*Journal of Pharmaceutical & Biomedical Analysis*
Vol. 11, No. 11/12, pp. 1121–1127, 1993
Printed in Great Britain

0731-7085/93 $6.00 + 0.00
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# Determination of endogenous ions in intercellular fluid using capillary ultrafiltration and microdialysis probes*

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**Abstract**: Capillary ultrafiltration probes are novel sampling tools for continuously monitoring small molecules in the extracellular fluid of awake animals. Capillary ultrafiltration uses a vacuum applied to hydrophilic membrane fibres and extracts intercellular fluid and quantitatively recovers many small hydrophilic molecules. The effects of continuously removing a small amount of fluid from the interstitial space are not known. The concentration of sodium, potassium, calicium and inorganic phosphorus were determined in the collected ultrafiltrates from subcutaneous tissue. These values were compared to literature values and to concentrations determined for the same animals using microdialysis. The concentrations of sodium, potassium, calcium and inorganic phosphorous were found to be 140 ± 4, 3.7 ± 0.1, 1.1 ± 0.1 and 1.7 ± 0.1 mM, respectively, in the subcutaneous ultrafiltrates obtained from rats. These corresponded very well with literature values and microdialysates obtained, using pure water as the perfusate, in subcutaneous tissue. The concentration of sodium and potassium were determined to be 142 ± 2 mM and 3.6 ± 0.2 mM, respectively, for the dialysates. Hyperinsulinemic-induced decrease in intercellular potassium levels under a euglycemic clamp were monitored using capillary ultrafiltration probes in rats to further validate this technique for monitoring small molecule dynamics in the intercellular space. The intercellular level of potassium in rats decreased from 3.6 ± 0.5 to 2.6 ± 0.3 mM after an acute dose of pork insulin.

**Keywords**: *Capillary ultrafiltration; microdialysis; intercellular ion concentration;* in vivo *sampling; subcutaneous tissue; potassium uptake.*

## Introduction

Capillary ultrafiltration probes (UF probes) are unique compared to all other *in vivo* sensors or sampling probes due to the active collection process. All other sensors rely on diffusion of the analytes to the probe through the interstitial space. Diffusion has been found to be the rate limiting factor [1, 2]. UF probes actively extract fluid and analytes from the interstitial space. The ultrafiltrate collected is thus a representative sample of the intercellular fluid [3, 4]. Capillary ultrafiltration has been shown to be a useful sampling method for monitoring therapeutic drug dynamics and pharmacokinetics in awake, freely-moving animals [4]. Capillary ultrafiltration probes are useful because they provide greater than 95% recoveries for many small analytes. The *in vivo* recovery can be corrected with *in vitro* calibration. The recovery is flow rate independent. The impact of removing intercelluilar fluid on the physiological balance is an obvious concern during this process.

The tissue of choice for capillary ultrafiltration studies has been the subcutaneous tissue due to the large area available and high degree of vascularization, thus providing a significant amount of fluid to be sampled. Blood capillaries are rarely more than 20 μm away from any single functioning cell [5]. The interstitial fluid is constantly in communication with the blood stream through the capillaries. A continuous transfer of fluid and small molecules between the blood and intercellular fluid takes place. In addition, the intercellular fluid is also in constant flux with the intracellular fluid of the cells and the terminal lymphatic ducts are involved in the removal of wastes from the interstitial space. This dynamic system insures the delivery of nutrients and removal of wastes from the body on a cellular level. The processes involved are diffusion, pressure differentials (both osmotic and

*Presented at the 'Fourth International Symposium on Pharmaceutical and Biomedical Analysis', April 1993, Baltimore, MD, USA.
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hydrodynamic), and active transport mechanisms. All of these processes are part of a well regulated, but delicately balanced system [6].

Figure 1 illustrates a typical ultrafiltration probe used for subcutaneous tissue studies. The introduction of a capillary ultrafiltration probe into the interstitial space adds an additional dimension to the system. Figure 2 illustrates the possible processes taking place. The applied vacuum of the ultrafiltration probe removes fluid from the interstitial space requiring the blood capillaries to constantly replenish the fluid. The UF probes are quite large when compared to the blood capillaries and cells in the tissue being sampled, the relative size is shown in Fig. 3. The membrane itself is four to five times as thick as the diameter of a single blood vessel. The bulk flow out of a capillary

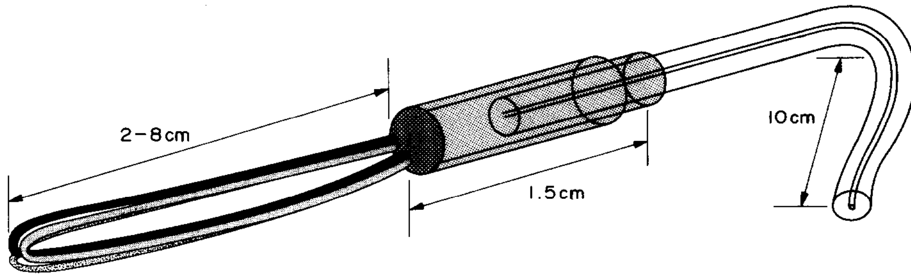


**Figure 1**
An illustration of a typical capillary ultrafiltration probe used for implantation into subcutaneous tissue.

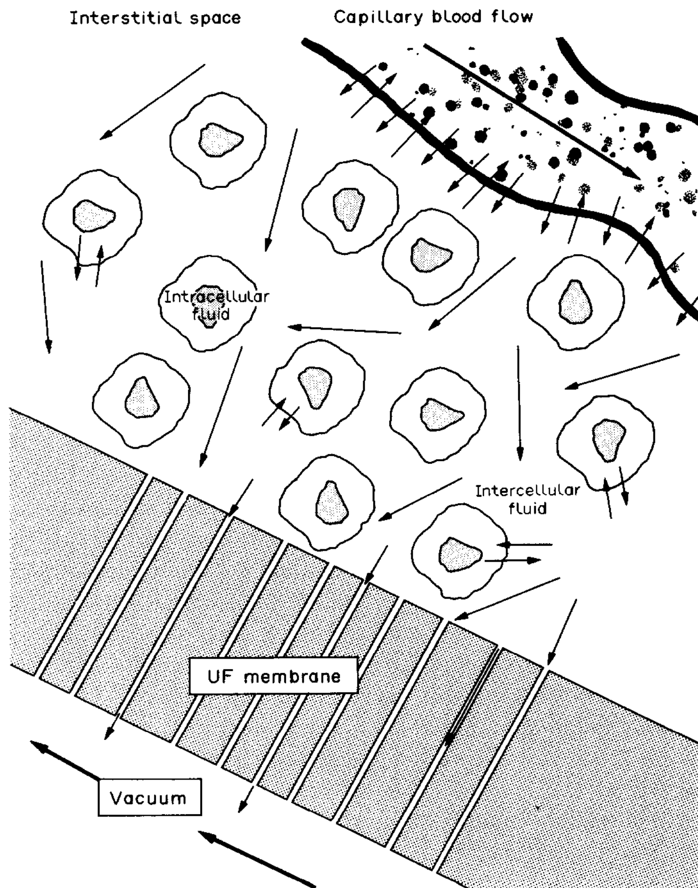


**Figure 2**
A schematic illustration of the interface between the blood capillaries, interstitial space and a capillary ultrafiltration probe. (The components are not drawn to scale.)

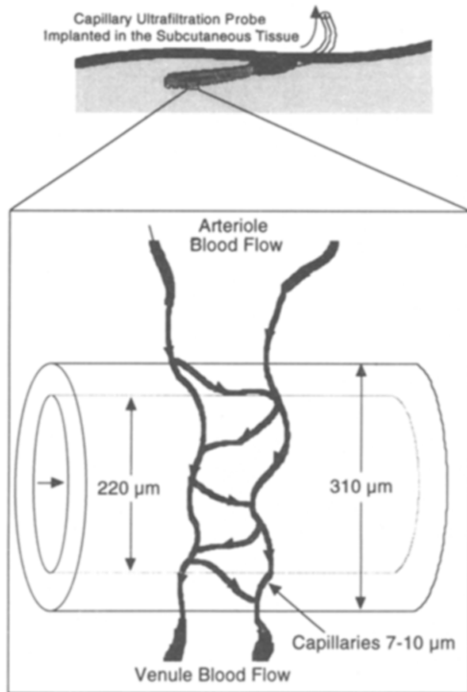


**Figure 3**
A schematic illustration of the relatively large size of the ultrafiltration membrane in comparison to blood capillaries.

ultrafiltration probe is between 1 and 3 µl min$^{-1}$ when used in subcutaneous tissue, however the amount of fluid removed from a specific volume of tissue is quite small. The ultrafiltration process removes 50 pl min$^{-1}$ for every µm of membrane. The fluid removed is from the intercellular space and the correlation to blood levels is very good for many compounds [3]. One concern is whether the ultrafiltration process will disrupt the equilibrium between the blood capillaries and intercellular fluid, introducing alterations to the ion content of the interstitial fluid.

The concentration of many ions in the intercellular and intracellular fluids are quite different. Sodium and calcium are present at very low concentrations in the intracellular fluid when compared to the intercellular fluid. On the other hand, potassium and inorganic phosphates are present in much higher concentrations inside the cell. The amount of protein in the blood stream is fairly large, whereas in the intercellular fluid it is very low. The approximate values for these substances in the three fluids are listed in Table 1. Potassium, sodium and calcium are very well regulated in all animals, with calcium being considered one of the few 'universal' constants in the body [7]. The regulation of potassium and sodium are closely related and active transport mechanisms for the two are dependent on each other [8]. Analysis of the ion concentrations in ultrafiltrates would reveal if the active transport process of ultrafiltration caused the rupture of cells or blood capillaries.

The objectives of this study were four-fold. The first concern was the determination of sodium, potassium, calcium and inorganic phosphorous concentration in capillary ultrafiltrates obtained from the subcutaneous tissue of rats. Suitable methods of analysis for the ions were needed that required less than 15 µl of sample. Second, a comparison of known values for blood and intercellular fluid for the above ions was made with values determined using capillary ultrafiltration. Third, insulin-dependent potassium uptake was monitored in non-diabetic animals to insure controlled dynamic measurements could be observed. Finally, a comparison of capillary ultrafiltration and microdialysis (a diffusion-dependent technique) using pure water as a perfusate

**Table 1**
Endogenous ions in the intracellular and intercellular fluid compared with intercellular fluid ion concentrations determined by capillary ultrafiltration

| Ion* | Intracellular† | Plasma‡ | Intercellular† | Capillary ultrafiltration |
|---|---|---|---|---|
| Sodium | 10 | 135–145 | 146 | 140 ± 4§ |
| Potassium | 150 | 3.5–5 | 4 | 3.7 ± 0.1‖ |
| Calcium | <0.5 | 2.1–2.6 | 1.25 | 1.1 ± 0.1¶ |
| Phosphates | 70 | 1–1.5 | 1 | 1.7 ± 0.1** |

*All values reported in mM.
†From ref. 7.
‡From ref. 21.
§Determined in 10 animals (mean ± SEM).
‖Determined in 17 animals (mean ± SEM).
¶Determined in five animals (mean ± SEM).
**Determined in six animals (mean ± SEM).

for the monitoring of intercellular ions was made.

## Experimental

*Determination of sodium and potassium*

The determination of sodium and potassium was accomplished using ion selective electrodes (ISE). 'Cardy' sodium and potassium meters (Fisher Scientific, PA) were used. Sodium samples were diluted 50 times in double distilled water that showed no response when applied alone. Potassium samples were diluted three times in distilled water. Sodium samples (4 μl) were diluted to a total volume of 200 μl. Potassium samples (15 μl) were diluted to a total volume of 45 μl. The ion meter was calibrated using a three point calibration before each use. After applying the sample the meter was allowed to stabilize for 20–30 s before taking a reading.

*Determination of calcium*

Calcium was determined using the spectrophotometric quantitation of the calcium–cresolphthalein–complexone complex at 574 nm. A calcium determination kit (Sigma, St Louis, MO) was used. The kit contained the calcium binding reagent (*o*-cresolphtalein complexone 0.024%, 8-hydroxyquinoline 0.25%) and calcium buffer (2-amino-2-methyl-1,3 propanediol, 500 mM). These were mixed in a 1:1 ratio. Sample (10 μl) was added to 1 ml of the mixture and agitated. The absorbance was monitored at 574 nm for a 30 s period using a Hewlett–Packard 8450 UV–vis diode array spectrophotometer. Calibration was done using three calcium standards of 5, 10 and 15 mg $dl^{-1}$.

*Determination of inorganic phosphorous*

Inorganic phosphorous was determined using the spectrophotometric quantitation of the unreduced phosphomolybdate complex in acid solution at 340 nm. A phosphorous determination kit (Sigma, St Louis, MO) was used. The kit contained the phosphorus reagent (ammonium molybdate 0.40 mM in sulphuric acid with surfactant). The reagent (1 ml) was mixed with 10 μl of sample. The absorbance was monitored at 340 nm for a 30 s period using a Hewlett–Packard 8450 UV–vis spectrophotometer. Calibration was done using three phosphorous standards of 1, 5 and 15 mg $dl^{-1}$.

*Surgical procedures*

All animal experiments were conducted using 4–6 week old Hooded Long Evans rats (Harlan-Sprague Dawley, Indianapolis, IN). The surgical procedures, drug administration, and euthanasia were all approved by the Purdue Animal Use and Care Committee. The animals were housed at the small animal facility, in the Department of Biology, in a temperature controlled room under a 12 h light–dark cycle. Food and water were provided *ad libitum*.

Subjects were anaesthetized with a 10:1 (100 mg $ml^{-1}$) mixture of ketamine–xylazine (1 ml $kg^{-1}$). For the implantation of capillary ultrafiltration (UF-3-16 PAN, Bioanalytical Systems, BAS) or microdialysis probes (prototype DL-6-RC, BAS) a 5 mm incision was made in the back between the shoulders. A second incision was made in the back of the animal 8–10 cm posterior to the shoulders. A thin walled 13 gauge needle was inserted through the two incisions. The probes were conditioned in dilute solutions of ethanol–isopropanol in water in order to hydrate the membrane, remove the glycerol, and sterilize the probe before implantation. The probe was inserted into the needle with the conduction tubing toward the head of the animal. While holding the probe in place by the Teflon tubing, the needle was then carefully removed through the distal incision, leaving the probe in the subcutaneous tissue. The probe was sutured to the skin to secure it. The two incisions were then sutured closed.

For the implantation of a jugular cannulae a 10 mm incision was made between the centre of the chest and right shoulder. The underlying tissue was cleared so that the pectoral muscle and jugular vein were exposed. A 5 cm length of suture was threaded through the pectoral muscle distal to the jugular vein. A split-tubing introducer, with an introducer needle (BAS/CMA) placed inside, was inserted through the muscle into the jugular vein. A 30 cm piece of PE-10 tubing (Scientific Products) was inserted into the split tubing and sutured to the muscle. The split tubing was then removed by pulling it apart around the PE-10 tubing. The PE-10 tubing was placed through a thin-walled 13-gauge needle inserted through the subcutaneous tissue exiting between the shoulders of the animal. The tubing was sutured to the skin to secure it. The incision made on the chest was closed using a combination of sutures

and Veg Bond (3M, Minneapolis, MN). The animal was then transferred to an awake animal sampling system [3, 4] and connected to the wire tether. The vacuum was applied for at least 1 h to the ultrafiltration probe and Ringers solution perfused through the cannula. The animals were allowed to recover from surgery for 4–12 h before any experiments were conducted.

*Reagents*

Regular Iletin I (pork-bovine, Lilly), Humulin R insulin (human recombinant, Lilly), and regular Insulin (pork, Novo Nordisk) all at 100 U $ml^{-1}$ were used as received. The calcium and phosphorus reagent kits, glucose, and standard solutions of calcium and phosphorous were purchased from Sigma (St Louis, MO). Double distilled water filtered, 0.45 μm, with no detectable sodium, calcium or potassium was used for all dilutions.

## Results and Discussion

*Sodium and potassium determinations using an ISE*

Potassium and sodium determinations were performed using ISE-based electrodes. These electrodes are potentiometric based and the response can be effected by the matrix sampled [9]. Ultrafiltrate samples are void of protein and other potentially interfering large molecules, but contain ions and other small compounds that might alter the response of the sensors. Ultrafiltration probes were observed to achieve quantitative recovery for both sodium and potassium when tested. Therefore it would be expected that the concentration in ultrafiltrates would be a good measure of intercellular values. Due to the size of the sensors used, a total sample volume required was between 30 and 200 μl. The sensors report the concentration in parts per million (ppm) on a logarithmic scale. Dilution of samples in pure water was performed to increase the total volume of sample and to have the meters read on the scale of 1–100 ± 1 ppm. Both of the meters were always calibrated immediately prior to use. Using this method both meters gave responses in the desired range under both dilution and standard addition calibrations. The sodium meter gave different standard addition calibration slopes for ultrafiltrates in plasma samples. The sodium meter showed much lower than expected results when plasma samples were measured. When a single sample was analysed multiple times with either meter an error of ±0.5 pm (0.1%) was observed. From these results it was concluded that these ISE meters were a practical solution for estimating the concentration of sodium and potassium in small volume capillary ultrafiltrate samples.

*Ionic composition of the intercellular fluid*

Using the ISE electrodes for sodium and potassium and the spectrophotometric assays of calcium and phosphorous the quantity of each ion was determined in capillary ultrafiltrate samples obtained from the subcutaneous tissue of rats. Table 1 lists the results of what was observed compared with the known literature values for both plasma and intercellular fluid. The values obtained show that indeed the fluid obtained in an ultrafiltrate is intercellular and not intracellular. An excellent correlation between all four substances was found. Each ion was determined in a large number of animals with a minimum of five samples obtained from any single animal. From these results it appears that the active collection process of the capillary ultrafiltration probes does not have an adverse effect on the cells and capillaries in the near vicinity. This would suggest that ion dynamics in the intercellular fluid could be monitored using this technique. One of the key ions, calcium, is much more matched with the intercellular fluid than the plasma value. In addition, the values obtained correlated well with plasma values for the other ions. This suggests that sampling the intercellular fluid is representative of the blood, with the selectivity that is imposed due to the differences in protein content of the intercellular fluid. In order to confirm this, a direct comparison of potassium and sodium in blood and capillary ultrafiltrate samples was made.

The literature suggests that there is a slight difference for the levels of potassium and sodium in blood (plasma) and the intercellular fluid [7]. This is due to the difference in osmotic and concentration of protein in the two matrices. In three animals the observed results obtained show a potassium concentration of 3.7 ± 0.1 mM in the intercellular fluid and 3.8 ± 0.3 mM (mean ± SEM) for plasma. These two values compare very well and there is no statistical difference between them. The intercellular sodium level was

140 ± 4 mM, whereas the plasma level was 128 ± 2 mM (mean ± SEM). The plasma levels were thus quite low in relation to both the literature value and the intercellular value obtained using capillary ultrafiltration. A possible explanation for this phenomenon is the plasma samples interacted with the ion selective electrode and reduced the signal [9].

*Monitoring insulin-dependent potassium uptake*

The effect of insulin on potassium metabolism and regulation is well known [10–14]. High levels of insulin (hyperinsulinemia) causes the transfer of potassium from the intercellular space into the intracellular space of the cell. This is of importance to insulin-dependent diabetics [12–14]. Both increased or decreased levels of potassium can have fatal effects. An increase in the extracellular level of potassium (hyperkalimea) can cause cardiac arrest and a decrease (hypokalemia) can cause muscle paralysis [8]. Capillary ultrafiltration probes implanted in the subcutaneous tissue have been shown to have the ability to monitor potassium in the intercellular fluid. The introduction of insulin will cause the flux of potassium into the cells dropping the intercellular levels. The objective was to monitor this phenomenon to substantiate the conclusion that the active sampling process of capillary ultrafiltration probes does not cause cell rupture and does monitor the intercellular fluid.

Under euglycemic conditions non-diabetic humans have shown a decrease in extracellular potassium levels of up to 1.4 mM after infusion of insulin [12, 15, 16]. Using the ISE potassium meter combined with capillary ultrafiltration it is possible to monitor the magnitude of this change. Initially no change in potassium was observed using pork-bovine insulin. However, since rats were being utilized the response to this type of insulin was very minimal. Attempts were made with other insulins, including recombinant human and pure pork. With the pure pork insulin at high acute doses (100 U $kg^{-1}$) under euglycemic clamp conditions a change in potassium concentration was observed. The euglycemic clamp was obtained using 20% glucose in Ringers solution with potassium omitted, perfused at a flow rate of 2 $\mu l$ $min^{-1}$ into the jugular vein. Figure 4 illustrates the mean change for insulin-induced potassium dynamics in three subjects. The potassium level changed from 3.6 ± 0.05 mM

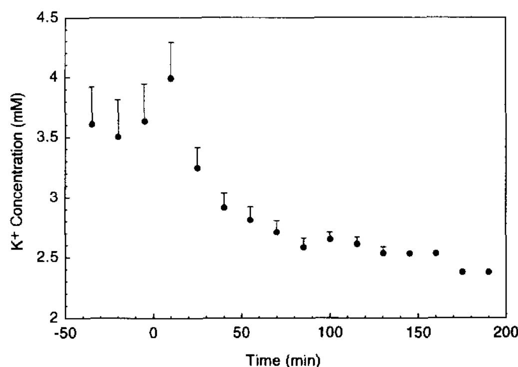


**Figure 4**
The monitoring of insulin-induced potassium dynamics by capillary ultrafiltration in three rats after an acute dose of 100 U $kg^{-1}$ of pork insulin.

to 2.6 ± 0.03 mM (mean ± SEM) in the three subjects studied. In each of the subjects the potassium peaked in the first sample after the injection of insulin. This is thought to be due to the matrix the insulin was dissolved in. These results confirm that capillary ultrafiltration is a suitable method for the monitoring of potassium dynamics in the intercellular fluid and that intracellular levels are not observed in the samples obtained. However, it does show that the technique can be used to monitor the flux of ions in and out of cells.

*Comparison of intercellular ultrafiltrates and microdialysates*

The technique of microdialysis sampling does not remove any fluid from the sampled tissue and is based solely on the diffusion of molecules across a concentration gradient [1, 2, 17, 18]. Microdialysis can be used as both a sampling tool and a delivery tool [17]. Substances in the perfusate will diffuse into the animal if they are at a higher concentration than the surrounding tissue. In many microdialysis experiments the choice perfusion fluid can be very critical. For example, the calcium concentration of the perfusate has been shown to cause adverse effects and changes in neurotransmitter levels [19, 20]. Under other circumstances, such as monitoring acetaminophen pharmacokinetics in subcutaneous tissue, the effects should be negligible. Using loop microdialysis probes with a perfusion fluid of only pure water, or void of a single ion desired, should provide a technique for determination of intercellular levels similar to capillary ultrafiltration [18]. Even though microdialysis probes with increased membrane lengths can

achieve quantitative recovery, no net fluid is removed from the tissue. Microdialysis probes with 60 mm regenerated cellulose membranes were implanted into the subcutaneous tissue and perfused with pure water at 1 μl $min^{-1}$. Microdialysates obtained contained 3.6 ± 0.2 mM ($n$ = 4) potassium and 142 ± 2 mM ($n$ = 5) sodium (mean ± SEM). These results compare very well with the capillary ultrafiltrates. The results suggest that quantitative recovery of potassium and sodium are obtained under these conditions, and microdialysis is also a suitable method for monitoring ion dynamics in the intercellular fluid. Modification of the flow rate to values greater than 1 μl $min^{-1}$ showed minor changes for both sodium and potassium up to 2 μl $min^{-1}$. A significant drop in recovery was observed at 2.5 μl $min^{-1}$ for both ions.

## Conclusions

The ability to monitor chemical dynamics in the intercellular fluid by both capillary ultrafiltration and microdialysis have been observed. Using ISE sensors and spectroscopic methods potassium, sodium, calcium and phosphorous in microlitre volumes of capillary ultrafiltrates were shown to be in the range reported in the literature, 3.7 ± 0.1, 140 ± 4, 1.1 ± 0.1 and 1.7 ± 0.1 mM (mean ± SEM) respectively. The vacuum applied showed no adverse effects to the sampling tissue. Microdialysis using pure water as the perfusate also resulted in similar values for sodium (3.6 ± 0.2 mM) and potassium (142 ± 2 mM). Capillary ultrafiltration probes were shown to be a useful method for monitoring chemical dynamics in the intercellular fluid by monitoring the hyperinsulinemic dependent uptake of potassium into the cells under an euglycemic clamp. The potassium level changed from 3.6 ± 0.05 mM to 2.6 ± 0.03 mM (AVE ± SEM) afer an acute injection of 100 U $kg^{-1}$ of pork insulin.

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[*Received for review 14 April 1993; revised manuscript received 9 June 1993*]