# Insulin Increases the Maximum Velocity for Glucose Uptake without Altering the Michaelis Constant in Man

## EVIDENCE THAT INSULIN INCREASES GLUCOSE UPTAKE MERELY BY PROVIDING ADDITIONAL TRANSPORT SITES

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ABSTRACT  The present studies were undertaken to assess the mechanism by which insulin increases glucose uptake in man. Because glucose uptake in most mammalian tissues occurs predominantly by a facilitated transport system that follows Michaelis-Menten kinetics, glucose uptake was measured isotopically in normal volunteers over the physiologic range of plasma glucose and insulin concentrations and was subjected to Lineweaver-Burk and Eadie-Hofstee analysis. With both methods, increases in plasma insulin from 18 $\mu$U/ml to 80 and 150 $\mu$U/ml were found to increase the maximum velocity ($V_{max}$) for glucose uptake nearly three- and fivefold, respectively, ($P < 0.025$ and $P < 0.001$) without significantly altering the Michaelis constant ($K_m$). Because an increase in the affinity or molecular activity of transport sites or provision of additional transport sites that differed from those present basally should have altered the $K_m$, whereas a mere increase in the number of transport sites would have only increased the $V_{max}$, our results indicate that in man, insulin may increase glucose uptake merely by providing additional transport sites.

## INTRODUCTION

Glucose uptake in man is a function of both plasma glucose and plasma insulin concentrations (1, 2). In most mammalian tissues, glucose uptake occurs predominantly by a facilitated transport system that follows Michaelis-Menten kinetics (3, 4). Insulin increases the action of this system but the mechanism is presently controversial (5, 6). In vitro studies using adipocytes and fibroblasts suggest that insulin may act simply by increasing the availability of transport sites that are similar to those present in the absence of insulin (6–16). However, in vitro studies using muscle, the predominant tissue accounting for insulin-stimulated glucose uptake in vivo (17, 18), are compatible with either an insulin-induced increase in the affinity or in the molecular activity of transport sites, or an insulin-induced addition of sites that differ from those present basally (19, 20).

The present studies were therefore undertaken to further evaluate the mechanism by which insulin increases glucose uptake in vivo. Glucose uptake was measured isotopically in normal human volunteers during infusions of insulin and glucose, which produced cohorts of plasma insulin and glucose concentrations spanning the physiologic range. Plots of 1/glucose uptake vs. 1/plasma glucose (Lineweaver-Burk analysis) (21) and plots of glucose uptake vs. glucose uptake/plasma glucose (Eadie-Hofstee analysis) (22) both yielded straight lines compatible with the concept that whole body glucose uptake follows Michaelis-Menten kinetics. Also, with both methods of analysis, insulin increased the maximum velocity ($V_{max}$) without altering the Michaelis constant ($K_m$) for glucose uptake. Because increases in the affinity or the molecular activity of transport sites, or the addition of functionally different sites should have altered the $K_m$ for glucose transport, our results indicate that in man, insulin may accelerate glucose uptake simply by increasing the number of available transport sites.

## METHODS

48 experiments were performed in 15 normal volunteers (4 male, 11 female, age 20–28) from whom informed written

consent was obtained. All subjects were within 10% of their ideal body weight (tables, Metropolitan Life Insurance Company, New York) and had no family history of diabetes mellitus. Subjects were studied after an overnight fast on two to four occasions separated by at least 48 h.

Between 700 and 800 h, primed-continuous infusion of [3-³H]glucose (New England Nuclear, Boston, MA) was begun, and 2 h were allowed for isotope equilibration. Somatostatin (250 μg/h, courtesy of N. Ling and R. Guillemin, Salk Institute, San Diego, CA) and crystalline insulin (Iletin II pork insulin, Eli Lilly & Co., Indianapolis, IN) were then infused for 3 h, during which time plasma glucose was clamped as previously described (1, 2). Insulin was infused at a rate of 0.2 mU/kg per min into five volunteers on four separate occasions, during which time plasma glucose concentrations were clamped at ~60, 95, 130, and 165 mg/dl. Glucose utilization and clearance data from these experiments have been previously published (1). In another set of experiments, insulin was infused into five different volunteers at a rate of 1 mU/kg per min, during which time plasma glucose concentrations were clamped at ~60 ($n$ = 5), 95 ($n$ = 4), and 165 ($n$ = 5) mg/dl. In the third set of experiments, insulin was infused into five different volunteers at a rate of 2 mU/kg per min, during which time plasma glucose was clamped at ~60 ($n$ = 5), 95 ($n$ = 4), and 165 ($n$ = 5) mg/dl.

*Analyses and calculations.* Arterialized-venous blood samples were obtained at 15-min intervals for determination of plasma glucose and insulin concentrations and glucose specific activity (1, 2). Glucose uptake was determined isotopically as previously described (1, 2). Steady-state values over the last 30 min of each clamp were used for statistical analysis. Data in text and figures are expressed as means±SEM and were evaluated using either analysis of variance or two-tailed unpaired and, when appropriate, paired $t$ tests. The intercepts of Lineweaver-Burk (21) and Eadie-Hofstee (22) plots were determined by least squares linear regression.

## RESULTS

*Plasma insulin and glucose concentrations (Table I).* Base-line plasma glucose (≃90–95 mg/dl) and insulin concentrations (≃9–12 μU/ml) were found to be comparable before each set of experiments. Plasma insulin concentrations during the 0.2, 1.0, and 2.0 mU · kg⁻¹ · min⁻¹ insulin infusions were 18±1, 78±7, and 151±5 μU/ml, respectively, and for a given insulin infusion rate, were not significantly different during the different glucose clamps. During the experiments designed to achieve plasma glucose concentrations of 60, 95, 130, and 165 mg/dl, plasma glucose concentrations were 60±1, 92±2, 129±1, and 162±2 mg/dl, respectively; their coefficients of variation averaged <5%. Plasma glucose concentrations for a given glucose clamp were not significantly different during different insulin infusions.

*Glucose uptake (Table I).* Glucose uptake increased as a function of both plasma glucose and plasma insulin concentrations. At each plasma glucose concentration studied, glucose uptake at ≃150 μU/ml plasma insulin exceeded that at ≃80 μU/ml ($P$ < 0.01), which in turn exceeded that at ≃18 μU/ml ($P$ < 0.01). At each plasma insulin concentration studied, glucose uptake at ≃165 mg/dl plasma glucose exceeded that at ≃95 mg/dl ($P$ < 0.05), which in turn exceeded that observed at ≃60 mg/dl ($P$ < 0.05).

*Kinetic analysis (Fig. 1).* Lineweaver-Burk and Eadie-Hofstee analysis yielded similar results, indi-

### TABLE I
*Plasma Glucose and Insulin Concentrations and Rates of Glucose Uptake*

| Insulin infusions | Glucose clamps (*mg/dl*) | | | |
| --- | --- | --- | --- | --- |
| | 60 | 95 | 130 | 160 |
| 0.2 mU · kg⁻¹ · min⁻¹ | | | | |
| Plasma glucose, *mg/dl* | 60±1 | 93±2 | 129±1 | 165±2 |
| Plasma insulin, *μU/ml* | 18±1 | 18±1 | 19±2 | 18±1 |
| Glucose uptake, *mg·kg⁻¹·min⁻¹* | 1.8±0.03° | 2.3±0.12 | 2.6±0.09° | 3.1±0.16° |
| 1.0 mU · kg⁻¹ · min⁻¹ | | | | |
| Plasma glucose, *mg/dl* | 60±1 | 91±1 | — | 161±2 |
| Plasma insulin, *μU/ml* | 81±8 | 79±18 | — | 75±14 |
| Glucose uptake, *mg·kg⁻¹·min⁻¹* | 4.1±0.50°‡ | 5.8±0.52‡ | — | 7.4±0.92°‡ |
| 2.0 mU · kg⁻¹ · min⁻¹ | | | | |
| Plasma glucose, *mg/dl* | 59±1 | 93±2 | — | 160±1 |
| Plasma insulin, *μU/ml* | 153±9 | 145±6 | — | 153±12 |
| Glucose uptake, *mg·kg⁻¹·min⁻¹* | 6.8±0.30°‡ | 8.4±0.54‡ | — | 12.5±1°‡ |

° $P$ < 0.05 vs. 95 mg/dl.
‡ $P$ < 0.05 vs. 0.2 mU · kg⁻¹ · min⁻¹.

FIGURE 1 Lineweaver-Burk analysis of effect of insulin on glucose uptake.

and other tissues follows Michaelis-Menten kinetics (3, 4), we analyzed our data according to the methods of Lineweaver-Burk (21) and Eadie-Hofstee (22) to derive estimates of $K_m$ and $V_{max}$ for glucose uptake. Plots of 1/glucose uptake vs. 1/plasma glucose yielded straight lines at the three different plasma insulin concentrations studied (18, 80, and 150 U/ml), suggesting that whole body glucose uptake could be described by Michaelis-Menten kinetics. This conclusion is supported by the fact that analysis of our data using Eadie-Hofstee plots, which provide a more sensitive method for detecting deviations from Michaelis-Menten relationships (22), also yielded straight lines. Application of these methods of analysis in the context of the mobile carrier model (25–27) requires that the glucose uptake measured represents unidirectional glucose flux (28). Because the glucose isotope used in the present studies does not recycle (29), and because the minimal accumulation of intracellular glucose in tissues such as muscle (30, 31) precludes appreciable backflux, this requirement appears to be satisfied.

Theoretically, insulin could accelerate glucose uptake by increasing the efficiency of the glucose transport system (i.e., its affinity for glucose or its molecular activity) and/or by increasing the number of transport sites available. A mere increase in affinity would result in a decrease in the $K_m$ for glucose transport with no change in $V_{max}$, whereas an increase in molecular activity should result in an increase in both $K_m$ and $V_{max}$, because for a particular transport site, carrier mobility is the rate-limiting step for glucose transport (27). A mere increase in the number of transport sites should
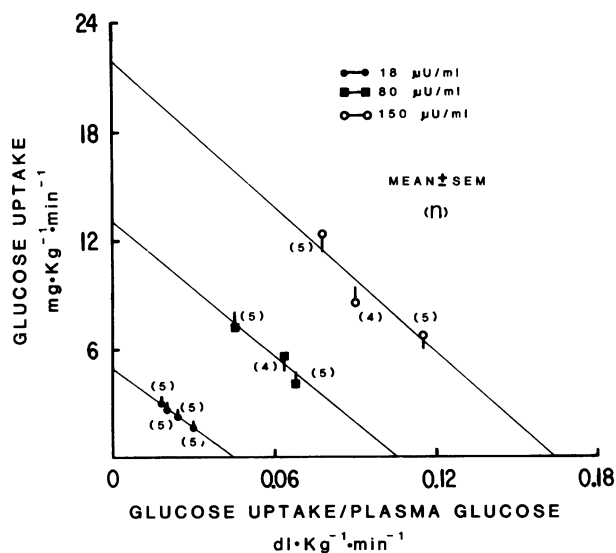
cating that insulin increased the $V_{max}$ for glucose uptake without affecting the $K_m$. Reciprocals of glucose uptake vs. plasma glucose concentration (Lineweaver-Burk analysis) were well-fitted to straight lines for each plasma insulin concentration studied; $r = 0.99$ ($P < 0.01$), $r = 0.98$ ($P < 0.01$), and $r = 0.98$ ($P < 0.01$) for $\cong 18$, 80, and 150 $\mu$U/ml, respectively (Fig. 1). The $K_m$ for glucose uptake at each plasma insulin concentration (range 6.2–8.7 mM) were not significantly different ($F_{12}^2 = 0.80$). The $V_{max}$ at plasma insulin concentrations of $\cong 150$ $\mu$U/ml ($23\pm4$ mg$\cdot$kg$^{-1}\cdot$min$^{-1}$) and $\cong 80$ $\mu$U/ml ($17\pm3$ mg$\cdot$kg$^{-1}\cdot$min$^{-1}$) were both significantly greater than that at $\cong 18$ $\mu$U/ml ($5.0\pm0.4$ mg$\cdot$kg$^{-1}\cdot$min$^{-1}$) ($F_{12}^2 = 49, P < 0.001$). Plots of glucose uptake vs. glucose uptake/plasma glucose (Eadie-Hofstee analysis) were well-fitted to straight lines for each plasma insulin concentration studied (Fig. 2); $r = 0.96$ ($P < 0.01$), $r = 0.94$ ($P < 0.05$), and $r = 0.90$ ($P < 0.05$) for $\cong 18$, 80, and 150 $\mu$U/ml, respectively. The $K_m$ at each plasma insulin concentration (range 5.9–7.6 mM) were not significantly different ($F_{12}^2 = 1.2$), whereas the $V_{max}$ at $\cong 80$ $\mu$U/ml ($13\pm3$ mg$\cdot$kg$^{-1}\cdot$min$^{-1}$) and 150 $\mu$U/ml ($22\pm6$ mg$\cdot$kg$^{-1}\cdot$min$^{-1}$) were both significantly greater than those at $\cong 18$ $\mu$U/ml ($5.0\pm0.4$ mg$\cdot$kg$^{-1}\cdot$min$^{-1}$) ($F_{12}^2 = 6.1, P < 0.025$).

## DISCUSSION

Glucose uptake measured isotopically in vivo represents the sum of glucose uptake by various tissues. However, it is likely that under our experimental conditions, which included suppression of glucagon secretion, 60–70% of the glucose uptake occurred in muscle (17, 18, 23, 24). Because glucose uptake in this



FIGURE 2 Eadie-Hofstee analysis of effects of insulin on glucose uptake.

result in only an increase in $V_{max}$. However, if the additional transport sites differed from those present basally, $K_m$ would also be altered.

In the present study, Lineweaver-Burk analysis indicated that an increase in plasma insulin from ~18 $\mu$U/ml to ~150 $\mu$U/ml increased the $V_{max}$ for glucose uptake nearly fivefold (from $5.0\pm0.4$ to $23\pm4$ mg$\cdot$kg$^{-1}\cdot$min$^{-1}$, $P < 0.001$) without significantly altering the $K_m$ ($6.4\pm0.6$ vs. $6.9\pm1.2$ mM). Similar results were obtained using Eadie-Hofstee analysis. Although a subtle change in $K_m$ may not have been detected, these results nevertheless support the hypothesis that insulin increases glucose uptake by providing additional glucose transport sites and suggest that these sites are similar to those present basally. Theoretically, insulin could make additional glucose transport sites available by unmasking transport sites already present in the plasma membrane or by recruiting to the plasma membrane intracellularly sequestered sites. The recent observations that insulin increases plasma membrane glucose transport activity (32) and cytochalasin B binding sites (33), which are thought to refect glucose transport sites, while decreasing intracellular glucose transport activity and cytochalasin B binding sites provide evidence for the latter mechanism.

Our finding that insulin increases the $V_{max}$ but not the $K_m$ for glucose uptake in man is similar to results derived from in vitro studies using human fibroblasts and both rat and human adipocytes (6–16), but differs from those using rat muscle in which either a decrease in $K_m$ with no increase in $V_{max}$ or an increase in both $K_m$ and $V_{max}$ were observed (19, 20). An explanation for these discrepancies is not apparent but might in part involve differences in the accessibility of glucose or insulin to individual cells in whole muscle preparations as opposed to accessibility in isolated cell preparations and under in vivo conditions.

The $K_m$ for glucose uptake in our in vivo study ($\simeq$6–9 mM) is similar to values reported for human adipocytes and fibroblasts in vitro (10, 13–15). This suggests that fibroblasts and adipocytes may be satisfactory models for evaluating glucose uptake in man even though muscle is the predominant tissue responsible for glucose uptake in vivo. Nevertheless, in vitro glucose uptake in adipocytes or fibroblasts (which must be cultured before study) may not always be representative of glucose uptake in vivo. Application of Lineweaver-Burk and/or Eadie-Hofstee analysis to measurements of whole body glucose uptake, determined at different plasma insulin concentrations, not only permits evaluation of glucose uptake kinetics in vivo but also an assessment of the appropriateness of insulin action on glucose uptake. Such an approach, combined with insulin receptor binding studies, may

prove useful in delineating mechanisms responsible for insulin resistance.

In summary, application of Lineweaver-Burk and Eadie-Hofstee analysis to measurements of glucose uptake in normal volunteers determined over the physiologic range of plasma glucose and plasma insulin concentrations indicated that insulin increases the $V_{max}$ for glucose uptake without altering its $K_m$. These results support the hypothesis that insulin increases glucose uptake merely by increasing the number of transport sites.

## ACKNOWLEDGMENTS

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