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# Effect of Flow Rate and Glucose Concentration on Glucose Uptake Rate by the Rat Limb (39597)

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Skeletal muscle is responsible for about 30% of the resting oxygen consumption (1), and thus the rate of nutrient uptake by this tissue can greatly influence the pool of nutrients available to other tissue. Free fatty acids are the great source of fuel, but resting skeletal muscle also consumes glucose. While only 10–37% of the oxygen consumption of resting skeletal muscle has been attributed to glucose oxidation (2, 3), the importance of muscle as a "glucose sink" can be realized in diabetes mellitus when the glucose uptake rate of skeletal muscle is markedly reduced.

*In vivo* a number of factors can influence the rate of glucose uptake by skeletal muscle. The permeability of muscle to glucose and the effect of insulin have undoubtedly received the most attention. No studies could be located in which the effect of flow rate on the glucose uptake rate by skeletal muscle was studied, although this parameter has been investigated in the brain (4, 5).

A growing number of metabolic studies employ perfused muscle preparations in which flow rate to the muscle is set to maintain the desired arterial pressure (6–9). Often these flow rates are much above the physiological range for resting muscle blood flow. What effect, if any, this has on the glucose uptake rate of muscle has not been ascertained. The purpose of the present investigation is to determine the influence of glucose concentration and flow rate on the rate of glucose uptake by the perfused rat hindlimb (basically a muscle preparation).

*Materials and methods. Surgical preparation.* Male rats (Charles River), 321 ± 12 g (SD) ($n$ = 31), were fasted overnight prior to surgery but were given free access to water. Vessels not supplying the hindlimb were ligated (only one limb was perfused), and the aorta and vena cava were then cannulated. More extensive details of the surgical preparation can be found elsewhere (10).

*Perfusion apparatus.* The environmental chamber and the arterial reservoir-oxygenator have previously been described by Zivin and Snarr (5). The perfusate was pumped at the desired flow rate by a variable speed microinfusion pump (Holter Model RL 175) from the arterial reservoir through a glass filter packed with Dacron. The Dacron was replaced at the beginning of every experiment and all tubing was flushed with 0.9% NaCl to remove loose particles of Dacron. A few centimeters beyond the filter, a bubble trap was inserted in the line. The perfusate was then pumped through the arterial cannula into the rat's hindlimb. The bubble trap contained a side arm connected to a Statham pressure transducer. Perfusion pressure was recorded on a Beckman RB Dynograph.

The venous effluent flowed from the vena cava into the venous cannula. This cannula passed outside the chamber and the venous perfusate flowed into a venous reservoir. Perfusate samples were taken from the arterial reservoir and venous cannual for analysis. The perfusate was not recirculated. An oxygen electrode (Beckman) was inserted in the venous line in preliminary experiments to determine if the limb could maintain a steady state rate of oxygen consumption.

*Perfusion medium.* The perfusion medium consisted of Krebs Ringer bicarbonate buffer, bovine albumin (Armour, fraction V), dextran (Sigma, MW 70,000–80,000), and washed dog erythrocytes (5). The artificial blood had a hematocrit of 32–34%, 2% albumin, 2.4% dextran, and 50–400 mg% glucose. Porcine insulin, 700 μU/ml, was also added to the perfusate. This dose of insulin should given muscle a near maximal glucose permeability (6).

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*Analysis of perfusate.* The glucose concentration in arterial and venous "plasma" samples was determined using a Technicon autoanalyzer employing the ferricyanide reduction method (11). The glucose uptake rate was calculated by multiplying the arteriovenous plasma glucose difference by the plasma flow rate. The plasma values were used in this calculation since it was found that 98% of the glucose added to the perfusate was recovered in the plasma several hours after adding the glucose, irrespective of whether the perfusate was at 37 or 4°. This recovery was found to be constant and independent of the exogenous glucose concentration. The reason the glucose did not penetrate the erythrocytes is unknown; however, it may be related to the fact that the cells were stored in an ACD solution and thus were exposed to a high glucose concentration for several days prior to use. There was no drop in the perfusate glucose concentration during its passage through the perfusion system, indicating no significant glucose utilization by the erythrocytes. The perfusate samples were centrifuged and analyzed for glucose immediately after the samples were taken.

All glucose uptake rates were expressed per 22.5 g of tissue, which was the average weight of the perfused limb tissue. The glucose uptake rates showed no correlation with leg weights over the narrow range of rat body weights employed.

*Results. Development of a stable preparation.* Preliminary studies were carried out in order to ascertain the stability of the preparation with respect to glucose uptake rate, venous oxygen tension, and vascular resistance. It was found that inclusion of insulin in nonrecirculated perfusate resulted in a preparation that reached a steady state rate of glucose uptake after 30 min and remained stable for an additional 270 min.

The venous oxygen tension was found to remain in the *in vivo* range, over a 5-hr perfusion period, and did not show a progressive increase as may be expected in a deteriorating preparation.

Vascular resistance (perfusion pressure/flow) remained stable for the first 3 hr of perfusion, but began to rise slowly after this time. Thus, the length of all experiments reported in this study was no longer than 3 hr.

*Effect of flow rate on glucose uptake rate.* The data relating the effect of plasma flow to the rate of glucose uptake by the limb are shown in Fig. 1. The relationship appeared hyperbolic and was therefore plotted as reciprocal uptake versus reciprocal flow for possible rectification (undoubtedly other equations could be fitted to the curve). A straight line resulted and when fitted to these rectified data by linear regression, had an intercept of 1.51 and a slope of 0.724. From these parameters, the coefficients of the hyperbola were calculated:

$$\text{Glucose uptake rate} = \frac{0.662\ (\text{plasma flow})}{0.478 + \text{plasma flow}}$$

*Effect of glucose concentration on the rate of glucose uptake.* Four different flow rates were employed to define the relationship between the glucose concentration and the rate of glucose uptake, the flow rate in any one experiment being kept constant. The glucose uptake rate was found to be linearly related to the arterial plasma glucose concentration at all four flow rates (Fig. 2 A–D). Linear regressions were done on each set of data, with slopes and intercepts indicated in Fig. 2. The intercepts were found to

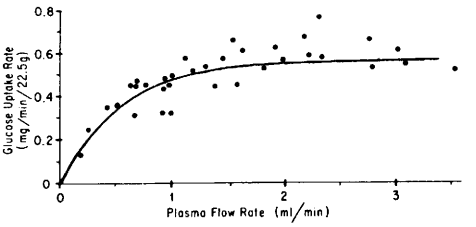


FIG. 1. Relationship between plasma flow rate and the rate of glucose uptake by the perfused rat hindlimb. The perfusate was not recirculated, plasma glucose concentration was 100 mg/100 ml, and the perfusate contained 700 μU/ml of insulin. Data shown are from six rats. Each point is an average of three determinations (taken at 5-min intervals) at the indicated flow rate for one rat. Approximately six different flow rates were used for each rat. These data, as well as those shown in Fig. 2, were collected between 30 and 180 min of perfusion. The flow rates were randomized as to the time from the start of the experiment in which a particular flow rate was chosen.

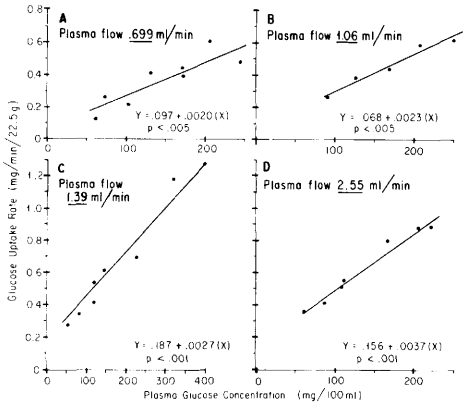


FIG. 2. Linear relationship between the rate of glucose uptake and the plasma glucose concentration at four flow rates. Each point is an average of three glucose determinations from one rat. Data shown at each flow rate are from two rats. Each point is an average of three glucose determinations taken at 5-min intervals during an experiment. The glucose concentration was changed 3–4 times in each experiment. The concentrations were randomized as to the time from the start of the experiment a glucose concentration was chosen to be run. The flow rate in each experiment was kept constant.

be significantly different from zero ($P < 0.005$) at the four flow rates studied, indicating that at very low glucose concentrations, the uptake rate is probably curvilinear. However, since no data were taken in this range, only the straight part of the curves were analyzed. In panel C, Fig. 2, it will be noted that the plasma glucose concentration was raised substantially higher than for the other three flow rates tested; hence, the scale on the abscissa is slightly different. It can be seen (Fig. 2C) that the glucose uptake rate remains linearly related to plasma glucose concentration up to 400 mg%.

*Discussion.* It has been shown that both the glucose concentration and the flow rate influence the rate of glucose uptake by muscle.

The steady state glucose uptake rates reported in this study are a result of glucose transport across the capillary membrane and transport into the muscle. Data from this study do not permit separation of the component of glucose transport due to muscle membrane from that due to the capillary membrane. However, the influence of the capillary on glucose transport is likely to be small compared to the influence of the muscle membrane on glucose uptake (12).

The glucose uptake vs plasma glucose concentration relationship was linear in the narrow range of glucose concentration chosen in this study (*in vivo* range). Daniel *et al.* (13) have shown that raising the concentration of glucose in the plasma will cause saturation of the mechanism by which glucose is transported into muscle. This group found the $K_t$ (glucose concentration at which the glucose entry rates were half maximal) was about 600 mg/100 ml, whereas in the insulin-treated animal, the rate of glucose entry into the muscle rose in direct proportion to the plasma glucose concentration up to a concentration of at least 1440 mg/100 ml (13). Thus, in our study it is probable that the linear relationship between glucose uptake rate and concentration will hold well above the glucose concentrations tested.

The saturating relationship between glucose uptake and flow rate may be due to several possibilities. The first possibility is that as flow rate increases, the venous glucose concentration and interstitial glucose concentration approach a maximum, this being equal to the arterial concentration.

Another possibility to explain the saturating relationship between flow and uptake rate is arteriovenous shunting (14). It has been suggested that muscle may contain two vascular circuits situated in parallel, composed of vessels of two different types. It is hypothesized that one type, which receives most of the flow at low flow rates, is composed of capillaries where exchange takes place (14–16). As the rate of flow increases it is thought that more blood may be shunted through a parallel vascular circuit, this circuit being composed of vessels not thin enough for solute exchange. Thus, it may be that as flow rate increases, more blood transverses the nonexchangeable vessels, and glucose uptake rate reaches a maximum.

It is not possible to determine which of these hypotheses or combination of hypotheses is responsible for the relationship seen between flow rate and glucose uptake rate in the present investigation. But one

point is certain, the limitation of the flow-dependent increase in the rate of glucose uptake is not caused by saturation of a transport step at the muscle cell, for a saturable step would also be affected by an increase in arterial glucose concentration.

The hyperbolic relationship between blood flow and glucose uptake rate appears to be of physiological importance. The best estimate of blood flow in the femoral artery in the resting rat hindlimb is 1.4 ml/min for a 500-g rat (17) (leg wt about 30 g), which is a plasma flow of approximately 0.8 mg/min/30 g (assuming a hematocrit of 42%) or about 0.6 ml/min/22.5 g. At this flow rate, glucose uptake rate falls at the "knee" of the hyperbola (Fig. 1). Doubling the *in vivo* flow would by itself give a 25% increase in the rate of glucose uptake according to the results of this investigation. Thus, deviation from the resting flow rate *in vivo* should give corresponding changes in the rate of glucose uptake and may be partially responsible for the increased rate of glucose uptake seen during exercise. However, the rate of glucose uptake by skeletal muscle has been reported to increase as much as 10 times during exercise (18), and therefore an increase in blood flow rate would account for only a small part of the increased glucose uptake rate seen during exercise.

It should be pointed out that while in the rat it appears that increasing the blood flow beyond the *in vivo* resting blood flow will increase the glucose uptake rate, this may not be true for the skeletal muscle of all species. Indeed, it depends where on the curve (Fig. 1) the resting "operating point" falls for the skeletal muscle of each species.

In light of these results, the practice of maintaining a desired arterial pressure and allowing flow rate to change passively when doing metabolic studies on perfused rat muscle preparations should be reevaluated.

*Summary.* This investigation has defined a relationship that glucose concentration and flow rate have upon the rate of glucose uptake by the resting perfused rat hindlimb. The glucose uptake rate is linearly related to the perfusate glucose concentration within the range of glucose concentration studied (50–400 mg%). It has also been determined that glucose uptake rate is hyperbolically related to the perfusate flow rate. It appears as though the glucose uptake rate is flow limited for rat skeletal muscle at rest.

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