min of upright sitting prior to any intervention, at supine rest prior to each intervention and at the end of each intervention. At least 30–45 min of supine rest separated each intervention. Three supine resting samples were obtained over 2.5 hr for the calculation of the in-vivo half-life of \(^{99m}\)Tc-labeled RBCs. After collection of resting baseline data, epinephrine was continuously infused for 10 min/dose at 10, 25, and 50 ng/kg/min. Following an additional 30 min of supine rest, symptom-limited supine bicycle exercise was performed starting at a workload of 200 kpm and increasing 200 kpm every 3 min until exhaustion. The last completed stage was considered the symptom-limited peak workload. The protocol was approved by the University of Washington human subjects research committee and each subject gave informed consent.

Data Analysis

The blood samples were positioned 5 cm from a high-sensitivity collimator of a GE300 A/M gamma camera and counted for 5 min using a 20% energy window. The entire field of view was used as the region of interest and the counts were corrected for ambient background activity using a separate 5-min acquisition without blood samples.

The blood sample counts were decay-corrected to the time they were drawn by the equation:

\[ C_0 = C e^{-\lambda t}, \]

where \( C_0 \) = decay-corrected counts, \( C \) = decayed counts, \( \lambda = 0.693/6.02 \) hr physical half-life of \(^{99m}\)Tc, and \( t \) = the time between when the sample was drawn and when it was counted.

The three decay-corrected blood samples obtained during supine rest prior to the epinephrine infusion and exercise versus the time the blood samples were obtained were plotted on a semilog scale to calculate the in-vivo half-life of \(^{99m}\)Tc. The in-vivo half-life, determined for each individual, was used to decay correct the intervention blood samples to the time the resting blood sample was obtained using Equation 1. Differences between the resting and intervention blood counts were evaluated with the paired t-test. ANOVA for repeated measures was used to test for differences between the young and older group.

By assuming that the endogenous epinephrine increase during exercise has a similar effect on the blood radioactivity as that induced by exogenous epinephrine infusion, the predicted increase in blood radioactivity during exercise due to endogenous epinephrine (Pred INCR End-Epi) was calculated by:

\[ \text{Pred INCR End-Epi} = \frac{\text{blood radioactivity increase during epinephrine infusion}}{\text{(epinephrine level during exercise)}} \times \frac{\text{endogenous epinephrine level at rest}}{- \text{epinephrine level at rest}}. \]

RBC labeling efficiency was performed as previously described (18). Results are expressed as the mean ± s.e.m. Significance was defined as \( p \leq 0.05 \). To assess variables potentially responsible for the increase in blood radioactivity during exercise, a multivariate regression was performed. Age, height, weight, body surface area, 22 hemodynamic variables, peak workload, in-vivo half-life and the predicted increase in blood radioactivity due to endogenous epinephrine as estimated by the subject's response to exogenous epinephrine were entered in the univariate model. Univariate predictors with a \( p \leq 0.10 \) were entered into a multivariate regression.

RESULTS

In-Vivo Half-Life

For all subjects, the measured in-vivo effective half-life of \(^{99m}\)Tc was 4.40 ± 0.12 hr. The effective half-life was significantly shorter than the 6-hr physical half-life (\( p \leq 0.0001 \)). There was no difference between the young (4.33 ± 0.16 hr) or older (4.47 ± 0.19 hours) subjects (Fig. 1). The effective half-life varied considerably between subjects, ranging from 3.34 to 5.72 hr. An inefficient RBC label will result in a shortened in-vivo half-life. To exclude this possibility, in five subjects we measured the in-vitro RBC labeling efficiency, which was high at 97.5% ± 1.8%.

Epinephrine Infusion

The blood radioactivity concentration increased 4.1% ± 1.0% (\( p \leq 0.001 \)) during the epinephrine infusion in all subjects. There was a trend (\( p = 0.16 \)) for a greater increase in the younger subjects when compared to the older subjects (5.5% ± 1.9% versus 2.6% ± 0.8% (Fig. 2A).

The predicted increase in blood radioactivity during exercise due to the increase in endogenous epinephrine as estimated by the subjects' responses to the exogenous
epinephrine infusion was $2.5\% \pm 1.1\%$ ($n = 14$) in the younger subjects, and $0.4\% \pm 0.2\%$ in the older subjects ($n = 11$, young versus older).

**Sitting Position**
Among all subjects, the blood radioactivity concentration increased $12.3\% \pm 3.0\%$ ($p \leq 0.0002$) during sitting when compared to the supine position. The increase was similar in both groups ($12.2\% \pm 5.3\%$ in the younger and $12.5\% \pm 2.9\%$ in the older subjects) (Fig. 2B). The response was highly variable among subjects, ranging from $-22\%$ to $+63\%$.

**Supine Exercise**
The blood radioactivity concentration increased $11.0\% \pm 1.7\%$ ($p \leq 0.0001$) during peak supine exercise in all subjects. There was a significantly greater increase in the peak exercise blood radioactivity concentration in the younger subjects when compared to the older subjects ($15.0\% \pm 2.3\%$ versus $6.3\% \pm 2.0\%$, $p \leq 0.01$) (Fig. 2C).

**Univariate and Multivariate Analysis—Exercise**
In the univariate analyses, the increase in blood radioactivity concentration during peak exercise was positively correlated with the predicted increase due to endogenous epinephrine (as estimated by the subjects' responses to exogenous epinephrine infusion), peak workload, peak heart rate and weight. The increase in blood radioactivity with exercise was inversely correlated with age and the in-vivo half-life of $^{99m}$Tc-labeled RBCs (Table 1).

In a multivariate analysis, the only independent predictors of the peak supine exercise blood radioactivity were the predicted blood radioactivity increases due to endogenous epinephrine (as estimated by the subjects' responses to the exogenous epinephrine infusion), the in-vivo half-life and the peak workload.

**DISCUSSION**
We have shown that $^{99m}$Tc counts/ml of blood increase significantly in both young and older male subjects during sitting versus supine posture, during an epinephrine infusion of 50 ng/kg/min and during peak supine bicycle exercise. The older subjects had a blunted increase in blood radioactivity concentration during peak supine exercise and a trend towards a blunted increase during the epinephrine infusion compared with young subjects. The independent predictors of the increase in blood radioactivity during peak exercise were the responses due to endogenous epinephrine (as estimated by the subjects' responses to the exogenous epinephrine infusion), the in-vivo half-life of $^{99m}$Tc-labeled RBCs and the peak workload. We also determined that the 4.4-hr in-vivo effective half-life of $^{99m}$Tc-RBCs is significantly shorter than the 6-hr physical half-life. Because we did not study female subjects, it is uncertain if these results are applicable to female subjects, which is a limitation of this study.

**In-Vivo $^{99m}$Tc Half-Life**
Since the physical decay of $^{99m}$Tc is not changed by attachment to RBCs, the shorter in-vivo half-life must be due to in-vivo removal of the $^{99m}$Tc from the circulating RBC mass. The biological factors that might lead to such a shortening of the half-life include an inefficient labeling
of the RBCs (20), splenic sequestration of RBCs damaged during the labeling process (17,21) or the intravascular loss of free pertechnetate (21), which should be minimal if the tagging efficiency is high. Since we determined a 97% RBC labeling efficiency in five of the subjects (data not shown), inefficient labeling of RBCs was not the cause of the shorter in-vivo half-life. Our findings of an effective half-life of 4.4 hr support reports by other groups of half-lives of 4.1 (15), 4.4 (16) and 5.7 (17) hr.

Blood Radioactivity

The increase in blood radioactivity concentration during an intervention reflects an increase in the 99mTc-RBC concentration (8,9). An increase in the RBC concentration can occur by the addition of hemoconcentrated RBCs to the circulating RBC mass or by the removal of plasma water. At rest, the splenic RBC pool is hemoconcentrated by 30%–35% (22,23) in comparison to the peripheral RBC pool, while other splanchnic organs are isoconcentrated. Thus, addition of hemoconcentrated RBCs from the spleen would increase the peripheral venous RBC concentration (24).

Epinephrine

Animal studies have established that epinephrine causes a decrease in splenic size that is associated with an increase in peripheral WBC, platelet and RBC concentrations (22, 25–28).

There are fewer data for humans. Splenic size decreased in humans with intra-arterial (29) or subcutaneous (30) epinephrine, and intra-arterial epinephrine during surgery increased the splenic vein RBC count by 35% (23). This is the first study, to our knowledge, that has shown an increase in human 99mTc-blood radioactivity concentration with epinephrine. In-vitro (31) and in-vivo (32,33) human studies have established that the splenic pooling of RBCs is proportional to the splenic blood flow. Alpha agonists (norepinephrine) reduce splenic blood flow and release RBCs, while beta agonists (isoproterenol) increase blood flow and cause retention of RBCs (32,33).

Thus, we would postulate that the majority of the increase in blood radioactivity concentration during the epinephrine infusion is due to the direct effect of epinephrine on the splenic release of hemoconcentrated RBCs. It is possible that there was also a loss of plasma volume associated with the increased cardiac output during the epinephrine infusion, which would also have increased the blood radioactivity concentration.

Upright Posture

With standing, there is an 11%–16% loss of plasma water (12) due to increased hydrostatic forces. This loss of plasma water will cause an estimated 7%–11% increase in the RBC concentration, assuming a normal hematocrit. Sandler found a 10% increase in blood radioactivity concentration with standing (8). There are less data on plasma volume changes from the supine to the sitting position, but the plasma volume loss is estimated at 5%–9% (13, 14), with an estimated 3%–6% increase in RBC concentration. We found a 12.3% increase in blood radioactivity concentration with sitting. This is presumably due to the loss of plasma water from increased hydrostatic forces in the upright position and possibly by the addition of RBCs from the spleen mediated by the increased catecholamine levels during sitting (34).

Exercise

The effects of exercise on plasma volume, hemoconcentration and splenic function have been previously evaluated in dogs and humans. The peripheral hematocrit increases in dogs by 39% with exercise (25) and this increase is abolished by splenectomy (35). In humans, splenic 99mTc-RBCs counts decrease by 37%–49% during exercise (8,9,24,26,36,37). This is associated with a 6.6%–10.5% increase in blood radioactivity (8,9) and a 6.2%–10% increase in the hematocrit (8,9,11). Unlike in dogs, the increase in RBC concentration during exercise in humans is not significantly altered by splenectomy (38).

The human spleen has been estimated to able to release 62 ± 18 ml of RBCs with exercise (26). The increase in the peripheral hematocrit due to the addition of 62 ml of hemoconcentrated RBCs is estimated at only 2%, which is well below the measured 6%–14% measured increase in the hematocrit during exercise (8,9,11–13). Intravascular loss of plasma volume has been estimated at 14%–20% during supine bicycle exercise (13,14), with an estimated 11%–14% increase in the RBC concentration. Thus, the release of RBCs from the spleen can account for only a small portion of the increase in blood radioactivity during exercise. The remaining increase must be due to the loss of plasma volume.

The multivariate analysis suggests that the increased blood radioactivity concentration with exercise can be predicted by the increase in the endogenous epinephrine levels during exercise (as estimated by the subjects' blood radioactivity responses to the exogenous epinephrine infusion), the in-vivo half-life of the 99mTc-RBCs and the peak workload. The shorter in-vivo half-life of 99mTc-RBCs, as discussed above, probably reflects splenic sequestration of RBCs damaged during the labeling process. Atkins (21) found that 7% of the 99mTc-labeled RBCs were in the spleen at 2 hr, but this increased to 14% at 3 hr, presumably due to further sequestration of damaged RBCs in the spleen. Thus, the hemoconcentration of 99mTc-labeled RBCs in the spleen may increase over time and is probably greater than the 30% hemoconcentration of the unlabeled RBCs. These 99mTc-labeled RBCs, which are more hemoconcentrated in the spleen than normal RBCs, can be released from the spleen during exercise or during other interventions and may result in a greater increase in the blood radioactivity concentration than the hematocrit (8,9,24).

The predicted increase in blood radioactivity associated with endogenous epinephrine as estimated by the subjects'
responses to the exogenous epinephrine infusion is 2.3% in the younger subjects and 0.4% in the older subjects. This is only 7%–15% of the total increase that occurred during exercise. However, norepinephrine (an alpha agonist) increases during exercise (39) and may also contribute to the splenic release of RBCs. Therefore, the total effect of circulating catecholamines during exercise may be substantial.

**Aging**

It is known that older subjects have a reduced vasodilatory, inotropic and chronotropic response to catecholamines (40–43). Whether the trend we noted towards a blunted increase in the blood radioactivity concentration with epinephrine in the older subjects is due to a decreased splenic response to catecholamines is uncertain.

Several investigators have reported that the loss of plasma volume (12,44,45), increase in hematocrit (11,12) and the reduction in splenic radioactivity (11) is proportional to the percent maximal VO₂ (relative peak workload), but they did not report whether the maximum VO₂ (absolute peak workload) was also a predictor of the changes.

We would postulate that older subjects may have a decreased splenic response to both endogenous and exogenous catecholamines. Also, the older subjects probably have a reduced loss of plasma volume during peak exercise due to the lower absolute maximal workload achieved.

**Implications**

The use of the 6-hr physical half-life to correct for the in-vivo decay of ⁹⁹mTc-RBCs will result in a spurious decrease in cardiac volumes over time using methods that require blood radioactivity sampling. This may be minimal (≈1.3%) during 20 min of exercise, but during a longer intervention, such as our 33-mm epinephrine infusion, it may be substantial (≈2%). Use of a supine resting sample to calculate cardiac volumes during upright exercise will result in a 12% mean overestimation of cardiac volumes.

Similarly, failure to correct for changes in blood radioactivity concentration during interventions will also result in significant errors in the calculated cardiac volumes. The use of a resting blood sample to calculate cardiac volumes during exercise will cause a 15% mean overestimation in young subjects. If the calculated stroke volume, using only a resting blood sample, is reported to increase by 30% with exercise, fully 20%–50% of the increase is due to a 6.5%–15% increase in the blood radioactivity concentration and not to an actual increase in stroke volume.

Equally important to these mean changes, however, is the fact that the responses varied relatively widely among subjects. For example, the in-vivo half-life varied from 3.34 to 5.72 hr, the change in blood radioactivity with supine to sitting from −24% to +62% and during peak supine exercise from −5% to +35%. Thus, in an individual subject, the estimates of volume changes during interven-

tions could be markedly affected unless blood radioactivity changes are measured.

Thus, it is necessary to obtain blood samples at rest and during peak intervention to accurately calculate any change in cardiac volumes using methods that require blood samples such as those reported by Links et al. (6) and Starling et al. (7). The count-based ratio method of Massardo et al. (46) and Levy et al. (47) do not have this limitation because they do not require a blood sample to calculate cardiac volumes.

**CONCLUSION**

We have shown that epinephrine, posture and exercise cause significant increases in blood radioactivity concentration. Older males had a blunted increase in blood radioactivity concentration during supine bicycle exercise and possibly during epinephrine infusions. The mechanism of the increased blood radioactivity concentration is uncertain, but presumably reflects mobilization of sequestered RBCs from the spleen and/or the loss of plasma volume.

Failure to correct for the increased blood radioactivity concentration during exercise or pharmacological interventions will result in significant errors in the calculated cardiac volumes. This potential pitfall can be avoided by the measurement of blood radioactivity both at rest and during the intervention or by the use of a method that does not require blood sampling to calculate cardiac volumes, such as the count-based ratio methods (46,47).

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