Annals of Biomedical Engineering, Vol. 31, pp. 659–666, 2003
Printed in the USA. All rights reserved.

0090-6964/2003/31(6)/659/8/$20.00

Effect of Blood Flow on Thermal Equilibration and Venous Rewarming

QINGHONG HE,[1] LIANG ZHU,[1] and SHELDON WEINBAUM[2]

[1]Department of Mechanical Engineering, University of Maryland Baltimore County, Baltimore, MD
and [2]Departments of Mechanical and Biomedical Engineering, City College of New York, New York, NY

(Received 17 October 2002; accepted 20 February 2003)

Abstract—In this study we have explored the feasibility of using an isolated rat limb as an animal model for studying countercurrent arterial thermal equilibration and venous rewarming in muscle tissue. Unlike *in vivo* experiments in which animal models have been used for studying thermoregulation or temperature response in tissue under various physiological conditions, isolated organ or tissue provides for better control and more accurate measurement of the blood perfusion rate. It has been shown that the induced perfusion rate in the rat limb can vary from 3 ml/(min·100 g) at normal physiological conditions to 25 ml/(min·100 g) during hyperemic conditions. Temperature distributions along the countercurrent arteries and veins have been measured using fine thermocouple wires. We observed a 25%–78% thermal equilibration along the femoral artery and its branches in intermediate size vessels between 700 and 300 μm diameter. This equilibration depends strongly on the local perfusion rate. In comparison, local perfusion rate plays a minor role in determining the overall venous rewarming in the rat hind limb. Approximately 70%–80% of the heat leaving the artery is recaptured by the countercurrent vein. This agrees well with our previous theoretical and experimental results, which show a dramatic shift in thermal equilibration between the supply artery and vein tissue cylinder and the secondary vessel tissue cylinder as the flow rate changes. © *2003 Biomedical Engineering Society.*
[DOI: 10.1114/1.1569265]

Keywords—Bioheat transfer, Isolated rat hind limb, Thermal equilibration, Venous rewarming.

INTRODUCTION

Although theoretical models have shown that local blood flow distribution and vascular geometry have a profound effect on heat exchange between blood and tissue,[4,12,19,20] there are relatively few experimental studies to validate the theoretical predictions at the level of the supply artery vein (SAV) vessels. Experimental studies have to be designed not only to test the hypotheses introduced in the theoretical models, but also to evaluate the general behavior of blood–tissue heat transfer predicted by the theory. In this study, an experimental approach is developed using isolated rat hind limbs where the local blood perfusion rate in the rat hind limb can be well controlled. To our knowledge, this is the first time the isolated rat hind limb is used in bioheat transfer research.

Several *in vivo* experimental approaches have been developed to study the thermal effect of blood perfusion in tissue. Among them, a variety of two-dimensional tissue preparations have been extensively used to study temperature response to hyperthermia and thermal equilibration along the major blood vessels in these microvascular tissue preparations.[16,17,23,24] Although two-dimensional tissue preparations have the unique advantage of transparency and uniform thickness, the maximum blood vessel size in the tissue is less than 200 μm in diameter. In a recent study by our research group[8] *in vivo* thermal equilibration along mid-sized blood vessels from 300 to 1000 μm in diameter in rat hind limb has been measured. As in most *in vivo* experiments, the range of blood perfusion was not fully controlled, which led to a limited range of blood perfusion rates [3–6 ml/(min·100 g)]. Isolated biological organs such as kidney,[5,10,22] and bovine tongue[6,13] have been used to study the blood flow effect on the tissue temperature field. In some of these organs the primary vascular structure is not countercurrent blood vessel pairs. Therefore, the primary vascular arrangement of their thermally significant vessels is not suitable for studying the contribution of countercurrent heat exchange between closely paired vessels in muscle, the organ which produces most of the body heat. Of particular interest from a conceptual standpoint is the shift in thermal equilibration from the SAV vessels (1000–300 μm diameter) to the secondary (s) vessels[20] (<300 μm diameter) as the flow rate is increased.

The isolated rat hind limb, where most of the thermally significant blood vessels occur as countercurrent pairs, is a good animal model to simulate countercurrent

Address correspondence to Liang Zhu, Ph.D., Department of Mechanical Engineering, University of Maryland Baltimore County, 1000 Hilltop Circle, Baltimore, MD 21250. Electronic mail: zliang@umbc.edu

heat exchange in a tissue cylinder. Previously, the isolated rat hind limb was extensively used as a model for studying skeletal muscle metabolism. The model provides for exquisite control of the metabolism and contractile activity.[3,7,9,14,15,21] In these studies, rat hind limb muscle was perfused via its own vascular network by infusing cell-free media or erythrocyte solution into a major artery such as the abdominal aorta or femoral artery. The exact amount of perfusate can be directly measured by collecting the effluent via the inferior vena cava or the femoral vein so that the error associated with other blood flow measurement techniques in *in vivo* experiments can be minimized. In addition, unlike *in vivo* experiments where temperature varies significantly on the surface of the rat hind limb due to physiological regulation, it is feasible to achieve a relatively uniform surface temperature along the tapered rat limb in *in situ* experiments.

In this study, both the femoral artery and vein are cannulated and the rat hind limb is perfused with a temperature-controlled perfusate. The flow rate in the feeding femoral artery and vein is controlled and measured by timed collection of the perfusate. Temperature variation along the major countercurrent blood vessel pairs and the skin surface of the limb is recorded using thermocouples. The relationship between the thermal equilibration along the major blood vessels and the average perfusion rate in the limb is examined. The feasibility of using isolated rat hind limb as an animal model in bioheat transfer study is evaluated and discussed.

METHODS AND MATERIALS

Animals

Male Sprague–Dawley rats weighting [mean $\pm$ standard deviation (SD)] 473 ± 10 g ($n = 8$) and 287 ± 9 g ($n = 7$) obtained from Charles River Laboratory (Boston, Massachusetts) were used for the experiments. The rats were housed one per cage, in a temperature-controlled room and had access to water and food until the experiments were performed. Each rat was anesthetized with intraperitoneal injections of pentobarbital sodium (40 mg/kg) and was put on a water-jacketed heating pad to keep it warm. The rectal temperature was monitored with a thermocouple inserted into the rectum. Once unconscious, a tracheal tube was inserted to maintain a patent airway. An L-shaped skin incision over the medial aspect of the groin and thigh region was made. The right femoral artery and vein were carefully exposed and separated from the surrounding fascia. The femoral artery and vein were then cannulated with a 26 gauge and 16 gauge Teflon tube (Omega Engineering, Inc., Stamford, Connecticut). Initially, a very slow perfusate flow rate (~ 1 ml/min) through the femoral artery was maintained and controlled by a high-resolution peristaltic pump (World Precision Instruments, Inc., Sarasota, Florida). The rat was then euthanized by a pentobarbital sodium overdose I.P. (150 mg/kg). The experimental protocol was reviewed and approved by the IACUC at University of Maryland Baltimore County.

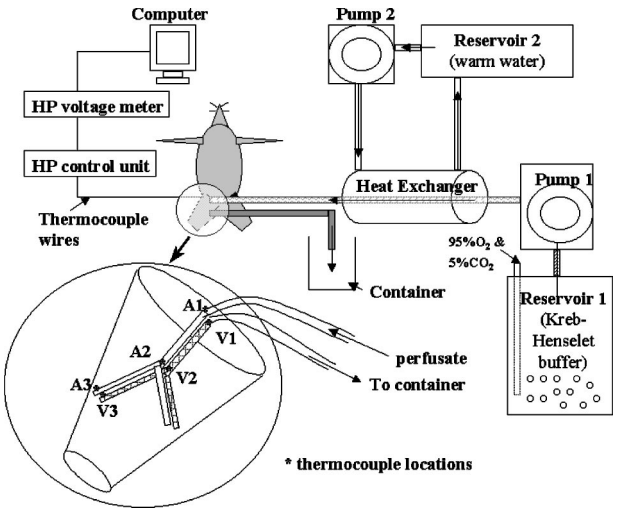

FIGURE 1. Experimental setup of the heat transfer study on an isolated rat hind limb.

Perfusate

Cell-free medium, specifically, Krebs–Henseleit buffer containing 2% bovine serum albumin (BSA) was used as perfusate for the isolated rat hind limb perfusion. The Krebs–Henseleit buffer[21] consists of (in mM): NaCl, 118; KCl, 4.74; KH_2PO_4, 1.18; $MgSO_4.7H_2O$, 1.18; $CaCl_2.2H_2O$, 2.54; $NaHCO_3$, 25; and D-glucose, 11.1. HCL was added to the perfusate to adjust its pH value to 7.3. The perfusate also contains sodium nitroprusside ($10^{-5.5}$ M), which was added to induce a maximal dilation of the vascular bed in the rat hind limb. During the experiment, the perfusate was continuously bubbled with $95\%O_2 - 5\%CO_2$ gas mixture provided by Air Gas, Inc. (Baltimore, Maryland). The perfusate was freshly prepared for each experiment.

Perfusion Apparatus

The experimental set up is illustrated in Fig. 1. The perfusion apparatus consists of a perfusate reservoir placed in a temperature controlled water bath (PB-1400, Boekel Scientific, Inc., Feasterville, PA). The perfusate is pumped by the peristaltic pump and is passed through a countercurrent heat exchanger before it is delivered to the limb. The countercurrent heat exchanger is used to maintain the temperature of the perfusate at 37 °C before it is delivered to the femoral artery. The perfusate is drained via the femoral vein and collected by a plexiglass container. The venous effluent is not pumped back

TABLE 1. Average vessel diameter (mm) for both rat groups before the cannulation.

	A1 (mm)	V1 (mm)	A2 (mm)	V2 (mm)	A3 (mm)	V3 (mm)
500 g	0.70±0.15	1.62±0.28	0.45±0.09	0.58±0.18	0.37±0.07	0.45±0.12
300 g	0.43±0.20	1.20±0.50	0.31±0.03	0.47±0.11	0.30±0.05	0.39±0.09

to the perfusate reservoir. The flow rate of the perfusate is controlled by adjusting the pump speed setting. As reported previously[7] and confirmed in our preliminary study, more than 95% of the perfusate entering the femoral artery perfuses the hind limb and is drained from the femoral vein. Since no edema was observed, the unaccounted for 5% could also be absorbed by tissue other than the perfused hind limb since the limb was not cut from the rat body during the experiment. The steady state flow rate of the perfusate is determined by timed collections of the venous effluent.

Temperature Measurements

The experiment was designed to measure the temperature along the femoral vessels and their subsequent vessel branches. Six high-resolution thermocouples made with individual 50 μm copper and constantan wires are used to measure the vessel temperature at three axial locations along the artery and vein, respectively. Figure 1 gives the schematic diagram of the temperature measuring sites. Among them, one thermocouple, denoted as A1, is inserted into the tip of the tubing cannulated to the femoral artery before the cannulation. The temperature of the femoral vein is measured by another thermocouple (V1) inserted into the tubing cannulated to the femoral vein. The third thermocouple (A2) is placed at the beginning of the saphenous artery where the femoral artery takes a deeper course to the popliteal space approximately 10 mm from location A1. The fourth thermocouple (A3) is attached to the saphenous artery surface approximately 12 mm downstream from A2, as shown in Fig. 1. Another two thermocouples (V2 and V3) are attached to the countercurrent vein at the same axial locations to measure the thermal equilibration in the veins. In addition, two thermocouples are used to measure the limb skin temperature. All thermocouples except A1 and V1 are attached to the surface using cyanoacrylate (''Super Glue'').

Experimental Protocol

After the femoral vessels were cannulated and the perfusate was circulated in the hind limb, the subsequent branches of the femoral vessels were carefully exposed. Thermocouples were inserted into or attached to the blood vessels using cyanoacrylate as described previously.[8] The skin was closed. The rat was then placed in a chamber which was at normal room temperature ($T_{env}=21.5\pm0.2\,^{\circ}\mathrm{C}$). All the temperature data were acquired and recorded with a LABVIEW® program running on a personal computer.

There are seven trials in each experiment representing seven perfusate flow rates in the femoral vessels. Since the rat limbs used in the experiments have a similar size in each rat weight group, each trial also represents a specific average perfusion rate in the limb. Initially, the perfusate flow rate was set at approximately 1.2 ml/min for the 500 g rat group (0.7 ml/min for the 300 g rat group). Previous experiments[7] and preliminary experiments by the authors suggested that a steady state flow rate through the hind limb would be established after approximately 5 min at a given pump speed setting. The temperatures were then recorded continuously by the computer. The perfusate that was drained from the femoral vein was collected and the flow rate was calculated. After all the temperature readings were stable, the perfusate flow rate was then increased approximately 1 ml/min for the 500 g rat group or 0.7 ml/min for the 300 g rat group by adjusting the pump speed setting. After each increment, the temperature would establish a new equilibrium and the procedures described above would be repeated until the final trial corresponding to a perfusion rate w of approximately $25\ \mathrm{ml/(min\cdot 100\ g)}$ was completed.

Statistical Analysis

Perfusate flow rate and temperature at each pump speed setting were analyzed and expressed as mean ±SD. Statistical comparisons were performed using analyses of variance for repeated measures between the initial flow rate ($Q\approx1$ ml/min) and any other perfusate flow rate. Significance was evaluated at the 5% confidence level.

RESULTS

The measured diameters of the arteries for both rat mass groups are listed in Table 1. Before the femoral vessels were cannulated, the femoral artery was approximately 0.43 mm for the 300 g group, while it was 0.7 mm for the 500 g group.

The average perfusion rate w [ml/(min·100 g)] in the rat hind limb is estimated by the mean flow rate (ml/min) in the femoral vessels divided by the mass (g) of the rat hind limb. Table 2 gives the calculated perfusion rates in

TABLE 2. Measured flow rate and the average perfusion rate at different pump speed settings. The average mass of the limb is 32.0 g for the 500 g rat group (19.2 g for the 300 g rat group).

Flow rate (ml/min)		Perfusion rate [ml/(min·100 g)]		Skin temperature (°C)	
300 g	500 g	300 g	500 g	300 g	500 g
0.67±0.02	1.18±0.03	3.5	3.7	28.1±0.9	30.5±1.1
1.37±0.01	2.35±0.08	7.1	7.3	29.3±1.0	30.7±0.8
2.07±0.03	3.29±0.15	10.8	10.3	31.2±0.5[a]	31.0±0.8
2.73±0.03	4.33±0.09	14.2	13.5	31.7±0.7[a]	31.3±0.9
3.38±0.05	5.41±0.07	17.6	16.9	31.7±1.1[a]	31.5±1.0[a]
4.11±0.02	6.34±0.24	21.4	19.8	31.9±0.9[a]	31.7±1.0[a]
4.79±0.08	7.20±0.12	25.0	22.5	32.4±0.4[a]	31.6±1.2[a]

[a]$p<0.05$.

the limb. Experimentally controlled perfusate flow rate in the feeding femoral artery provides a much larger range of the average perfusion rates than in our *in vivo* model.[8] The lower limit of the perfusion rate, 3.5 (or 3.7) ml/(min·100 g), can be viewed as within the normal physiological conditions[2] in skeletal muscle, while the upper limit, 25.0 ml/(min·100 g), can be achieved during hyperthermic treatment in skeletal muscle.[1]

Peripheral flow resistance of the rat hind limb can be evaluated by the arterial–venous pressure difference divided by the perfusate flow rate in the femoral artery. Due to the extremely small perfusate flow rate (1.2–7 ml/min, see Table 2) in the experiment, the calculated Reynolds numbers inside the blood vessels or in the tubes are much smaller than the critical Re_D of 2300 for transition to turbulent flow. Thus, the flows both inside and outside the limb are laminar. Considering that flow resistance in a rigid tube in laminar flow is inversely proportional to the fourth power of the tube diameter, one can draw the conclusion that the flow resistance outside the limb is much smaller than that inside the limb. Figure 2 plots the relationship between the pump speed setting and the perfusate flow rate at each trial for the 500 g rat group. In this experiment, we consider that the speed setting of the peristaltic pump is proportional to the arterial–venous pressure difference. The correlation factor R, which is close to 1, implies a relatively constant overall flow resistance from trial to trial in the rat hind limb, since flow resistance outside the hind limb is much smaller than that inside the limb.

The skin temperatures at different perfusate flow rates are listed in Table 2. The measured skin temperature was elevated as a result of the increase in the perfusion, since more of the warm perfusate can be delivered to the superficial layer of the limb. Figures 3 and 4 give the measured temperature variations along the arteries and veins for the 500 g rat group. Note that no significant temperature difference is found at the arterial entrance at location A1 from trial to trial. The arterial temperature decreases from 37.1 °C at A1, to 34.5 °C at A2, and to 31.9 °C at A3 when the flow rate is 1.2 ml/min. Temperature decay along the arteries from A1 to A3 is only 1.6 °C when Q is equal to 7.2 ml/min. Rewarming in the veins is observed in Fig. 4 in which most of the temperature increase occurs in the smaller vessels between location V2 and location V3, and no significant temperature increase is observed for veins larger than 580 μm diameter (V2).

Figure 5 shows the dimensionless temperature distribution along the femoral artery and its branches for different perfusion rates for the 500 g rat group. The dimensionless temperature T^* is defined as $T^*=(T-T_{skin})/(T_{A1}-T_{skin})$, where T_{A1} is the femoral arterial temperature at location A1 in each trial, and T_{skin} is the mean temperature of the skin at the specific perfusion rate. Since the femoral artery temperature and the skin temperature represent the maximal and minimal temperatures, respectively, within the hind limb, the dimensionless temperature varies from 1 (at location A1) to 0 (at the skin surface). The results show that under normal physiological conditions of 3.7 ml/(min·100 g) (perfusate flow rate $Q=1.2$ ml/min), the temperature decays 78% from location A1 to location A3. In comparison, the

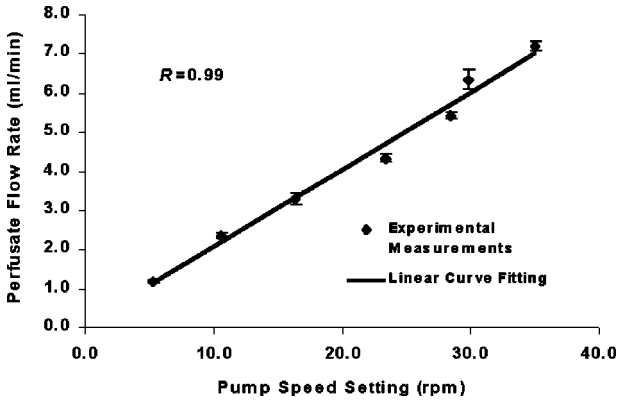

FIGURE 2. Perfusate flow rate (ml/min) in the perfused rat hind limb as a function of the pump speed setting (rpm). Perfusate flow rate is expressed as mean±SD for the 500 g rat group ($n=8$). Line represents the linear regression curve for the perfusate flow rate.

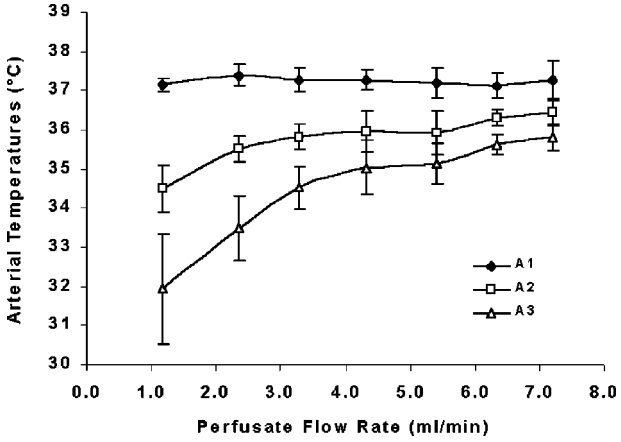

FIGURE 3. Effect of the perfusate flow rate (ml/min) on the artery temperatures at three axial locations: the femoral artery (A1) and its saphenous branches (A2 and A3) for the 500 g rat group. The temperature values are expressed as means±SD (n=8).

temperature decays only 26% when the perfusate flow rate is 7.2 ml/min, which is equivalent to an average perfusion rate of 23 ml/(min·100 g) in the rat hind limb. The temperature distribution along the femoral vein and its subsequent branches is shown in Fig. 6. It is interesting to notice that the dimensionless temperatures at the femoral vein (location V1) do not vary significantly, although the perfusion rates are quite different from trial to trial. The measurements suggest that there is an approximately 70%–80% venous rewarming during countercurrent heat exchange between the artery and vein in the rat limb and the local perfusion has only a minor effect on this value. As already noted most of the rewarming occurs before the blood reenters the femoral vein at location V2 where the venous diameter is approximately 580 μm.

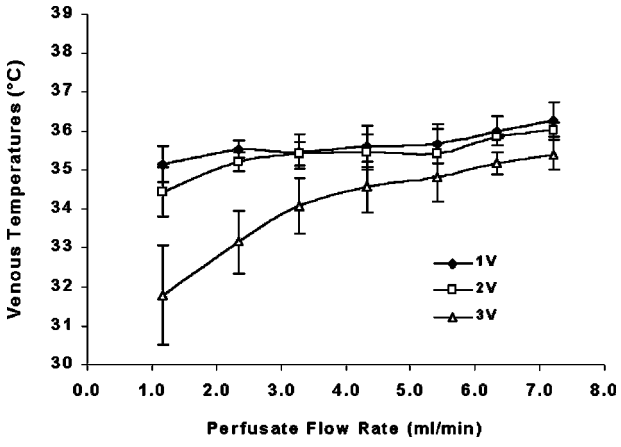

FIGURE 4. Effect of the perfusate flow rate (ml/min) on the vein temperatures at three axial locations: the femoral vein (V1) and its saphenous branches (V2 and V3) for the 500 g rat group. The temperature values are expressed as means ±SD (n=8).

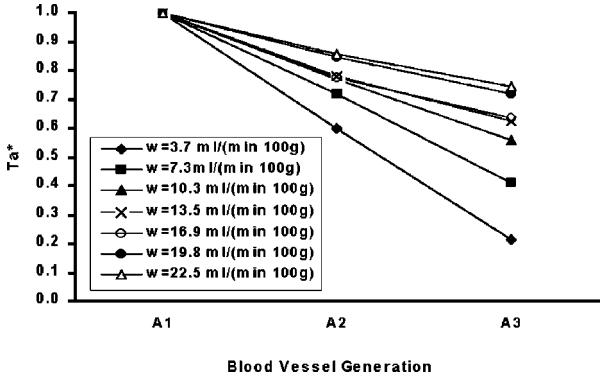

FIGURE 5. Effect of the perfusion rate in the feeding artery on the dimensionless arterial temperatures at different vessel generations for the 500 g rat group. Note that the dimensionless temperature at location A1 is always equal to 1.

Dimensionless temperatures for the 300 g rat group are shown in Figs. 7 and 8. The temperature distribution is similar to that of the 500 g rat groups despite the difference in vessel sizes. The temperature decay from location A1 to location A3 decreased from 81% to 30% when the perfusion rate changes from the normal physiological condition [3.5 ml/(min·100 g)] to the hyperemic condition [25 ml/(min·100 g)]. The overall venous rewarming for the 300 g rat group is similar to that for the 500 g rat group, if one compares Figs. 6 and 8. This is reasonable since the local temperature field is mainly determined by the perfusion rate and the dimensionless vascular geometry.

DISCUSSION

In this study we have explored the feasibility of using isolated rat limb as a possible animal model for exam-

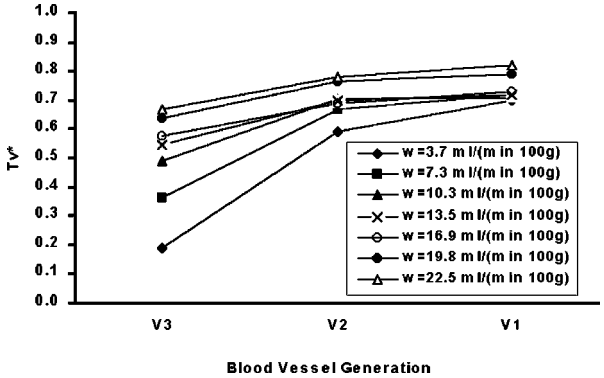

FIGURE 6. Effect of the perfusion rate in the feeding artery on the dimensionless venous temperatures at different vessel generations for the 500 g rat group.

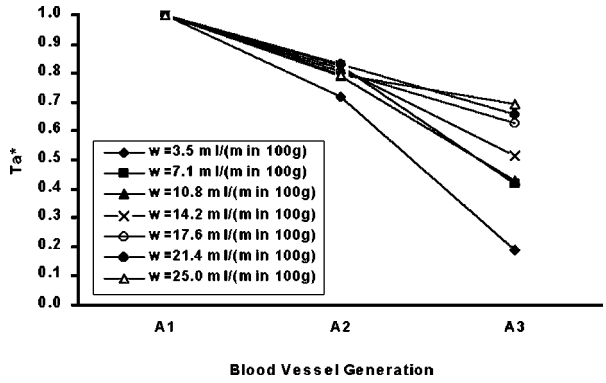

FIGURE 7. Effect of the perfusion rate in the feeding artery on the dimensionless arterial temperatures at different vessel generations for the 300 g rat group. Note that the dimensionless temperature at location A1 is always equal to 1.

ining countercurrent heat exchange in thermally significant blood vessels between approximately 1000 and 300 μm diameter. Unlike *in vivo* animal models that have been used for studying thermoregulation or temperature response in tissue under different physiological conditions, isolated organ or tissue provides for a better control and more accurate measurement of the perfusion rate. Our experimental study has shown that the perfusion rate can vary from normal physiological conditions $[3\ \mathrm{ml/(min\cdot 100\ g)}]$ to hyperemic conditions $[25\ \mathrm{ml/(min\cdot 100\ g)}]$ similar to that induced during hyperthermia therapy.

Under the current experimental setting, preparation of the isolated rat hind limb is easy and the surgery is completed in approximately 15 min. Once prepared, the limb can maintain its freshness for several hours.[3] In this experiment, cell-free perfusate is used to avoid the tedious preparation of erythrocytes and to allow direct control of perfusate flow without the interference of erythrocyte metabolism.[3] BSA is added to maintain the blood vessel permeability for a relatively long time. Previous experiments[21] have tested the effect of different concentrations of BSA including 2%, 4.7%, and 7%, and have shown that a higher concentration of BSA induces a greater flow resistance. Their results suggest that a Krebs–Henseleit buffer containing 2% BSA is a suitable perfusate for most studies of the isolated rat hind limb perfusion.[21] Thus, 2% BSA concentration was selected in our experiments, which provided for a wide range of flow rates and also protected capillary permeability at the same time. Sodium nitroprusside has been added to the perfusate to maximally dilate the vascular bed. Blood tissue thermal interaction is usually determined by both the perfusion rate and vascular geometry. The relatively small change in vascular geometry after maximum dilation during the bioheat transfer experimental study enables one to isolate the effect of the perfusion rate on tissue temperature. Our experimental results indicated a linear relationship between the entrance flow rate and the pump speed setting. This suggests that a relatively constant flow resistance is maintained in the vascular bed in the rat hind limb during the experiments, confirming that there is little change in vascular diameter after sodium nitroprusside is added.

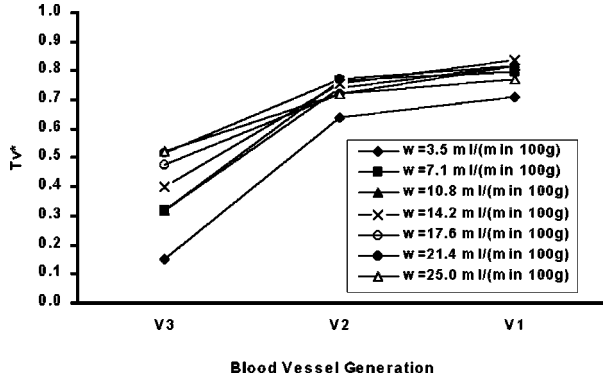

FIGURE 8. Effect of the perfusion rate in the feeding artery on the dimensionless venous temperatures at different vessel generations for the 300 g rat group.

Several possible limitations are associated with using the rat hind limb as an animal model in bioheat transfer studies. The measured perfusion rate in the rat limb is an average value. Other techniques such as the thermal pulse decay method can be used to quantitatively determine the regionally varying perfusion rate in tissue. In this study temperature distributions along the countercurrent artery and vein pairs were measured using 50 μm diameter thermocouple wires. At the locations A1 and V1, thermocouples are inserted directly inside the tubing to measure the perfusate temperature. At other locations, perfusate temperatures are measured on the blood vessel surface. Contact thermal resistance between the probe and the blood vessel surface has been minimized by applying a small amount of cyanoacrylate between them. However, there is still the question as to whether this surface temperature can represent the average temperature inside the blood vessel. This could be investigated by inserting a thermocouple inside the femoral artery and at the same time measuring the surface temperature of the femoral artery. This approach is not very accurate since the cannulated tube made of Teflon is not very thin. The conductive resistance added by the Teflon tube will certainly make the surface temperature of the femoral vessel inaccurate. In our previous *in vivo* experiments,[8] the measured surface temperature at location A1 ($37.72 \pm 0.44\,^{\circ}\mathrm{C}$, $n=9$) was very close to the measured rectal temperature ($37.76 \pm 0.41\,^{\circ}\mathrm{C}$, $n=9$). If the rectal temperature can be viewed as the average blood temperature upstream of the femoral artery, this illustrates that the contact and convection resistance is very small; thus, the measured temperature at the blood vessel surface is a

close estimate of the perfusate temperature.

The temperature distribution measured in this study agrees in general with previous theoretical predictions[25] and *in vivo* experimental data.[8] Thermal equilibration along the femoral artery and its branches varies from 78% ($Q=1.18$ ml/min) to 26% ($Q=7.2$ ml/min) for the 500 g rat group. In the previous *in vivo* experiment, we observed a 64% thermal equilibration from location A1 to location A3 when the blood flow rate was 1.0 ml/min.[8]

Surface temperature can vary significantly in the axial direction (3–4 °C) in the *in vivo* experimental setup.[8] In the current experimental setup the rat was euthanized before the temperature measurements, and thus, the limb temperature was relatively uniform to within 1 °C at the skin surface. Our present measurements agree more closely with our previous theoretical predictions[25] in which a uniform temperature is assumed at the tissue cylinder surface.

Isolated rat limb allows one to observe the shift of tissue–vessel thermal interaction from the small sized vessels to intermediate sized blood vessels when the flow condition is changed. During normal conditions ($Q=1.18$ ml/min), less than 20% of the thermal equilibration occurs in the region containing vessels less than 370 μm in diameter in the 500 g rats. This observation is consistent with the *in vivo* measurment[8] of 24% and the theoretical prediction[25] of 16% in the smaller *s* vessels (<300 μm in diameter). In contrast, the relative contribution of the smaller vessels changes significantly during hyperemic conditions. At the two highest perfusion rates in Fig. 5, approximately 75% of the total thermal equilibration occurs in vessels <370 μm diameter. Our theoretical model[25] predicts that nearly 90% of the tissue–blood thermal equilibration occurs in the *s* vessel tissue cylinders (300–100 μm in diameter) and the microcirculation (<100 μm in diameter) when $w=24$ ml/(min $\cdot$ 100 g). This shift of the primary thermal interaction to smaller vessels in the tissue is also clearly evident in the veins, see Figs. 6 and 8.

One of the important conclusions we have drawn by comparing the experimental data in Figs. 5 and 6 is that the perfusion rate has a much smaller effect on the dimensionless venous return temperature at location V1 than on the arterial temperature decay. The current experiment confirms that the venous return temperature is primarily determined by the local vascular geometry and is nearly independent of the local perfusion rate. In addition, the scaling law used in Zhu *et al.*[25] suggested that the venous rewarming is also independent of the rat weight.

As shown in the newly developed modified Pennes source term,[20] a correction coefficient ε should be introduced into the original Pennes perfusion source term. A correction coefficient ε close to 1 implies no rewarming in the veins, while an ε close to zero implies a perfect countercurrent heat exchange between the countercurrent artery and vein. Our results suggest that between 70% and 80% of the heat leaving the femoral artery and its branches is recaptured by their countercurrent veins in the rat hind limb. The remaining 20%–30% of the heat is absorbed by the tissue. Thus, for the vasculature in the rat hind limb, a correction coefficient between 0.2 and 0.3 should be used in the modified Pennes perfusion source term when this model is applied to simulate the temperature distribution under different physiological conditions. A small value of the correction coefficient also indicates the important role played by the countercurrent heat exchange between the paired artery and vein. In a previous *in vivo* experimental measurement[11] of temperature disturbance in the vicinity of blood vessels between 50 and 500 μm diameter in rabbit hind limbs, Lemons *et al.* reported temperature differences in these primary heat exchange vessels of less than 0.5 °C. These observations were used to support the hypothesis in the Weinbaum–Jiji bioheat theory[19] as to the importance of countercurrent microvascular heat exchange. The current experimental measurement of the temperature difference of the artery and vein agrees well with the previous experimental data[11] in vessel generations (A2 and A3) of <500 μm diameter. In blood vessels >500 μm in diameter, temperature differences between A1 and V1 as large as 2 °C were observed. A relatively large temperature difference indicates a large departure from a nearly perfect heat exchange in the large countercurrent vessels.

For the human muscle, the correction coefficient used in the modified Pennes perfusion term could differ significantly due to the difference in vascular geometry. Previous theoretical analysis[25] suggests a correction coefficient of 0.58 for human limbs. The correction coefficient is largely determined by thermal equilibration along the countercurrent artery and vein pair. Several dimensionless parameters may affect the thermal equilibration along the countercurrent vessels. Those parameters may include the ratio of the vessel length L to the vessel radius r, and the dimensionless tissue cylinder radius, R/r. L/r (120 mm/0.5 mm = 240) and R/r (20.1 mm/0.5 mm = 40.2) in human limb[8] are larger than in the rat hind limb ($L/r=27/0.35=77$, and $R/r=15/0.5=30$). The discrepancies in these dimensionless lengths, as well as differences in the thermal equilibration in the smaller *s* vessels, between human and rat limbs may lead to different correction coefficients. In general, one expects less venous rewarming in the long SAV vessels where the flow rate is high. However, we believe that the correction coefficient for human muscle will also be independent of the blood perfusion rate and the body size. This feature should allow one to use an accurate modified Pennes perfusion term in theoretically

predicting the temperature field in various clinical applications for a given muscle tissue.

In conclusion, this study demonstrates the feasibility of using the rat hind limb to measure heat exchange in thermally significant intermediate sized countercurrent vessels. The isolated rat hind limb is easy to prepare and can maintain its freshness for several hours for experimental study.[3] Good agreement has been shown between the current experimental study and previous theoretical[25] and *in vivo* experimental results.[8] The perfusion rate in the limb is well controlled and the perfusions are accurately measured using the current experimental setup. Perfusion rates could be controlled to simulate normal physiological conditions or hyperemic conditions under hyperthermia treatment.[18]

ACKNOWLEDGMENTS

This research was supported by a Whitaker Biomedical Research Grant to Liang Zhu. It was performed in partial fulfillment of the requirements for the PhD degree from University of Maryland Baltimore County by Q.H.

REFERENCES

[1] Akyurekli, D., L. H. Gerig, and G. P. Raaphorst. Changes in muscle blood flow distribution during hyperthermia. *Int. J. Hyperthermia* 13:481–496, 1997.

[2] Anderson, P., and B. Saltin. Maximal perfusion of skeletal muscle in man. *J. Physiol.* 366:233–249, 1985.

[3] Bonen, A., M. G. Clark, and E. J. Henriksen. Experimental approaches in muscle metabolism: Hindlimb perfusion and isolated muscle incubations. *Am. J. Physiol.* 266:E1–E16, 1994.

[4] Chato, J. Heat transfer to blood vessels. *ASME J. Biomech. Eng.* 102:110–118, 1980.

[5] Crezee, J., and J. J. W. Lagendijk. Experimental verification of bioheat transfer theories: Measurement of temperature profiles around large artificial vessels in perfused tissue. *Phys. Med. Biol.* 35:905–923, 1990.

[6] Crezee, J., J. Mooibroek, C. K. Bos, and J. J. W. Lagendijk. Interstitial heating: Experiments in artificially perfused bovine tongues. *Phys. Med. Biol.* 36:823–833, 1991.

[7] Gorski, J., D. A. Hood, and R. L. Terjung. Blood flow distribution in tissues of perfused rat hindlimb preparations. *Am. J. Physiol.* 250:E441–E448, 1986.

[8] He, Q., L. Zhu, D. E. Lemons, and S. Weinbaum. Experimental measurement of the temperature variation along artery-vein pairs from 200 to 1000 μm diameter in rat hind limb. *ASME J. Biomech. Eng.* 124:656-661, 2002.

[9] Hettiarachchi, M., K. M. Parsons, S. M. Richards, K. A. Dora, S. Rattigan, E. Q. Colquhoun, and M. G. Clark. Vasoconstriction-mediated release of lactate from the perfused rat hindlimb. *J. Appl. Physiol.* 73:2544–2551, 1992.

[10] Kolias, M. C., A. E. Worthington, M. D. Sherar, and J. W. Hunt. Experimental evaluation of two simple thermal models using transient temperature analysis. *Phys. Med. Biol.* 43:3325–3340, 1998.

[11] Lemons, D. E., S. Chien, L. I. Crawshaw, S. Weinbaum, and L. M. Jiji. The significance of vessel size and type in vascular heat transfer. *Am. J. Physiol.* 253:R128–R135, 1987.

[12] Pennes, H. H. Analysis of tissue and arterial blood temperatures in the resting human forearm. *J. Appl. Physiol.* 1:93–122, 1948.

[13] Raaymakers, B. W., J. Crezee, and J. J. Lagendijk. Comparison of temperature distributions in interstitial hyperthermia: Experiments in bovine tongues versus generic simulations. *Phys. Med. Biol.* 43:1199–1214, 1998.

[14] Ruderman, N. B., C. R. S. Houghton, and R. Hems. Evaluation of the isolated perfused rat hindquarter for the study of muscle metabolism. *Biochem. J.* 124:39–651, 1971.

[15] Shiota, M., and T. Sugano. Characteristics of rat hindlimbs perfused with erythrocyte- and albumin-free medium. *Am. J. Physiol.* 251:C78–C84, 1986.

[16] Song, J., L. X. Xu, S. Weinbaum, and D. E. Lemons. Enhancement in the effective thermal conductivity in rat spinotrapezius due to vasoregulation. *ASME J. Biomech. Eng.* 119:461–468, 1997.

[17] Song, J., L. X. Xu, S. Weinbaum, and D. E. Lemons. Microvascular thermal equilibration in rat spinotrapenius muscle. *Ann. Biomed. Eng.* 27:56–66, 1999.

[18] Valvano, J. W., S. Nho, and G. T. Anderson. Analysis of the Weinbaum–Jiji model of blood flow in the canine kidney cortex for self-heated thermistors. *ASME J. Biomech. Eng.* 116:201–207, 1994.

[19] Weinbaum, S., and L. M. Jiji. A new simplified bioheat equation for the effect of blood flow on local average tissue temperature. *ASME J. Biomech. Eng.* 107:131–139, 1985.

[20] Weinbaum, S., L. X. Xu, L. Zhu, and A. Ekpene. A new fundamental bioheat equation for muscle tissue: Part I–blood perfusion term. *ASME J. Biomech. Eng.* 119:278–288, 1997.

[21] Wu, Z. Y., L. P. Rivory, and M. S. Roberts. Physiological pharmacokinetics of solutes in the isolated perfused rat hindlimb: Characterization of the physiology with changing perfusate flow, protein content, and temperature using statistical moment analysis. *J. Pharmacokinet Biopharm.* 21:653–688, 1993.

[22] Xu, L. X., M. M. Chen, K. R. Holmes, and H. Arkin. The theoretical evaluation of the Pennes, the Chen–Holmes and the Weinbaum–Jiji bioheat transfer models in the pig renal cortex. *ASME, WAM, Atlanta,* HTD-189:15–22, 1991.

[23] Zhu, L., D. E. Lemons, and S. Weinbaum. A new approach for prediction the enhancement in the effective conductivity of perfused muscle tissue due to hyperthermia. *Ann. Biomed. Eng.* 23:1–12, 1995.

[24] Zhu, L., D. E. Lemons, and S. Weinbaum. Microvascular thermal equilibration in rat cremaster muscle. *Ann. Biomed. Eng.* 24:109–123, 1996.

[25] Zhu, L., L. X. Xu, Q. He, and S. Weinbaum. A new fundamental bioheat equation for muscle tissue: Part II–temperature of SAV vessels. *ASME J. Biomech. Eng.* 124:121–132, 2002.