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# Modest hyperglycemia prevents interstitial dispersion of insulin in skeletal muscle☆

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ABSTRACT

Insulin injected directly into skeletal muscle diffuses rapidly through the interstitial space to cause glucose uptake, but this is blocked in insulin resistance. As glucotoxicity is associated with endothelial dysfunction, the observed hyperglycemia in diet-induced obese dogs may inhibit insulin access to muscle cells, and exacerbate insulin resistance. Here we asked whether interstitial insulin diffusion is reduced in modest hyperglycemia, similar to that induced by a high fat diet.

*Methods.* During normoglycemic (100 mg/dl) and moderately hyperglycemic (120 mg/dl) clamps in anesthetized canines, sequential doses of insulin were injected into the vastus medialis of one hindlimb; the contra-lateral limb served as a control. Plasma samples were collected and analyzed for insulin content. Lymph vessels of the hind leg were also catheterized, and lymph samples were analyzed as an indicator of interstitial insulin concentration.

*Results.* Insulin injection increased lymph insulin in normoglycemic animals, but not in hyperglycemic animals. Muscle glucose uptake was elevated in response to hyperglycemia, however the insulin-mediated glucose uptake in normoglycemic controls was not observed in hyperglycemia. Modest hyperglycemia prevented intra-muscularly injected insulin from diffusing through the interstitial space reduced insulin-mediated glucose uptake.

*Conclusion.* Hyperglycemia prevents the appearance of injected insulin in the interstitial space, thus reducing insulin action on skeletal muscle cells.



## 1. Introduction

Obesity, insulin resistance and hypertension are all components of the metabolic syndrome, and the links between each of the diseases involved are continually being investigated. Elevated fasting plasma glucose (>5.6 mmol/L) or impaired glucose tolerance is a diagnostic criterion for the metabolic syndrome put forward by several expert groups, including the World Health Organization, the International Diabetes Federation, the National Cholesterol Education Program and others, and fasting hyperglycemia is noted as a step on the progression to diabetes, often occurring after impaired glucose tolerance [1]. Raised fasting glucose levels are associated with impaired insulin secretion, while impaired glucose tolerance is thought to reflect insulin resistance [2].

Insulin action *in vivo* is frequently studied, as is insulin action at the cellular level *in vitro*. However the ability of insulin to get to the cell surface *in vivo* is essential for the cellular response to insulin, and there is a gradient in insulin concentration from the plasma to the interstitial space [3–6].

*Abbreviations:* BF, blood flow; LGU, leg glucose uptake; PKC, protein kinase C.

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Insulin transport to the interstitial space is rate limiting for whole body insulin action [5,7] and is impaired in insulin resistance [8]. Insulin injected into healthy muscle is rapidly detected within the interstitial space using lymph cannulation to sample interstitial fluid, and a similarly rapid effect on muscle glucose uptake can also be detected [9]. Insulin resistance induced by either acute lipid infusion or feeding of a high fat diet prevented insulin from reaching the interstitial fluid and prevented glucose uptake [10,11]. While both treatments induce insulin resistance by excess lipid, in contrast to lipid infusion the fat feeding intervention does not induce elevated plasma lipid during the study, therefore the factor preventing the access of insulin to the interstitial space is not known. It was noted that the fat-fed dogs had a modestly higher plasma glucose level (120 mg/dl compared to approximately 95 mg/dl in controls) during the experiment [11]; this was evident when the dogs were under anesthesia. Since this fasting glucose level is above that used in the diagnostic criteria for the metabolic syndrome, it is possible that the slight hyperglycemia could be responsible for limiting the access of insulin to the cell.

Glucotoxicity can have effects on many different cell types, and is known to cause endothelial dysfunction [12,13]. As insulin's ability to access tissue relies on healthy responses to insulin in the blood vessel wall, such as vasodilation, capillary recruitment and transendothelial transport, hyperglycemia and the resulting endothelial dysfunction may therefore impair the dispersion of insulin through muscle. A recent study has shown that obese women with postprandial hyperglycemia have impaired insulin delivery to both adipose tissue and skeletal muscle [14], an effect that may be due to the obesity or the postprandial hyperglycemia. Here we investigate the effect of modestly elevated glucose on the access of insulin to muscle. Surprisingly, we find that mild hyperglycemia is sufficient to impair the distribution of injected insulin through the interstitial space.

## 2. Materials and methods

### 2.1. Animals

Experiments were performed on anesthetized male mongrel dogs. Animals were housed in the University of Southern California Medical School Vivarium under controlled kennel conditions (12 h light:12 h dark) and fed standard chow (49% carbohydrate, 25% protein, 9% fat; Alfred Mills, Chicago, IL) once per day. Dogs were used for experiments only if they were judged to be in good health as determined by visual observation, body weight, hematocrit, and body temperature. Protocols were approved by the University of Southern California Institutional Animal Care and Use Committee.

### 2.2. Groups

In an effort to conserve animals, some of the animals from our previous publications have been incorporated into the control group [9], however we have expanded this control group to ensure that previous results still apply; as such the data are not identical to previous publications. The two groups for comparison within this manuscript were those maintained at normoglycemic levels (control), and those maintained at hyperglycemic levels. The level of hyperglycemia was chosen to match that of previously published high fat fed animals, approximately 120 mg/dl.

### 2.3. Protocol

Animals were fasted 15 h before the morning of the experiment at 0600 h. Dogs were preanesthetized with acepromazine maleate (Prom-Ace, Aueco, Fort Dodge, IA; 0.22 mg/kg) and atropine sulfate (Western Medical, Arcadia, CA; 0.11 mL/kg). Anesthesia was induced with sodium pentobarbital (Western Medical, Arcadia, CA; 0.44 mL/kg) and maintained with isofluorane (Western Medical, Arcadia, CA). Indwelling catheters were implanted in the right jugular vein for a continuous saline drip (~1 L for the first 60 min of surgery and a slow drip thereafter) and in the left carotid artery for sampling and blood pressure monitoring (Space Labs, Issaquah, WA; model #90603A). Intracatheters were inserted into the cephalic veins for glucose, insulin, somatostatin and tracer infusion. Indwelling catheters were also placed into both the right and left femoral arteries and veins for sampling. Two perivascular ultrasonic flow probes (2 mm diameter; Transonic, Ithaca, NY) were placed around both right and left femoral arteries proximal to the femoral catheter for measuring rates of blood flow. Left and right hindlimb lymphatic vessels were cannulated by placing polyethylene catheters (PE10) into the afferent lymphatic vessels of the deep inguinal lymph node. Lymph was collected by gently massaging the leg directly above the popliteal area, which has been shown to instantaneously increase lymph drainage without affecting lymph or plasma oncotic pressures. Blood pressure, heart rate, $O_2$ saturation and $CO_2$ were monitored continuously. At the conclusion of these experiments, animals were euthanized with an overdose of sodium pentobarbital (Eutha-6, Western Medical; 65 mg/kg).

### 2.4. Clamps

Immediately after starting surgical procedures, a basal insulin eugylcemic clamp was started (t = −180 min). Glycemia was maintained by variably infusing exogenous 20% glucose to maintain euglycemia (n = 8) or hyperglycemia (120 mg/dl) (n = 7); glucose infusion rate was chosen to maintain euglycemia in the femoral artery plasma of the injected leg. Simultaneously, somatostatin was infused (1 μg/min/kg; Bachem), and basal insulin replaced systemically (1.2 pmol/min/kg; Novo Nordisk, Bagsvaerd, Denmark) and continuously for the remainder of the study (Fig. 1). Samples were taken simultaneously from the right and left femoral arteries and veins. Left and right hindlimb lymph vessels were sampled by gently massaging the hindlimb distal to the site of catheterization from 2 min prior to 2 min after each blood sample point.

### 2.5. Intramuscular insulin

At times 0, 60, 120, 180 and 240 min, porcine insulin (0.3, 0.5, 0.7, 1, and 3 U, represented as I1, I2, I3, I4, and I5 respectively) was injected into the vastus medialis of the quadriceps