# Skeletal Muscle Blood Flow In Vivo: Detection with Rubidium-82 and Effects of Glucose, Insulin, and Exercise

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In order to assess the effects of glucose, insulin, and exercise on skeletal muscle blood flow in vivo, we measured positron emission from the thigh muscle of anesthetized rabbits after simultaneous aortic bolus injection of $^{82}$Rb and radiolabeled microspheres (15 $\mu$m diameter). Estimates of flow with $^{82}$Rb were based on first-pass regional extraction of $^{82}$Rb by skeletal muscle. Flow estimates were made serially as a function of variations in plasma glucose and insulin and changing the muscle contractile state by electrical stimulation. Flow ranged from 3.1 ml/min/100 g at rest to 71 ml/min/100 g during stimulation. There was good agreement between the two methods of flow measurement over the entire range of flows ($r = 0.96$ at a slope of 0.90). Flow measured by either method did not vary significantly from baseline over a range of plasma glucose from 5 to 30 m*M* and plasma insulin from 0 to 20 $\mu$U/ml. When flow was increased up to 20-fold by electrical stimulation there was a decrease in extraction of $^{82}$Rb proportional to the increase in flow. However, at pharmacologic levels of insulin (>150 $\mu$U/ml) flow was increased twofold as measured by radiolabeled microspheres, but not as measured by rubidium. There was no apparent decrease in extraction of $^{82}$Rb with high insulin. The discrepancy between the microsphere measured flow and rubidium measured flow with high plasma insulin levels can be explained by the assumption that the expected decrease in the extraction fraction was counteracted by an increase in $Na^+/K^+$-ATPase activity. It is concluded that the first-pass flow model gives valid estimates of skeletal muscle blood flow in vivo with $^{82}$Rb, provided that plasma insulin levels are normal.

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The use of positron emitting tracers in the study of myocardial and cerebral metabolism and blood flow has increased recently (*1*) due to the greater availability of isotope production facilities and the evolution of electronic and computing systems. One of the early biomedical applications of a positron emitting radionuclide was in the measurement of myocardial flow with the potassium analog rubidium-84 ($^{84}$Rb) as first proposed by Bing et al. (*2*). The method was further developed by Budinger et al. (*3*) and Mullani and Gould (*4*) who used rubidium-82 ($^{82}$Rb), which is easier to produce and has a shorter half-life than $^{84}$Rb.

Measurement of blood flow with a radioactive tracer is based on the Fick principle and requires knowledge of the fractional extraction of labeled tracers by the tissue under study (*5,6*). It is imperative that the extraction fraction be determined to derive accurate flow estimates since flow and tissue extraction of tracer are inversely related. The peak-counts modification of the first-pass model of regional blood flow has been shown to be valid for flow measurements in dog myocardium (*7,8*). We are assuming that the methodology can also be applied to tissues with a lower resting flow and a greater range of flow augmentation. A tissue in which these conditions exist is skeletal muscle.

The study of blood flow in vivo with tracer analogs is difficult because interpretation of the results is hampered by a number of variables, such as circulating hormones and metabolic activity of the tissue have a direct influence on the analog. The use of rubidium as a tracer for the in vivo study of blood flow requires that attention be paid to the effects hormones have on the behavior of rubidium in vivo. For example, the effect

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of insulin on increased membrane transport of $K^+$ and $Rb^+$ are well known (*9*). The mechanism of the effect of insulin is thought to reside in an increased $Na^+/H^+$ exchange, increasing the intracellular $Na^+$, which brings about the increase in the membrane bound $Na^+/K^+$-ATPase activity (*10*). In addition, insulin has been shown to increase skeletal muscle blood flow (*11*) and glucose transport (*12*).

The purpose of this study was first, to investigate the feasibility of measuring rabbit skeletal muscle blood flow using the positron emitter $^{82}Rb$ and second, to assess the effects of plasma glucose and insulin on blood flow in an in vivo preparation.

## METHODS

### Animal Preparation

New Zealand white rabbits weighing 2-3 kg were anesthetized by an intramuscular injection of a cocktail consisting of three parts ketamine (100 mg/ml), three parts xylazine (20 mg/ml), and one part acepromazine (10 mg/ml) (*13*). The initial dose was 0.5 ml/kg body weight followed by 0.2 ml/kg as necessary.

Figure 1 shows a schematic of the animal preparation. Catheters were placed in the left carotid and right femoral arteries with the animal in the supine position. The femoral line was necessary for reference blood withdrawal and plasma sampling and consisted of polyethylene tubing (0.03 × 0.05in). A 4F Lehman end-hole catheter was inserted into the carotid artery and the tip advanced retrograde until contact was made with the wall of the aorta. The tip was then withdrawn 2-3 mm and the catheter anchored. Heart rate and blood pressure were monitored with a Statham force transducer (P23 10) and recorded on a physiologic recorder. All glucose and insulin infusions as well as the simultaneous microsphere and $^{82}Rb$ injections were administered through the aortic cannula.

Positron emission from the left anteromedial thigh muscles was detected by a pair of beta radiation detectors (*14*) (plastic scintillator (NE104), 10 mm diam., 3 mm thick)* placed directly over and in contact with the exposed musculature. The face of one detector was shielded with lead (1 mm thick) to exclude beta activity and thereby allow for background radiation correction.

### Experimental Protocol

Initially, baseline measurements of blood flow with no intervention were performed. Rubidium-82 was eluted from a bench top generator (*15*) as RbCl from its parent isotope strontium-82 ($^{82}Sr$). A bolus of ~ 2-3 ml of $^{82}Rb$ (2-3 mCi) was injected rapidly (3-5 sec) along with the radiolabeled microspheres (100,000 - 300,000) and followed by a 5-ml flush of normal saline. Microspheres were 15 microns in diameter and labeled with niobium-95, $^{85}Sr$, cobalt-57, or scandium-46 ($^{46}Sc$).

After a baseline flow determination either a glucose and/or insulin challenge was administered, or the anteromedial thigh musculature was electrically stimulated. When insulin was administered, it was presented in a saline-albumin (0.1%) medium and infused at a rate of 5 mU/min. Glucose was infused at a rate of 0.5 or 5 μmol/min with or without insulin.

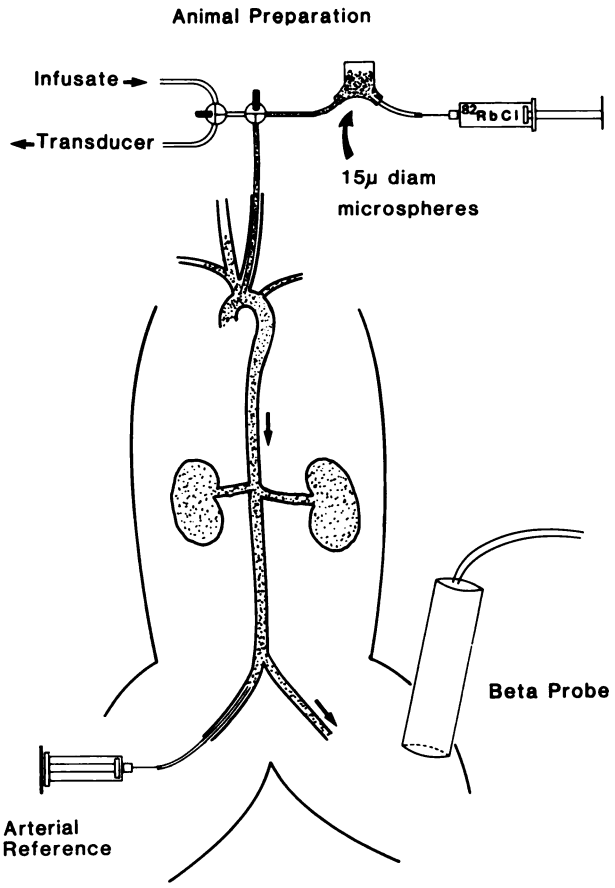


**FIGURE 1**
Schematic of the animal preparation. The rabbit is catheterized in the left carotid and tight femoral arteries. Monitoring of hemodynamic stability, glucose and/or insulin infusion and injection of microspheres and $^{82}Rb$ were through the carotid site. Blood and plasma samples were collected from the femoral artery. Right and left kidneys were sampled to ensure homogenous mixing of microspheres. The beta probes monitored positron emission from the anteromedial thigh musculature.

The muscle was stimulated to contract with a Grass SD9 stimulator at a rate of two pulses/sec, 100 msec duration, a 10 msec delay, and at 5-10 V intensity for 10-15 min. Immediately prior to $^{82}Rb$ and microsphere injection, both the infusion or stimulation were stopped.

### Biochemical Determinations

Arterial blood samples were taken from the femoral site and immediately centrifuged at 1,500 rpm (150 *g*) and 4°C. The plasma was then frozen for later analyses. Insulin in the plasma was determined by a commercial radioimmunoassay method.† Glucose and lactate in the deproteinized plasma were estimated by methods previously described (*16*).

### Rubidium Flow Determinations

Decay corrected time-activity curves for the tissue uptake of rubidium were generated by a VAX 11-780 computer interfaced to a pulse-height analyzer‡ that processed the detected positron counts. Tissue counts/sec were obtained by subtracting background gamma activity (shielded probe) from the total tissue activity (unshielded probe). Figure 2 shows

typical decay corrected time activity curves for rubidium uptake in skeletal muscle during baseline and low flows (A) and at high flow elicited by electrical stimulation of the muscle (B). Tissue activity at the plateau between 50 and 150 sec was used to calculate flow. Arterial blood was sampled by controlled withdrawal (3 ml/min) with a Harvard pump between 0 and 150 sec following intraaortic injection. Blood activity was counted by placing the blood in a 150 ml beaker and placing the probes directly over the surface of the blood. Since the spatial relationship between the detectors and the sample being counted (blood or tissue) was constant, errors due to detector geometry and sampling volume were held to a minimum.

Skeletal muscle blood flow was estimated using the equation of Ishii et al. (*17*):

$$FE = \frac{U(T)}{\int_0^t Ca(t)dt}, \quad (1)$$

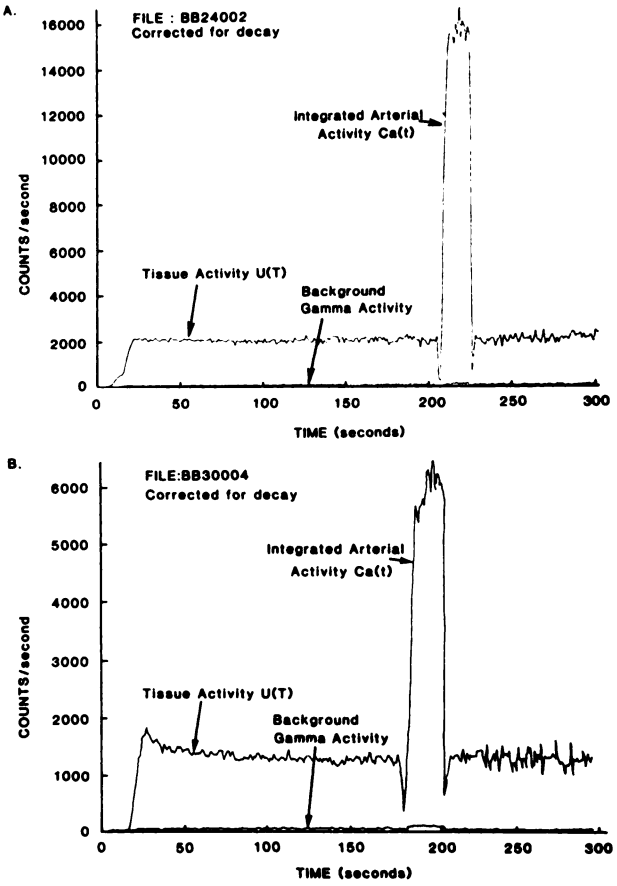


**FIGURE 2**
Time-activity curve for $^{82}Rb$. Tissue uptake was measured over the course of 5 min. Blood flow was calculated on the basis of tissue activity between 50 and 150 sec and the integral of arterial activity from 0 to 150 sec. A: Tissue uptake during low flow conditions as well as high insulin. B: Tissue uptake during high flow induced by electrical stimulation.

where F is skeletal muscle blood flow (ml/min/100 g), E is the extraction fraction, Ca is the arterial activity (counts/sec/ml), and U(T) is the tissue activity (counts/sec). All flow estimations were based on the derivation of the extraction fraction by the curve fitting algorithm developed by Mullani et al. (*7*). The method derives the extraction fraction from the ratio of peak counts in the trapped space at the time of peak counts to peak counts (Fig. 4 in Ref. *7*).

**Microsphere Flow Determinations**

Blood flow was determined by radiolabeled microspheres according to the method of Heymann et al. (*18*). The same blood sample used for obtaining $^{82}Rb$ activity was stored until the rubidium decayed away and then counted for gamma emitting microsphere activity. Tissue samples of the thigh musculature were obtained at the end of each experiment after the animal was killed by an injection of potassium chloride. Muscle directly under the beta probe, ~ 3 mm thick and 10 mm in diameter, was weighed and later counted for each gamma emitter injected. Both right and left kidneys were also removed, weighed and radiolabeled microspheres counted to estimate renal blood flow. This was done to insure homogeneous mixing of microspheres upon injection. Only those flow determinations in which the flows to each kidney were within 10% of their mean flow were used in the final data analysis.

**Data Analysis**

Comparison of the microsphere derived flow estimates with the rubidium flow estimates was made using the Student's t-test for paired observations. To detect any effects of circulating plasma glucose and insulin on skeletal muscle blood flow a one-way analysis of variance (ANOVA) with repeated measures was performed on those flow measurements in which glucose and/or insulin was infused. Groupings for the analysis were based on the combined glucose and insulin concentrations existing at the time of the flow measurement. A Newman-Keuls post hoc comparison for a significant F value followed (*19*). Flow measurements made after electrical stimulation were not included in the ANOVA because of the much greater flows that existed. Much higher flows would only serve to contribute a large variance to the analysis and thus mask subtle differences in flow that resulted from changes in the plasma glucose and insulin.

Linear regression analysis of the microsphere and rubidium flows was done on those measurements in which the extraction fraction was assumed to be unity. A separate regression analysis was done on those flow measurements that immediately followed electrical stimulation. This distinction was made to allow a more accurate assessment of the first-pass flow model's applicability to both low flow and high flow conditions in the muscle. A third regression analysis was done on those flows measured during high physiologic to pharmacologic levels of plasma insulin.

## RESULTS

Figure 3 shows the hemodynamic stability of the preparation. All flow measurements were made during the period of greatest stability (between 120 and 240 min).

Figure 4 shows the changes of plasma glucose over

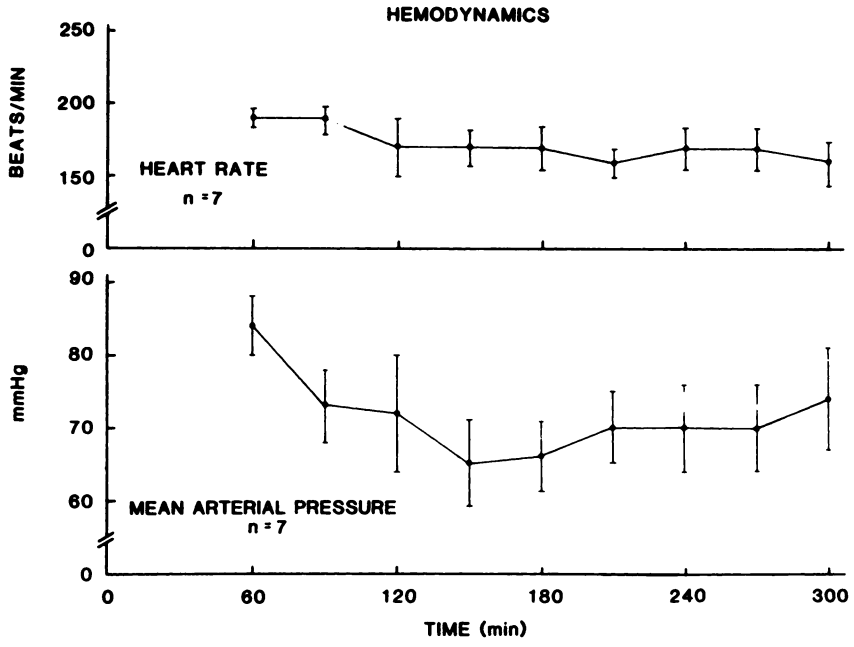


**FIGURE 3**
Hemodynamic stability for a group of seven animals subjected to high physiologic to pharmacologic levels of insulin. Values are means ± s.e.

the time course of those experiments in which glucose and/or insulin were infused. The initial rise in plasma glucose was most likely due to a catecholamine mediated increase in liver glycogenolysis brought on by the stress of anesthesia and surgical trauma. The rise was followed by normalization of plasma glucose which corresponded to a decrease in mean arterial pressure (Fig. 3). At this point the first flow measurement was taken. Also indicated in Figure 4 are the time points for the two subsequent flow determinations made after beginning constant infusion of glucose and/or insulin. Electrical stimulation experiments showed similar glucose responses early and up to the time of the baseline flow measurement (data not presented). The two subsequent flow estimates, each made after 10-15 min. of muscle contraction, occurred with plasma glucose averaging in the range of 10-15 m*M* and insulin levels of 5-7 $\mu$U/ml.

Figure 5 illustrates a typical curve fit under low flow conditions. The characteristics of the raw data are equivalent to the curve shown in Figure 2A in which the tissue activity simply rose to a plateau value and remained there during the period of counting. The rise and plateau of the curve fit (total tissue activity) is

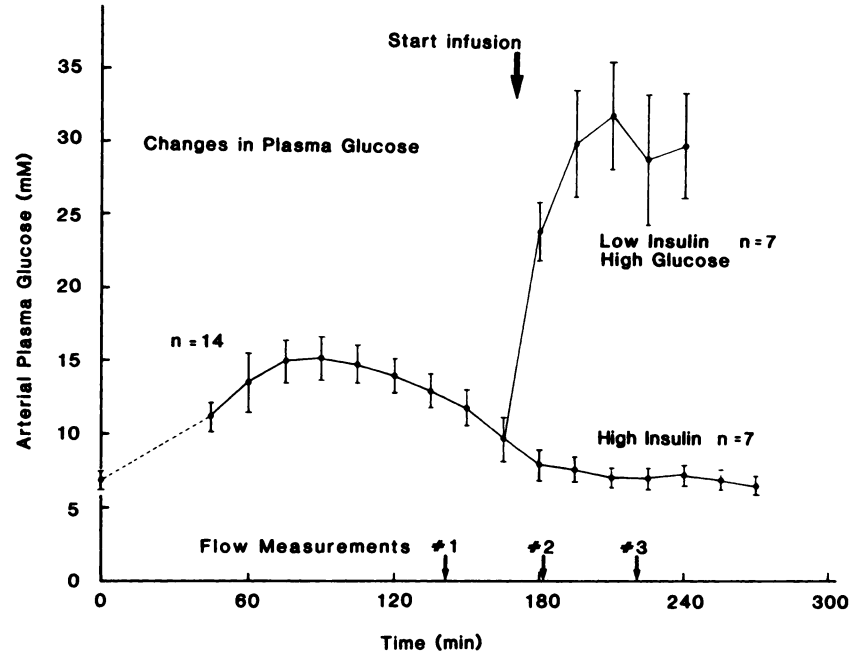


**FIGURE 4**
Changes of plasma glucose during the experimental procedure. Time zero is when the animals were first anesthetized and between 0 to 60 minutes the animals were undergoing surgical preparations. Values are means ± s.e.

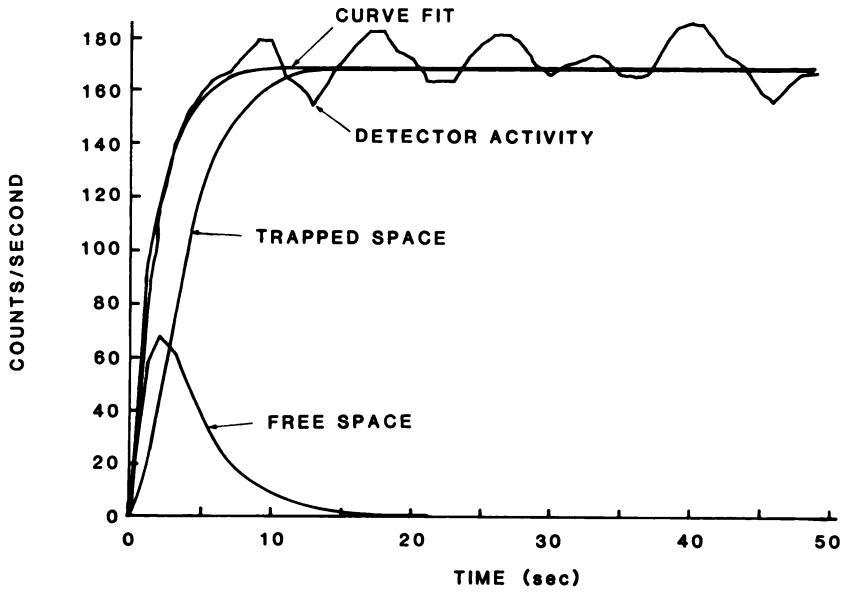


**FIGURE 5**
Time-activity curve illustrating a three-point smoothing of the raw data, curve fit, and the radioactivity in the trapped and free spaces. Extraction is equal to one based on the temporal equality of the curve fit peak counts and the trapped space peak counts.

followed closely by the rise and plateau of the trapped space activity. This curve is characteristic of low flow, low insulin conditions as well as high insulin conditions. We interpret this as an extraction fraction of one existing during the low flow conditions of resting skeletal muscle. Under high flow conditions in which the muscle was electrically stimulated there was a decline in extraction at high flow as indicated by the large difference between the first-pass peak counts and the steady state trapped counts (Fig. 4 in Ref. 7). The relationship between skeletal muscle blood flow and the extraction fraction of $^{82}$Rb is shown in Figure 6. As flow increases there is a nonlinear decrease in the first-pass extraction of rubidium.

In Table 1 data for all flow measurements are grouped according to the plasma glucose and insulin levels that existed at the time of the flow measurement. Group I consists of those flows that occurred with normal to slightly elevated plasma glucose with insulin levels typical of the fasted state. Group II are flows also occurring with low insulin but with high plasma glucose levels. Flows measured in Group III occurred during hyperglycemia with insulin levels typical of the 6-8 hr postabsorptive state. Group IV consists of those flows occurring under conditions of normal plasma glucose and high physiologic to pharmacologic levels of insulin.

Figure 7 shows the corresponding data for blood flow based on Groups I-IV defined in Table 1. These four groups were subjected to a one-way ANOVA. Blood flow did not differ significantly over a wide range of plasma glucose levels and relatively low plasma insulin levels. However, when insulin levels reached a high physiologic to pharmacologic range, flow increased significantly as measured by the radiolabeled microspheres. In the same group flow measured by $^{82}$Rb increased slightly, but the increase was not proportional to the increase in flow as measured by the microsphere technique. In addition, there was no apparent decrease in rubidium extraction based on the characteristics of the tissue time-activity curves and the modeling scheme.

Figure 8 shows the linear regression analyses of rubidium estimated flow versus microsphere estimated flow during conditions of low flow, high flow, and high

**TABLE 1**
Flow Groupings According to Plasma Glucose and Insulin

| Group | N | Glucose (m*M*) | Insulin (μU/ml) | Flow (ml/min/100 g) MS | Flow (ml/min/100 g) Rb | Rb extraction (First pass) |
|---|---|---|---|---|---|---|
| I | 7 | 9.6 ± 1.3 | 3 | 3.1 ± 0.70 | 3.5 ± 1.0 | 0.97 ± 0.03 |
| II | 12 | 23 ± 2.0 | 3 | 3.8 ± 0.53 | 4.4 ± 0.58 | 0.99 ± 0.01 |
| III | 8 | 34 ± 3.0 | 14 ± 1.0 | 3.6 ± 0.66 | 4.0 ± 0.31 | 1.0 ± 0.0 |
| IV | 11 | 6.7 ± 0.40 | 131 ± 30 | 7.3 ± 0.93 | 5.6 ± 0.53 | 1.0 ± 0.0 |
| E.S. | 7 | 12 ± 1.2 | 6 ± 0.3 | 28 ± 7.9 | 26 ± 7.3 | 0.38 ± 0.04 |

Values are mean ± s.e.
ES = electrical stimulation.

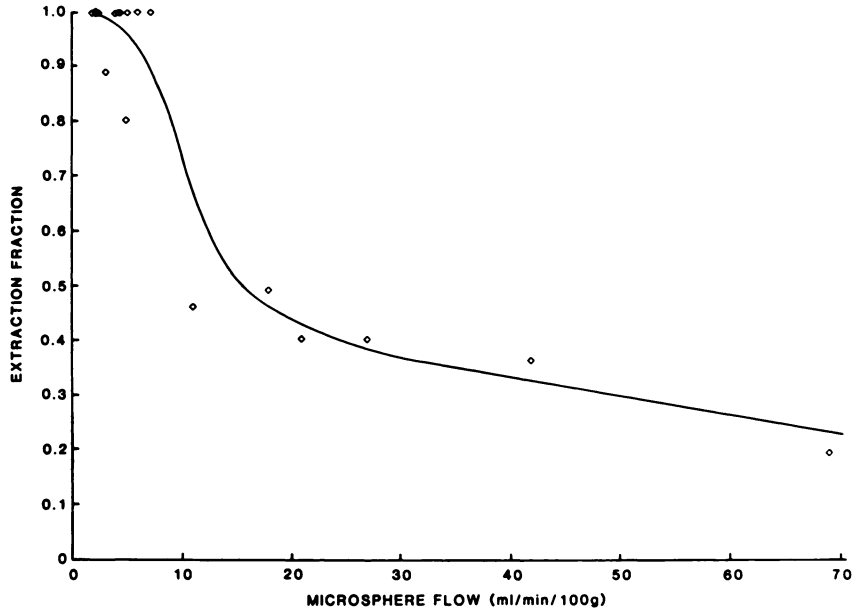


**FIGURE 6**
Nonlinear reciprocal relationship between microsphere determined flow and the extraction fraction for $^{82}$Rb.

insulin. Figure 8A relates microsphere and rubidium flow estimates in which the extraction fraction of $^{82}$Rb was estimated to be one. The best fit regression line force fit through the origin had a slope of 0.99 and r = 0.77 (n = 24). Figure 8B shows the relationship between microsphere flow and rubidium flow in which the tissue extraction was less than one. This included only the high flows elicited by electrical stimulation. The regression line force fit through the origin had a slope of 0.89 and r = 0.92 (n = 7). Comparison of the flow measurements made during high plasma insulin (Fig. 8C) yielded a slope of 0.73 and r = 0.82 (n = 11). When all the data were grouped together, excluding the high insulin experiments, the regression line had a slope of 0.96 and an r value of 0.90 (regression line not depicted in Fig. 8).

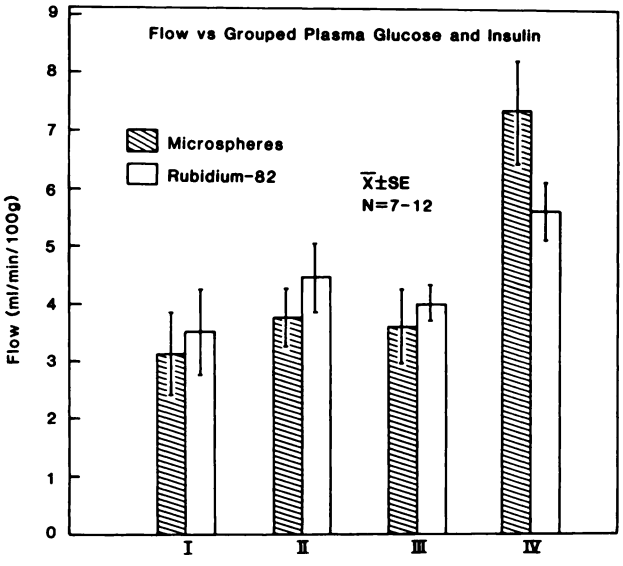


**FIGURE 7**
Skeletal muscle blood flow for Groups I-IV according to the plasma glucose and insulin in levels that existed at the time the flow measurements was made. Group I, low glucose low insulin; Group II, high glucose low insulin; Group II, high glucose moderate insulin; Group IV, low glucose high insulin.

## DISCUSSION

### General Comments

In the present study we have described a method to measure skeletal muscle blood flow in vivo using the positron emitting radioisotope $^{82}$Rb and validated it with radioactive microspheres. We paid special attention to substrate availability and plasma insulin levels, as these can modify not only blood flow but also the transport and extraction of monovalent cations. Both at low flows as well as high flows (20 times higher than control) there was good agreement between the $^{82}$Rb and the microsphere methods as long as plasma insulin levels were not excessively elevated. Furthermore, we found good agreement between values reported here and those of other investigators who have measured skeletal muscle blood flow with microspheres at rest (*20*), during exercise (*21*), and after insulin administration (*11*).

The measurement of blood flow with diffusible tracers requires that the extraction fraction of the tracer be known. We have applied a technique in which the extraction fraction is determined on first pass of the arterial bolus through the region of interest. This is made possible by the characteristics of the isotope, $^{82}$Rb (maximal energy = 3.350 MeV), and the rapid counting

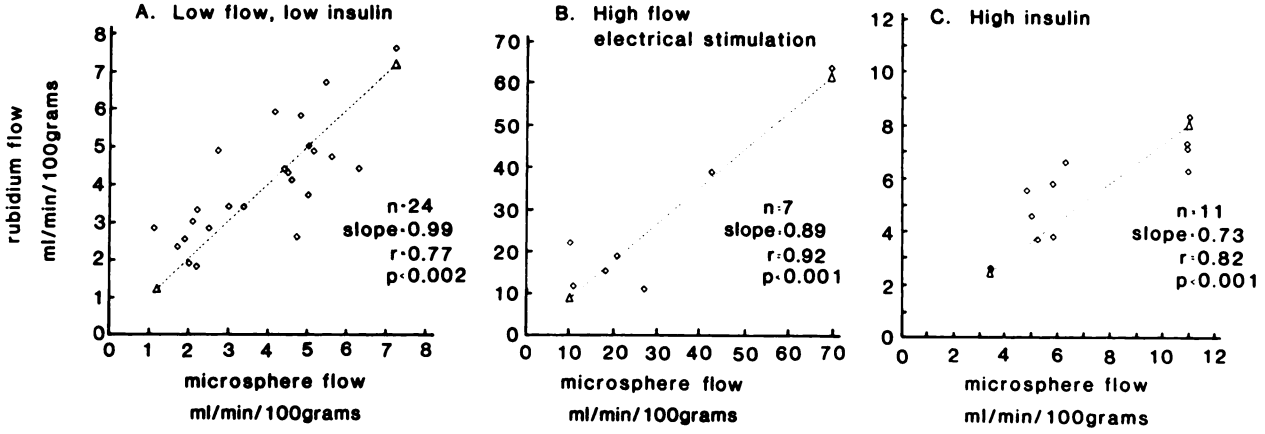


**FIGURE 8**
Linear regression of rubidium versus microsphere flow estimations. All lines are force fit through the origin. Standard error of the estimate (s.e.e.) A = 1.09, B = 7.71, C = 1.28.

and data acquisition system available. Previous applications of this technique have been in the measurement of myocardial flow which is 50 to 100 times greater than resting skeletal muscle flow. Extraction fractions in heart are slightly higher at equivalent flows due to continuous contractile activity necessitating a greater $Na^+/K^+$-ATPase activity. This comparison is somewhat limited however, due to the ischemic conditions necessary to produce myocardial flows equivalent to the high flows seen in postexercise skeletal muscle.

Extraction fractions for $^{86}Rb$ at varying flows have been determined specifically for skeletal muscle by a number of workers in the past (*22,23*). These studies have utilized the perfused, isolated muscle preparation under controlled flow conditions. The bulk of the extraction fraction estimations have been made during continuous infusion of tracer. Under these conditions the estimate of extraction is made under conditions of equilibrium and thus must be corrected for backdiffusion of tracer into the capillary. On the other hand, the first pass flow model provides an estimate of the extraction fraction within the first 20 sec after the tracer reaches the region of interest. This essentially negates the effects of backdiffusion resulting in a somewhat larger estimate of extraction at comparable flows. Friedman (*24*) compared extraction fractions derived from continuous perfusion experiments with those derived from a single injection of $^{86}Rb$ using the perfused hindlimb model. He found the single injection estimates of extraction to be slightly higher than continuous infusion at comparable flows. These values were also slightly higher than those reported by Sheehan and Renkin (*23*).

### Critique of Animal Model

The rabbit thigh muscle preparation used in the present work differs from other skeletal muscle preparations (mainly from rats) on several accounts. First, we used rabbits because they met the size requirements necessary for accurate detection of positron emission from skeletal muscle. Secondly, for accurate detection of positron emission the tissue under study has to be motionless during the time of the data acquisition. This necessitates anesthetizing the animal. General anesthesia was also necessary for catheter placement and muscle exposure.

Anesthesia and surgical trauma profoundly affect blood flow, circulating hormones and metabolites. Neutze et al. (*20*) found skeletal muscle blood flow in anesthetized rabbits to be one half that seen in awake animals. Our flow results agree with those previously reported in anesthetized rabbits. The present data revealed striking changes in circulating metabolites during the first 60-90 min of the experiment in which glucose levels rise dramatically and then return to baseline (Fig. 4). Insulin levels during this time are essentially zero. These changes are presumably due to a stress induced increase in circulating catecholamines and for this reason flow measurements were made after the stress response subsided.

The first-pass flow model requires that the transit time of the tracer bolus through the region of interest is sufficiently small to allow accurate estimates of the extraction fraction. To minimize the bolus transit time through the distal skeletal musculature we injected tracer into the arch of the aorta in small volumes of 2-3 ml. During conditions of high flow, flow was either underestimated by the rubidium method or overestimated by the microsphere method based on the slope of the line for the high flow measurements (Fig. 8B). The discrepancy could be due to the bolus size still being too large thus increasing transit time through the capillaries under the region of the interest.

### Critique of Rubidium Method

Rubidium readily passes through the capillary endothelium, diffuses throughout the extracellular space, and is actively transported across the cell membrane by

the enzyme $Na^+/K^+$-ATPase. For these reasons $Rb^+$ has been referred to as a marker of "capillary or nutritional flow" (*22*).

Flow measurements with rubidium using the first-pass flow model are based on a functional two-compartmental model in which there is a trapped rubidium space and a free rubidium space (*4*). A mathematical model based on the two compartments is used for estimating the extraction fraction of rubidium. The peak-counts modification of the first-pass model determines the extraction fraction by dividing the trapped counts at the time of peak counts by the peak counts (*7*). Under high flow conditions rubidium underestimated flow slightly as evidenced by the slight deviation from unity of the slope of the linear regression line. This could be due to the decreased transit time of the bolus through the capillary causing the venous egress of tracer to become significant and introducing a small error in the estimation of the extraction fraction. The discrepancy between the two methods became even greater when insulin levels were high even though the extraction fraction was determined to be one. When the extraction fraction of rubidium was one (Figs. 2A and 5) and insulin levels were low there was excellent agreement between the two independent methods.

**Effects of Insulin**

While hyperglycemia appeared to have no effect on flow as measured by either method, flow measured by radiolabeled microspheres was increased with administration of high physiologic to pharmacologic levels of plasma insulin. The increased flow response has been shown by others (*11,25*) and is thought to be due to an increase in tissue metabolism and secondary hyperemia (*26,27*). The increase in flow was, however, only significant when flow was measured by radiolabeled microspheres. Rubidium flow measurements did increase but not to the same extent that microsphere flow increased. Since rubidium is a potassium analogue, it was expected to behave similarly under conditions of high plasma insulin. It has been shown by many workers (*10,28,29*) that insulin stimulates the enzyme $Na^+/K^+$ ATPase in vitro which leads to an increase in the uptake of potassium and/or rubidium.

The present results are surprising in view of the above findings because of the underestimation of flow by rubidium as compared to the microsphere flow values. The discrepancy may, however be explained on the basis of the known effects of insulin on cellular function. As stated above, insulin enhances the uptake of rubidium by the cell which, in effect would increase the extraction of rubidium as it passes through the capillary by reducing any diffusion limitations. However, as flow increases, the extraction of rubidium, or any other diffusible tracer, decreases. If extraction is enhanced by insulin, it is likely that the decrease in extraction at higher flows is partially obscured by the enhanced $^{82}Rb$ uptake. The result is an apparent complete extraction of tracer yet an underestimation of flow. Thus, high physiologic to pharmacologic levels of insulin enhance $^{82}Rb$ uptake to such a degree that the normal decrease in extraction was not evident.

Insulin could also be contributing to the discrepancy between rubidium and microsphere estimated flow in another way. On theoretical grounds it is possible that insulin may have differential effects on microsphere flow and rubidium extraction. Because extraction is one, yet flow is underestimated when compared to the microsphere method, insulin may be exerting its influence on precapillary shunts in addition to its effect on the $Na^+/K^+$-ATPase.

## CONCLUSION

We have demonstrated the usefulness of using $^{82}Rb$ as a marker for skeletal muscle blood flow at both low and high flows. Because of the ultra-short half-life of $^{82}Rb$, flow measurements can be rapidly repeated thereby allowing the study of medical and/or physical interventions affecting skeletal muscle blood flow.

Given that rubidium is a monovalent cation and is subjected to the same cellular and molecular forces as potassium, attention must be paid to the circulating insulin levels at the time of the flow measurement. This may be particularly true for a very insulin sensitive tissue like skeletal muscle. In a less insulin sensitive tissue like the heart this may not be of great concern and in the brain it may not be a concern at all. Insulin sensitivity at present however is based on glucose transport and may not be applicable to monovalent cation transport.

We are encouraged by the results of the present study and are currently investigating the usefulness of $^{82}Rb$ tracer flow measurements using intravenous injection and coincidence gamma detection to monitor tissue uptake of radioactivity. Reliable estimation of skeletal muscle blood flow by the coincidence gamma counting technique could develop into a clinical tool for quantitative and functional assessment of peripheral vascular disease.

## NOTES

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