Diabetic State-Induced Modification of Insulin-Stimulated, Glucose Uptake into and Kinase Activity in, the Diaphragm Muscle of Genetically Diabetic KK-CA\textsuperscript{y} Mice

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The effect of insulin on glucose and 2-deoxyglucose uptake into isolated diaphragm was investigated in genetically diabetic KK-CA\textsuperscript{y} mice, and in part in alloxan-treated mice. Concentration-response curves of insulin for glucose uptake (8.3 mM in vitro) for 1 h were shifted to the left in two kinds of diabetic model mice. Insulin-stimulated glucose uptake was biphasic; it was high 1 week, and returned to the normal level 4 weeks, after alloxan injection despite high blood glucose levels. Insulin-stimulated glucose uptake was the same at higher glucose levels (25 mM) as at 8.3 mM glucose for 1 h, despite an increase in basal glucose uptake (without insulin) in KK-CA\textsuperscript{y} mice. Insulin-receptor kinase activity in diabetic KK-CA\textsuperscript{y} mouse diaphragm also changed biphasically as the glucose concentration increased: an increase at 8.3 mM, but no increase at 16.7 mM or 25.0 mM glucose for 3 h-pretreatment including 1 h-insulin treatment. These results suggest that although the initial state of diabetes enhances insulin action, the prolonged hyperglycemia rather suppressed the insulin action in vivo.

Keywords — insulin-stimulated glucose uptake; insulin-stimulated kinase activity; diaphragm; diabetic KK-CA\textsuperscript{y} mice; high-concentration glucose

Introduction

Insulin controls blood glucose levels by regulating glucose uptake into the skeletal muscle of rodents. The effect of the diabetic state on insulin-stimulated glucose uptake is controversial; some investigators have reported that the diabetic state enhances the effect of insulin on glucose uptake into skeletal muscle,\textsuperscript{1-4} and others have reported no effect\textsuperscript{5,6} or a decrease in insulin effect, on skeletal muscle.\textsuperscript{7-11} Insulin-stimulated 2-deoxyglucose uptake is dependent on insulin receptor phosphorylation in Chinese hamster ovary cells.\textsuperscript{12} Insulin also stimulates insulin-receptor kinase activity in different types of skeletal muscle.\textsuperscript{13} The tyrosine kinase activity of insulin receptors decreases, and the number of insulin receptors is doubled in the liver plasma membrane of streptozocin-diabetic rats.\textsuperscript{14} The findings of Kadowaki et al.\textsuperscript{14} concerning total insulin-receptor tyrosine kinase activity in the diabetic plasma membrane suggest the increased activity in the streptozocin-diabetic rat. Diabetic KK-CA\textsuperscript{y} mice have genetic non-insulin dependent diabetes mellitus (NIDDM), characterized by hyperglycemia, hyperinsulinemia, obesity and insulin-resistance in vivo.\textsuperscript{15} The insulin effects on skeletal muscle of diabetic KK-CA\textsuperscript{y} mice have not yet been investigated in vitro.

In the present paper we investigated whether the diabetic state enhances insulin-stimulated glucose uptake and its relation to tyrosine kinase activity using the diaphragms of genetically diabetic KK-CA\textsuperscript{y} mice, and furthermore whether higher glucose concentrations in vitro alters the diabetic state-induced change of insulin action.

Materials and Methods

Animals — Male genetically diabetic and prediabetic KK-CA\textsuperscript{y}, and alloxan-diabetic and age-matched ddY mice were used. The KK-CA\textsuperscript{y} mice were fed ad libitum and maintained in a constant temperature (23 ± 1 °C) environment with light from 8 a.m. to 6 p.m. Male ddY mice, 5 weeks of age, were bolus-injected with 85 mg/kg alloxan (Nacalai, Kyoto, Japan) in saline

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Table I. Strain, Age, Body Weight, Blood Glucose, and Diaphragm Protein Content and Basal Glucose Uptake of Diabetic Male Mice

<table>
<thead>
<tr>
<th>Strain</th>
<th>Age (weeks old)</th>
<th>Body weight (g)</th>
<th>Blood glucose level (mM)</th>
<th>Diaphragm (mg protein)</th>
<th>Basal glucose uptake of 8.3 mM (nmol/mg protein/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>KK-CA (^a) (fed)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prediabetic</td>
<td>15—46</td>
<td>35.0±1.6</td>
<td>6.7±0.3</td>
<td>10.4±0.5</td>
<td>50±3</td>
</tr>
<tr>
<td>Diabetic</td>
<td>28—69</td>
<td>39.4±1.3</td>
<td>21.6±0.6(^a)</td>
<td>10.6±0.3</td>
<td>48±2</td>
</tr>
<tr>
<td>ddY (fed)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-diabetic</td>
<td>5—9</td>
<td>33.4±0.8</td>
<td>6.8±0.2</td>
<td>11.8±0.5</td>
<td>55±1</td>
</tr>
<tr>
<td>Alloxan-diabetic</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>1 d</td>
<td>5</td>
<td>27.1±0.3(^a)</td>
<td>21.6±0.9(^a)</td>
<td>9.6±0.3(^b)</td>
<td>51±2</td>
</tr>
<tr>
<td>1 week</td>
<td>6</td>
<td>27.6±0.7(^a)</td>
<td>27.4±2.6(^a)</td>
<td>9.4±0.3(^c)</td>
<td>51±3</td>
</tr>
<tr>
<td>2 week</td>
<td>7</td>
<td>29.9±0.7(^b)</td>
<td>26.8±1.1(^c)</td>
<td>10.4±0.4</td>
<td>60±4(^c)</td>
</tr>
<tr>
<td>4 week</td>
<td>9</td>
<td>32.3±1.2</td>
<td>28.9±1.5(^c)</td>
<td>13.2±0.4</td>
<td>50±2</td>
</tr>
<tr>
<td>ddY (fasted)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-diabetic</td>
<td>6</td>
<td>27.5±0.5(^d)</td>
<td>4.4±0.4(^d)</td>
<td>11.5±0.8</td>
<td>63±3(^d)</td>
</tr>
<tr>
<td>Alloxan-diabetic</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 week</td>
<td>6</td>
<td>23.7±0.3(^d)</td>
<td>19.1±3.2(^d)</td>
<td>9.2±0.5</td>
<td>61±4(^d)</td>
</tr>
</tbody>
</table>

\(^a\) Prediabetic KK-CA \(^a\) vs. diabetic KK-CA \(^a\) (p<0.01), \(^b\) non-diabetic ddY, vs. diabetic ddY (p<0.05), \(^c\) (p<0.01) and \(^d\) fed vs. fasted (p<0.05), \(^e\) (p<0.01) (significant difference on the basis of the unpaired Student's t-test).

via their tail veins, and used 1 d, or 1-, 2- or 4-weeks after injection. Blood glucose levels were measured with a glucose analyzer (Type II, Beckman, CA, U.S.A.). The blood glucose levels, age, diaphragm protein content, and basal glucose uptake levels at 8.3 mM glucose in these mice are summarized in Table I. In some experiments, mice were fasted 18 h just before the experimental procedure.

Preparation of Diaphragm Segments — Mice were decapitated, their diaphragm with ribs were isolated, and placed in Krebs Ringer bicarbonate (KRB) solution consisting of 118.5 mM NaCl, 4.8 mM KCl, 2.5 mM CaCl₂, 1.3 mM KH₂PO₄, 1.2 mM MgSO₄, and 25.0 mM NaHCO₃ solution (pH 7.3) and containing 2.8 mM glucose through which a 95% O₂ and 5% CO₂ gas mixture was bubbled. After ribs and central tendons were removed, the diaphragms were cut into 4—8 segments in ice-cold KRB solution containing 2.8 mM glucose.

Assay for Glucose and 2-Deoxyglucose Uptake — A segment of diaphragm was incubated for 1 h at 37 °C in KRB solution containing insulin (Iszillin-40; Shimizu Seiyaku, Shimizu, Japan), 0.5% bovine serum albumin (BSA, Fraction V, Sigma), 8.3, 16.7 or 25.0 mM [U-¹⁴C]-glucose in vitro (14.8 kBq/ml, 9.99 GBq/mmol, Amersham Japan). Insulin-stimulated glucose uptake reached a peak after 1 h-incubation in fasted normal ddY mice, where the uptake curve was linear in that time (data not shown). In experiments concerned with 2-deoxyglucose uptake, the diaphragm was incubated for 1 h at 37 °C in KRB solution containing insulin, 0.5% BSA and 8.3 mM [¹³H]-2-deoxyglucose (14.8 kBq/ml, 592 GBq/mmol, Amersham Japan) and 2 mM pyruvate. The segment was washed 4 times with ice-cold KRB solution containing 0.5% BSA and 8.3 mM glucose or 8.3 mM 2-deoxyglucose and 2 mM pyruvate, and water was absorbed with filter paper. The segment was dissolved in 1 N NaOH and its radioactivity was determined using a liquid scintillation spectrometer (300C, Packard, U.S.A.). The protein concentration of aliquots was determined by the method described by Lowry et al.\(^{19}\) with BSA as the standard.

Assay for Insulin-Stimulated Kinase Activity — Both the method described by Burant et al.\(^{17}\) and Wang and Baltimore\(^{18}\) were used modified as follows: Diaphragms were incubated for 3 h at 37 °C in modified KRB solution containing 0.1 mM KH₂PO₄, [³²P]-orthophosphate (0.37 MBq/ml, carrier-free, Amersham Japan), 0.5% BSA and glucose (8.3, 16.7 and 25.0 mM glucose in vitro). After labeling the en-
dogenous adenosine triphosphate (ATP), the segment was incubated with or without insulin for 1 h at 37 °C in modified KRB solution containing 0.1 mM KH₂PO₄, 0.5% BSA and glucose, transferred to 0.3 ml of 4-(2-hydroxyethyl)-l-piperazineethane-sulfonic acid (HEPES) solution (50 mM HEPES, 1.5% Triton X-100, 10 mM NaF, 0.2 mM ammonium vanadate, 10 mM pyrophosphate, 40 mM ATP, 1 trypsin inhibitory unit/ml aprotinin, 100 μU/ml bacitracin and 1 mM phenyl methyl sulfonyl fluoride, pH 7.5), immediately frozen in liquid nitrogen, and homogenized using a sonicator (UR-20P, Tomy Seiko, Japan). A 0.2 ml of the sample of homogenate was precipitated by adding 1.8 ml of 10% trichloroacetic acid and 0.1 M pyrophosphate. The pellet was collected by centrifugation (2500 × g, 20 min) at 4 °C, dissolved in 0.2 ml of 1 N NaOH, incubated for 1 h at 37 °C to remove phosphoserine and phosphothreonine, and added to 0.2 ml of 1 N HCl. A 100 μl sample of neutralized solution was delivered to 3MM paper (Whatman, Clifton, U.S.A.), exposed to 0.1 M chilled pyrophosphate in 10% trichloroacetic

Fig. 1. Enhancement of Insulin-Stimulated Glucose Uptake into Diaphragm of Diabetic KK-CA⁺ Mice
A segment of diaphragm was incubated for 1 h at 37 °C in Krebs Ringer bicarbonate (KRB) solution with insulin (0.17—167 nM), 0.5% bovine serum albumin (BSA) and 8.3 mM [U-¹⁴C]-glucose. Δ-Glucose uptake mean insulin (0.17—167 nM)-stimulated glucose uptake minus basal glucose uptake shown in Table 1. Values represent means ± SEM of data from 8 to 18 experiments. a) p < 0.05 (unpaired Student's t-test); significant difference from the control values in prediabetic KK-CA⁺ mice. ○, prediabetic; ●, diabetic.

Fig. 2. Enhancement of Insulin-Stimulated 2-Deoxyglucose Uptake into the Diaphragm of Diabetic KK-CA⁺ Mice
A segment of diaphragm was incubated for 1 h at 37 °C in KRB solution with insulin (0.17—167 nM), 0.5% BSA and 8.3 mM [¹H]-2-deoxyglucose. Δ-2-deoxyglucose uptake means insulin (0.17—167 nM)-stimulated 2-deoxyglucose minus basal 2-deoxyglucose uptake (prediabetic KK-CA⁺; 25.0 ± 0.6, and diabetic KK-CA⁺; 24.2 ± 2.0 nmmol/mg protein/h). Values represent means ± SEM of data from 5 to 7 experiments. a) p < 0.05 (unpaired Student's t-test); significant difference from the control values in prediabetic KK-CA⁺. ○, prediabetic; ●, diabetic.

Fig. 3. Transient Enhancement of Insulin-Stimulated Glucose Uptake Associated with Duration of Alloxan Diabetes
Experimental procedure was the same as the legend in Fig. 1. Δ-Glucose uptake is the same as shown in Fig. 1. Values represent means ± SEM of data from 8 to 40 experiments. a) p < 0.05, b) p < 0.01 (unpaired Student's t-test); significant difference from corresponding values at respective concentrations of insulin in normal mice. ○, normal; ●, alloxan.
acid for 20 min, then boiled for 15 min. The 3MM paper was washed with ethanol–ether (1:1) and then with ether at room temperature. The 3MM paper was air-dried, and its radioactivity was measured using a liquid scintillation spectrometer (LS 3801, Beckman, CA, U.S.A.).

Results

Enhancement of Insulin-Stimulated Glucose or 2-Deoxyglucose Uptake into Diaphragm Muscles of Diabetic KK-CA$^\text{y}$ Mice

The diabetic state doubled glucose or 2-deoxyglucose uptake at 8.3 mM glucose by 16.7 nM insulin in KK-CA$^\text{y}$ mice, compared with prediabetic control mice. Insulin (up to 1.67 nM) concentration-dependently increased 2-deoxyglucose or glucose uptake at 8.3 mM by the diaphragm of diabetic KK-CA$^\text{y}$ mice than the prediabetic control mice (Figs. 1 and 2). The insulin-stimulated uptake in normal ddY mice was two times higher than in prediabetic KK-CA$^\text{y}$ mice (data not shown). The absolute amount of insulin-stimulated 2-deoxyglucose uptake was less than that of insulin-stimulated glucose uptake. The maximal effective concentration of insulin was 100-times higher in 2-deoxyglucose uptake than in glucose uptake. Both concentration-response curve for insulin-stimulated glucose and 2-deoxyglucose uptake was shifted to the left in the diabetic state. Diabetic state enhanced the insulin-stimulated glucose uptake in KK-CA$^\text{y}$ mice.

Effect of Interval after Alloxan-Injection on Insulin-Stimulated Glucose Uptake

We attempted to determine whether enhancement of glucose uptake depended on diabetic interval after alloxan-injection. The enhancement of insulin action tended to become evident on day 1, reach a peak 1—2 weeks after alloxan-injection, and then return to the control level (Fig. 3). The data of 1.67 nM insulin-stimulated glucose uptake in Fig. 3 are replotted in against interval after alloxan injection in Fig. 4. Neither insulin-stimulated glucose uptake nor blood glucose level changed with the diabetic duration in normal mice (Fig. 4). In the alloxan mice, blood glucose levels increased greatly (from 7.2 mM to 21.6 mM) 1 d and were maintained at high levels up to the end of week 4, after alloxan injection, although maximal glucose uptake by insulin

![Graph showing glucose uptake](image)

Fig. 4. Transient Enhancement of Insulin-Stimulated Glucose Uptake Associated with Duration of Alloxan-Diabetes Despite High Blood Glucose

Blood glucose levels of normal (□) and alloxan-diabetic (■) mice were compared with insulin-stimulated glucose uptake. Values for glucose uptake in the diaphragm of normal (○) and alloxan-diabetic (●) ddY mice were replotted from data at 1.67 nM insulin in Fig. 3. Values represent means ± SEM of data from 10 to 15 experiments. a) (glucose uptake), b) (blood glucose levels) $p<0.01$ (unpaired Student's t-test); significant difference from corresponding values in normal mice.

![Graph showing glucose uptake](image)

Fig. 5. Insulin-Stimulated Glucose Uptake at High Glucose Concentrations (8.3, 16.7 and 25.0 mM in Vitro) in the Diaphragm of Diabetic KK-CA$^\text{y}$ Mice

A segment of diaphragm was incubated for 1 h at 37 °C in KRB solution with insulin (0.17—167 nM), 0.5% BSA and 8.3, 16.7 or 25.0 mM glucose [U-14C]-glucose. Values represent means ± SEM of data from 5 to 18 experiments. The shaded area represents the difference value in glucose uptake at 8.3 mM (replotted from Fig. 1), 16.7 and 25.0 mM glucose from the control value without insulin.
Insulin Action in Diabetic Diaphragm

Fig. 6. Insulin-Stimulated Kinase Activity at High Glucose Concentrations (8.3, 16.7 and 25.0 mM in Vitro) in the Diaphragm of Diabetic KK-CA\(^\gamma\) Mice (○) Compared with Prediabetic KK-CA\(^\gamma\) Mice (□)

A diaphragm was incubated for 3 h in modified KRB solution containing 0.1 mM KH\(_2\)PO\(_4\), [\(^{32}\)P]-orthophosphate, 0.5% BSA and glucose (8.3, 16.7 or 25.0 mM). After labeling, the segment was incubated with or without insulin for 1 h at 37 °C in modified KRB solution containing 0.1 mM KH\(_2\)PO\(_4\), 0.5% BSA and glucose (8.3, 16.7 or 25.0 mM). Values represent means ± SEM of data from 3 to 9 experiments. a) \(p<0.05\), b) \(p<0.01\) (unpaired Student’s t-test); significant differences from corresponding values at respective insulin concentrations in prediabetic KK-CA\(^\gamma\) mice.

was lower after 4 weeks (Fig. 4). The enhancement was not generated with longer diabetic state despite continued high levels of glucose, suggesting that long-time hyperglycemia may rather suppress the enhancement of insulin-stimulated glucose uptake to the normal level.

Effect of High Concentrations of Glucose in Vitro on Insulin-Stimulated Glucose Uptake into the Diaphragm of Diabetic KK-CA\(^\gamma\) Mice

We attempted to determine whether hyperglycemia inhibited the diabetic enhancement of insulin-stimulated glucose uptake into the diaphragm of diabetic KK-CA\(^\gamma\) mice. Higher glucose concentrations for 1 h in vitro increased basal glucose uptake to a higher level (Fig. 5); the basal uptake level was 48 ± 2 nmol/mg protein/h (\(n=19\)) at 8.3 mM glucose and 100 ± 17 (\(n=5\)) or 125 ± 19 (\(n=5\)) nmol/mg protein/h at 16.7 or 25.0 mM glucose (Fig. 5). Insulin stimulated glucose uptake to the same extent in diabetic KK-CA\(^\gamma\) mice even at the severely high concentration of glucose. These findings indicate that high concentrations of glucose (1 h-treatment) enhance basal glucose uptake but do not change the insulin-stimulated ones.

Effect of High Glucose Concentration in Vitro on Insulin-Stimulated Kinase Activity in the Diaphragm of Diabetic KK-CA\(^\gamma\) Mice

In order to determine the direct effect of high glucose for longer time (4 h-treatment) on insulin receptors, we examined insulin-stimulated (1-h) kinase activity in the diaphragm of KK-CA\(^\gamma\) (Fig. 6). At 8.3 mM glucose, basal kinase activity was significantly higher than in prediabetic KK-CA\(^\gamma\) mice. The basal value of kinase activity was 1250 ± 140 cpm/mg protein (\(n=9\)) in diabetic mice and 717 ± 99 cpm/mg protein (\(n=6\)) in prediabetic mice (Fig. 6). The absolute value in insulin-stimulated kinase activity (the difference between with and without insulin) and basal activity tended to be higher in diabetic mice than in prediabetic ones. These changes in the diabetic state demonstrated the same pattern as in the case of the results of insulin-stimulated glucose uptake (Figs. 1 and 2).

We investigated the effect of high glucose concentration on insulin-stimulated kinase activity which was enhanced by diabetes. Maximal insulin-stimulated kinase activity at 8.3, 16.7 and 25.0 mM glucose was 2.07 ± 0.08 (\(n=4\)), 1.74 ± 0.18 (\(n=6\)) and 1.28 ± 0.18 (\(n=5\)) × 10^3 cpm/mg protein, respectively (Fig. 6). Insulin had little effect on the kinase activity of the diaphragm of diabetic KK-CA\(^\gamma\) mice under pretreatment with higher concentrations of glucose for 3 h at 16.7 mM. This suggests that high glucose concentrations rather suppress the diabetic state-induced enhancement of insulin-stimulated kinase activity. High glucose concentrations did not affect the ATP content of the diaphragm of diabetic KK-CA\(^\gamma\) mice (8.3 mM glucose; 5.57 ± 1.33, 16.7 mM glucose; 4.40 ± 0.97, 25.0 mM glucose; 7.44 ± 0.41 μg/mg protein). These parameters tended to behave in a manner similar to the results shown in Figs. 3 and 4.

Discussion

Insulin-stimulated glucose uptake in diabetic skeletal muscles is controversially reported. At normal glucose concentration in vitro, an appar-
ently similar enhancement by the diabetic state was observed in two murine models of diabetes; alloxan-mice and genetically diabetic KK-CA\(^v\) mice. KK-CA\(^v\) mice generated spontaneously NIDDM-diabetes.\(^{15}\) We also used alloxan-mice because the blood glucose levels of the mice increase at a greater rate than that of diabetic KK-CA\(^v\) mice\(^{19}\) and were compared with age-matched control. Present results furthermore demonstrated that insulin-stimulated glucose uptake was enhanced under restricted conditions such as in initial state of hyperglycemia. We confirmed that glucose uptake in either the fasted or fed state was enhanced by the diabetic state. Many investigators\(^{1,2,10,11}\) use 2-deoxyglucose of lower concentration than the prevailing glucose concentration for determination of glucose uptake because 2-deoxyglucose is not metabolized. As shown by many investigators, insulin-stimulated uptake of 2-deoxyglucose was the same as that of glucose uptake by insulin in the diabetic state.

The duration of the diabetic state affected insulin-stimulated glucose uptake in alloxan mice differently. A week after alloxan injection insulin action was enhanced, but after 4 weeks no enhancement of insulin action was detected. The longer the duration of hyperglycemia, the weaker insulin action became. This finding is not consistent with the report by Maegawa et al.\(^{10}\) concerning streptozocin-diabetic rats indicating that prolonged hypoinsulinemia induces a decrease in insulin action. Our results showed a biphasic insulin action and that the rising blood glucose levels by alloxan-injection preceded the enhancement of insulin-stimulated glucose uptake. The prolonged hyperglycemia in diabetic KK-CA\(^v\) mice apparently did not suppress the insulin action because the age-matched control was not obtained.

We suggest that the suppression of insulin-action by high concentrations of glucose in vitro needed 3 h at least, because 3 h-high glucose pretreatment suppressed insulin-stimulated kinase activity, but 1 h-high glucose treatment produced no change in insulin-stimulated glucose uptake in diabetic KK-CA\(^v\) mice diaphragms. Even normal rat skeletal muscle\(^{20}\) and cultured rat adipocytes\(^{21}\) become insulin-resistant after 5 and 24 h, respectively, in the presence of high concentrations of glucose in vitro. High concentrations of glucose may cause insulin resistance in the diabetic state, although 20 mM glucose-treatment for 24 h does not inhibit insulin binding in cultured normal rat adipocytes.\(^{21}\) We first found that high glucose treatment in vitro inhibited insulin-stimulated protein kinase activity in diaphragms of diabetic KK-CA\(^v\) mice. This may be due to glycosylation of insulin receptors because early nonenzymatic glycosylation in albumin (Shiff base and amidori products) forms time dependently in hours\(^{22}\) and excess glycosylated insulin receptor from streptozocin-diabetic rat skeletal muscle is deficient.\(^{23}\)

Increases in intracellular Ca\(^{2+}\) in adipocytes decrease insulin receptor kinase activity and insulin-stimulated 2-deoxyglucose uptake.\(^{24}\) In a previous paper, we have found that in normal skeletal muscles the increase in intracellular Ca\(^{2+}\) inhibits insulin-stimulated glucose uptake.\(^{25}\) As an abnormal increase in Ca\(^{2+}\) is observed in diabetic skeletal muscles,\(^{26}\) our present results may be related in part to the diabetic increase in intracellular Ca\(^{2+}\).

In conclusion, kinase activity is high in the genetically diabetic mouse, and is further enhanced by insulin in the presence of 8.3 mM, but not 25.0 mM glucose in vitro, suggesting that long duration of severely high concentrations of glucose in the diabetic state suppress the insulin-receptor activity in vivo.

References


Insulin Action in Diabetic Diaphragm


