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Research Article

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Effects of Aging on Glucose-mediated Glucose Disposal and Glucose Transport

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Abstract

To assess the effects of aging on glucose-mediated glucose disposal and glucose transport, glucose disposal rates were measured in 10 nonelderly (32±4 yr) and 11 elderly (64±4 yr) subjects at five different plasma glucose concentrations. Glucose disposal was decreased by 30–35% in the elderly at each level of glycemia (100–350 mg/dl) in the presence of similar levels of hyperinsulinemia ($\sim$100 μU/ml), and the 50% effective concentration (EC$_{50}$) was similar in both the nonelderly (100±9) and elderly (103±5 mg/dl). The Michaelis constant (K_m) of 3-O-methyl glucose transport in adipocytes was unchanged with aging (3.8±0.5 vs. 3.2±0.2 mM) while the maximum velocity of insulin stimulated transport was reduced by 34% in the elderly (8.3±1.3 vs. 12.6±1.5 pmol/5 × 10^4 cells per s, P < 0.05). The insulin resistance of aging is therefore due to a reduction in the capacity of the glucose uptake system, while the affinity of glucose utilization (EC$_{50}$ and K_m) is unchanged. This supports the hypothesis that a reduction in the number of glucose transport and metabolic units occurs with aging, but that each unit functions normally.

Introduction

Glucose intolerance is a well recognized characteristic of human aging (1). This glucose intolerance is generally accompanied by hyperinsulinemia, indicating an insulin-resistant state. Indeed, evidence in favor of insulin resistance was presented some 40 years ago by Himsworth and Kerr, who demonstrated a decrease in insulin sensitivity with aging using an oral glucose tolerance test with a simultaneous intravenous insulin injection (2). These conclusions have been substantiated over the years, primarily with the advent of the glucose clamp technique, which has enabled us to not only establish the existence of insulin resistance with aging, but also to quantitate the severity of the resistant state and to gain insights as to its mechanisms (3–6). Recent in vivo data using the euglycemic glucose clamp technique to construct insulin dose-response curves for glucose disposal have indicated that insulin resistance in aging manifests itself as a rightward shift in the insulin dose-response curve with maximal responsiveness ranging from normal to markedly decreased, depending on the degree of glucose intolerance present in the elderly (4, 5). When insulin binding to either adipocytes or monocytes has been simultaneously measured in these studies,

no changes could be demonstrated in the aged. Since insulin resistance exists in the face of normal insulin binding, the probable locus of the insulin resistance must reside at a postbinding step. We and others have demonstrated a decrease in glucose transport activity in adipocytes from elderly subjects (7, 8) that is consistent with this formulation. Furthermore, more distal defects in intracellular metabolism involving decreased maximal glucose oxidation have also recently been identified with aging (9). All these studies, therefore, point to a postbinding defect in insulin action that occurs with aging. However, they do not distinguish between the effects of insulin on the processes of glucose metabolism and a defect in the glucose metabolism processes themselves.

In an effort to further elucidate the mechanisms of insulin resistance in aging, we have utilized the glucose clamp technique to study insulin-stimulated glucose disposal rates over a wide range of glucose concentrations in younger and older individuals. The purpose of the study was to examine the in vivo kinetic characteristics of glucose utilization by measuring the effects of glucose in stimulating its own disposal and thus to determine whether there existed alterations in the efficiency or capacity of glucose disposal with aging. In addition, we have examined the kinetic characteristics of the glucose transport system in adipocytes from the same population to determine the maximum velocity (V_{max}) and the Michaelis constant (K_m) of glucose transport in young and old and to provide in vivo and in vitro correlates.

Methods

Materials

Subjects

The study group included a total of 21 nonobese healthy volunteers, divided into two groups on the basis of age. The nonelderly group consisted of 10 men aged 26–40 yr (mean±SD, 32±4 yr) and the elderly group consisted of 10 men and 1 woman aged 60–70 yr (mean±SD, 64±4 yr). All subjects were within 20% of ideal body weight, based on the Metropolitan Life Insurance Tables. They were in good general health and had no evidence of renal, hepatic, or cardiac dysfunction. None of the subjects was taking any medication during the study, with the exception of iron supplementation. After informed consent was obtained, subjects were admitted to the Special Diagnostic and Treatment Unit of the San Diego Veterans Administration Medical Center. Daily carbohydrate intake as determined by dietician-obtained histories was 150 g

in both nonelderly and elderly subjects in the week before the studies. Subjects were weighed daily and calories adjusted to maintain initial weight throughout the study. The diet contained 45% carbohydrate, 35% fat, and 20% protein. The clinical and metabolic characteristics of the subjects are summarized in Table I.

An oral glucose tolerance test (75 g) was performed before participation of each subject in the experimental protocol. Six of the elderly group and one of the nonelderly group had 2-h plasma glucose values greater than 140 mg/dl. Three of the six elderly subjects had impaired glucose tolerance while the other three and the one nonelderly individual had nondiagnostic glucose tolerance tests as defined by the National Diabetes Data Group (10). As a whole, the elderly group was significantly more glucose intolerant than the nonelderly group as indicated by the 2-h glucose values.

Total kilograms of fat was calculated in all subjects according to the formula of Rowe et al. (5) by measuring abdominal and triceps skin fold thickness, abdominal circumference, and 24-h urinary creatinine. Skin folds were measured with the use of Lange skin fold calipers. The kilograms of lean body mass (LBM) were then calculated as total weight minus the kilograms of fat.

Study protocol

Glucose clamp studies. The glucose clamp technique was used as previously described (4). Insulin was infused in a primed continuous fashion at a rate of 40 mU/M^2 per min in all of the studies. The plasma glucose was clamped at five different levels of glycemia in each patient, and the studies were all carried out on separate days. Thus, five separate glucose clamps were performed in each subject. The goal plasma glucose concentrations were 100, 150, 200, 250, and 350 mg/dl. During the hyperglycemic studies, glucose was infused in a priming dose to increase the plasma glucose to the desired level in a 10-min period, according to a modification of the protocol of DeFronzo et al. (11). SRIF was infused throughout each of the studies at a rate of 600 μg/h, to suppress endogenous insulin secretion. [3-^{3}H]glucose was infused in a primed continuous fashion only during the 100 and 150 mg/dl studies. 30 μCi of [3-^{3}H]glucose was injected as a bolus, followed by a continuous infusion at the rate of 0.6 μCi/min. It was not necessary to infuse [3-^{3}H]glucose during the hyperglycemic studies, since under the conditions of our protocol, i.e., hyperinsulinemia, hyperglycemia, and glucagon deficiency due to the infusion of SRIF, hepatic glucose output is completely suppressed. In fact, even during the studies done at the lowest plasma glucose concentration of 100 and 150 mg/dl, hepatic glucose output was completely suppressed in both age groups.

After the initiation of the glucose and insulin infusions, the desired level of glycemia was maintained for at least 180 min, or until the glucose infusion rate had not changed by more than ±10% for 60 min. The mean length for each study was 194 min with a range of 180–260 min. There was no difference between the two groups in the length of study (194±5 min vs. 194±2 min in the nonelderly and elderly, respectively). The glucose disposal rates for the last three 20-min intervals of each study were measured and used as the datum point for that particular study.

Table I. Metabolic Characteristics of Nonelderly and Elderly Groups

	Nonelderly (n = 10)	Elderly (n = 11)	
Age (yr)	32±2	65±2	
Age range	21–40	60–70	
BMI (kg/M^2)	23.5±0.5	23.5±1.0	NS
Fat cell size (pl)	361±44	419±40	NS
Fasting glucose (mg/dl)	93±1	94±2	NS
Fasting insulin (μU/ml)	10±1	12±1	NS
2-hour OGTT glucose (mg/dl)	118±4	144±8	P < 0.01

BMI, body mass institute; OGTT, oral glucose tolerance test.

Urinary glucose was measured during the studies and was subtracted from the glucose infusion rate to give the true glucose disposal rate.

Glucose transport studies. Adipose tissue was obtained from the lower abdominal region after a 14-h overnight fast, as previously described (12) and glucose transport was assayed by measuring initial rates of 3-O-methyl glucose influx according to a modification (13) of the method of Whitesell and Gliemann (14). The substrate, 3-O-methyl-D-[U-^{14}C]glucose (0.4 μCi/tube), was added to unlabeled 3-O-methyl glucose to give the final desired concentration as indicated. A 40% cell suspension was preincubated with and without 100 ng/ml insulin for 30 min. The reaction was started by the addition of 50 μl of the cell suspension to 20 μl of the substrate and subsequently terminated at time intervals ranging from 20 s to 3.5 min in the basal state, and from 10 to 60 s in the insulin-stimulated state, to ensure that initial uptake of 3-O-methyl glucose was being measured at all glucose concentrations. The reaction was terminated by the rapid addition of 400 μl of 0.1 mM phloretin. Aliquots were transferred to 550-μl microfuge tubes and centrifuged over silicone oil, after which the cell-associated radioactivity was counted. The portion of cellular uptake due to diffusion and trapping of the label in the extracellular water space was measured by performing parallel reactions with the cell suspension added directly to a mixture of phloretin and substrate. All values of transport were corrected for the trapping and extracellular water space and the data expressed as picomoles 3-O-methylglucose transport/5 × 10^4 cells per second. Adipocyte counts were performed according to a modification of method III of Hirsch and Gallian (15). Cell sizing was determined as previously described (16).

Analytical methods. Serum insulin levels were measured by a double-antibody radioimmunoassay according to the method of Desbuquois and Aurbach (17). C peptide levels were measured courtesy of Dr. A. Rubenstein at the University of Chicago, Chicago, IL, by a previously described assay (18).

Data analysis. Data presented represent the mean±SEM. Statistical analysis employed two-tailed paired and unpaired Student's *t* tests as indicated. Analysis of variance (ANOVA) for repeated measures was used to test for differences between groups and for changes over dose (for studies of glucose transport and glucose disposal rates). Correlation coefficients were determined using linear regression analysis. Statistical analyses were performed on a Digital PDP 11/24 computer (Digital Equipment Corp., Marlboro, MA) using the CLINFO data base management and analysis program (Bolt, Beraneck, and Newman, Inc., Cambridge, MA).

Results

Glucose clamp studies: plasma glucose, insulin, and C peptide (Table II): The goal glucose levels were attained in both groups with a coefficient of variation of <5% for each of the studies performed. Steady state serum insulin levels were similar (100 μU/ml) in both the elderly and nonelderly group at each level of glycemia, with the sole exception of the elderly group studied at a plasma glucose concentration of 350 mg/dl where the serum insulin level was somewhat higher (124±6 μU/ml) compared with the nonelderly group (109±6 μU/ml, P < 0.05). Despite the increasing plasma glucose levels attained during the clamp studies, endogenous insulin secretion remained suppressed due to the SRIF infusion. Even at a glucose concentration of 350 mg/dl, C peptide was suppressed by 40±11% in the nonelderly and 41±9% in the elderly group (Table II). Absolute basal C peptide values were similar in both groups as well (0.35±.04 vs. 0.38±.03 pmol/ml in the nonelderly and elderly, respectively) and no difference was observed in the ability of SRIF to inhibit endogenous insulin release in the two age groups. In both the elderly and nonelderly, however, SRIF resulted in a greater suppression of C peptide at lower glucose levels of 100 and 150 mg/dl compared with the hyperglycemic levels of 250 and 350 mg/dl (Table II).

Table II. Glucose and Insulin Concentrations during Glucose Clamp Studies

	Goal glucose				
	100 mg/dl	150 mg/dl	200 mg/dl	250 mg/dl	350 mg/dl
Plasma glucose (*mg/dl*)					
Nonelderly	100±1	150±1	200±1	250±1	350±1
Elderly	99±1	149±1	201±1	249±1	349±1
Serum Insulin ($\mu U/ml$)					
Nonelderly	97±5	96±5	106±5	93±5	109±6
Elderly	102±4	116±9	108±7	104±6	124±6*
C Peptide Suppression during glucose clamp studies (%)					
Nonelderly	95±1	92±1	84±3	66±9	40±11
Elderly	93±1	92±1	90±1	72±4	41±9

* $P < 0.05$ compared with nonelderly at 350 mg/dl. $P < 0.05$ compared with elderly at 100, 200, and 250 mg/dl.

Glucose disposal rates. Glucose disposal rates were significantly decreased by 30–35% in the elderly group, compared with the nonelderly group, at all steady state plasma glucose concentrations, ranging from 100 to 350 mg/dl, ($P < 0.05$, $F = 9.93$, ANOVA) (Fig. 1 *A,* Table III). This indicates that the insulin resistance of aging is expressed equally at all glucose levels and that it is the maximal capacity for glucose metabolism which is impaired. The decrease in glucose uptake in the elderly was similarly apparent when the data were analyzed on a per kilogram LBM basis (Fig. 1 *B,* Table III, $P < 0.05$, $F = 6.75$, ANOVA). Hepatic glucose output was completely and equally suppressed by 100% in both age groups during the studies done at plasma glucose concentrations of 100 and 150 mg/dl. Because of this complete suppression at lower glucose concentrations, [3-³H]glucose was not infused during studies done at higher plasma glucose levels of 200, 250, and 350 mg/dl.

Glucose utilization increased significantly ($P < 0.05$) in both the nonelderly and elderly subjects when the plasma glucose concentration was increased from 100 to 250 mg/dl during the 40 mU/M² per min insulin infusions. However, increasing the glucose concentration from 250 to 350 mg/dl resulted in no significant increase in glucose disposal rates in both groups (15.2±1.6 vs. 16.4±1.5 mg/kg per min in the nonelderly group and 10.9±1.0 vs. 11.3±0.9 mg/kg per min in the elderly group). This indicates that glucose utilization was almost totally saturated at a glucose level of 250 mg/dl during physiological hyperinsulinemia.

In order to more closely examine the ability of glucose to increase total glucose disposal, and to determine if aging resulted in an impaired ability of an increasing glucose concentration to increase glucose utilization, the glucose disposal rates were analyzed as a percentage of the maximal glucose disposal and the change in the percent increase in glucose disposal per change in plasma glucose (slope) (19) was examined in both groups (Fig. 2). This relationship was evaluated over the glucose concentrations where glucose uptake increased in a fairly linear fashion, i.e., from 100 to 250 mg/dl. This was confirmed by least squares linear regression analysis and indicated that the slope did not differ significantly between the nonelderly ($r = 0.91±0.03$, slope $= 0.25±0.03$) and elderly groups ($r = 0.90±0.04$, slope $= 0.26±0.03$, $P = NS$). The similar effectiveness of glucose in increasing glucose uptake in both the nonelderly and elderly subjects is further illustrated by the plasma glucose concentration
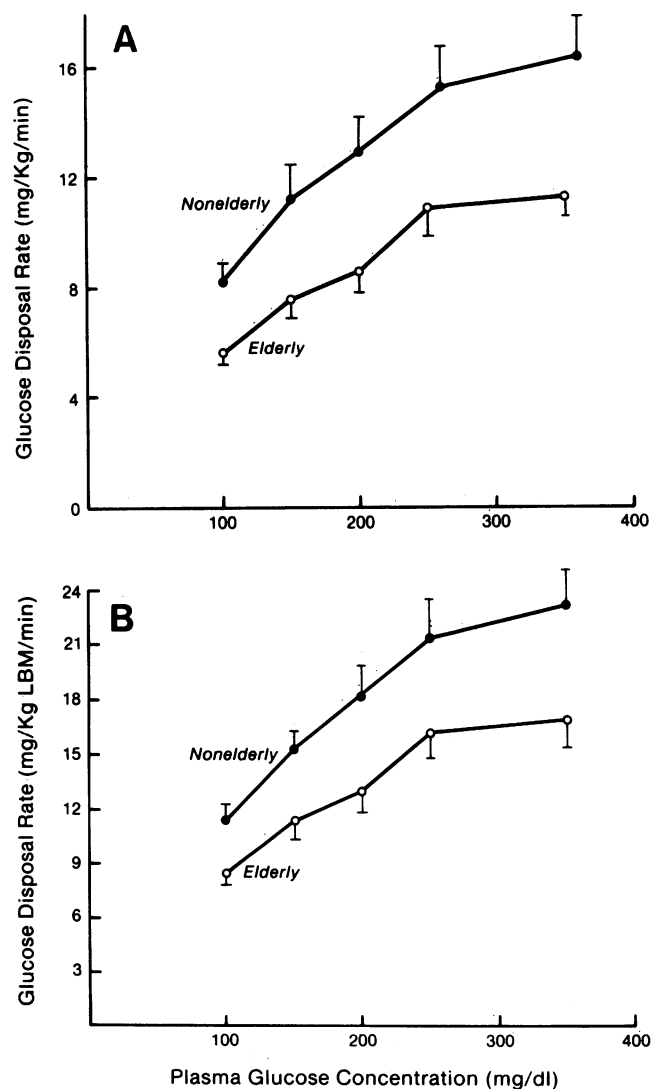

Figure 1. (*A*) Glucose disposal rates (milligram per kilogram per minute) at plasma glucose concentrations of 100, 150, 200, 250, and 350 mg/dl during insulin infusion rates of 40 mU/M² per min in nonelderly (●) and elderly (○) subjects. (*B*) Glucose disposal rates expressed as milligram per kilogram LBM per minute in nonelderly (●) and elderly (○) subjects.

Table III. Results of Glucose Clamp Studies

Glucose disposal rate	100 mg/dl	150 mg/dl	200 mg/dl	250 mg/dl	350 mg/dl
(mg/kg/min)					
Nonelderly	8.2±0.8	11.2±1.3	12.9±1.3	15.2±1.6	16.4±1.5‡
Elderly	5.7±0.4*	7.6±0.7*	8.6±0.7*	10.9±1.0*	11.3±0.9*§
(mg/kg LBM/min)					
Nonelderly	11.4±1.0	15.5±1.6	18.1±1.8	21.3±2.2	23.1±2.0‡
Elderly	8.5±0.7*	11.3±1.0*	13.0±1.2*	16.2±1.6	16.9±1.5*§

* $P < 0.05$ for differences between elderly and nonelderly subjects. ‡P = NS compared with nonelderly at 250 mg/dl. §P = NS compared with elderly at 250 mg/dl.

at which half the maximal glucose disposal rate was observed (EC_{50}). This was determined for each individual based on a plot of the percent of maximal glucose disposal versus plasma glucose concentrations. These values (Fig. 2) were virtually identical in the two groups, 100±9 mg/dl in the nonelderly subjects and 103±5 mg/dl in the elderly subjects (P = NS). As predicted by the similar EC_{50} values in the two groups, there was no significant relationship observed between the EC_{50} and the 2-h plasma glucose value during the oral glucose tolerance test ($r = 0.18$, nonelderly; $r = -0.27$, elderly; P = NS) or between the slope as derived in Fig. 2 and the 2-h glucose value ($r = -0.14$, nonelderly; $r = 0.21$, elderly; P = NS).

The glucose disposal curves were also analyzed using the assumption that in vivo glucose uptake follows Michaelis-Menten kinetics as suggested by Gottesman et al. (20). Eadie-Hofstee plots generated straight lines with similar K_m values of 296±54 vs. 296±25 mg/dl but with a 26% reduction in V_{max} in the elderly (21.9±2.1 vs. 29.4±3.8 mg/kg per min, $P < 0.05$).

Adipocyte glucose transport. (Fig. 3) Basal rates of glucose transport were similar in the nonelderly and elderly subjects at all glucose concentrations ranging from 0.1 to 50 mM. Insulin, however, resulted in a greater stimulation of glucose transport in the nonelderly compared with the elderly ($P < 0.05$, $F = 5.02$, ANOVA). Since glucose transport follows Michaelis-Menton kinetics, the data were analyzed by Eadie-Hofstee plots in order to determine the K_m and V_{max} of glucose transport in each group (Fig. 4). In the basal state, the K_m and V_{max} of glucose transport were not significantly different in the nonelderly (K_m = 3.1±0.2

mM, V_{max} = 5.0±0.6 pmol/5 × 10^4 cells per s) compared with the elderly (K_m = 3.6±0.3 mM, V_{max} = 4.1±0.5 pmol/5 × 10^4 cells per s). Insulin increased the V_{max} of glucose transport without changing the K_m in both groups. Therefore, in the presence of insulin, the decrease in glucose transport in the elderly group could be entirely accounted for by the 34% decrease in V_{max} (12.6±1.5 vs. 8.3±1.3 pmol/5 × 10^4 cells per s, $P < 0.05$) (Table IV). In addition, the effect of insulin to stimulate glucose transport was decreased in the fat cells from the elderly with a 20% reduction in the fold insulin stimulation (2.5-fold increase in the nonelderly compared with a twofold increase in the elderly). Since cell size was somewhat larger in the elderly (Table I), this reduction in the V_{max} of glucose transport is even more apparent when the data are expressed on a per cell surface area basis, rather than per cell number. In the basal state, the V_{max} of glucose transport was decreased by 28% in the elderly group, although this difference did not quite achieve statistical significance (6.2 vs. 8.6 pmol/2 × 10^9 μ^2 per s, $P = 0.07$ in the elderly and nonelderly, respectively). In the presence of insulin, however a 40% reduction in the V_{max} of glucose transport was observed in the elderly (13.2±2.5 vs. 21.2±2.6 pmol/2 × 10^9 μ^2 per s, $P < 0.05$ in the elderly and nonelderly, respectively).

Discussion

We have previously found that aging is associated with insulin resistance using euglycemic clamps across the dose-response
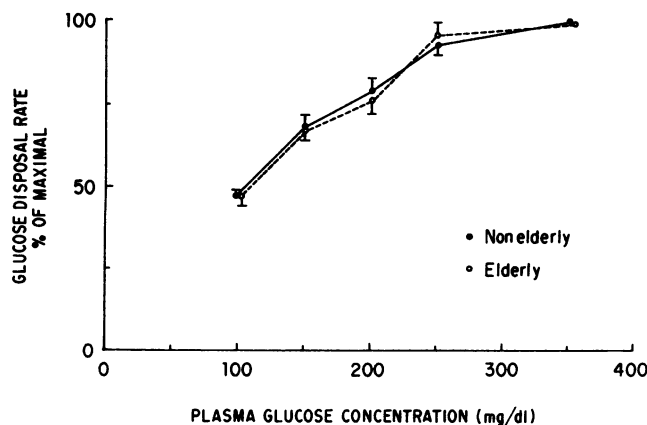

Figure 2. Glucose disposal rates expressed as a percentage of maximal glucose disposal in nonelderly (●) and elderly (○) subjects.
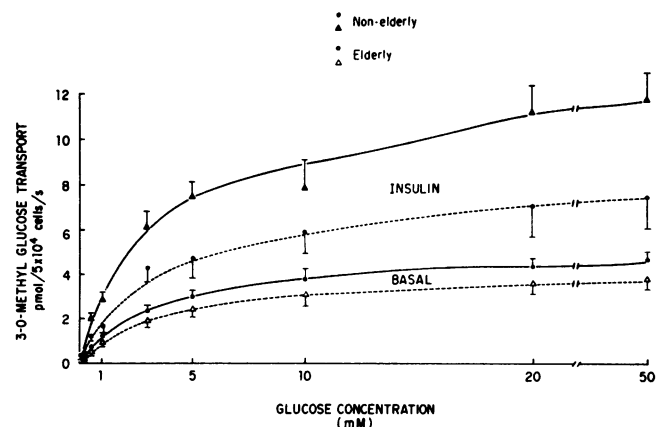

Figure 3. 3-*O*-methyl glucose transport in adipocytes from nonelderly (●, ▲) and elderly (○, △) subjects in the basal state and in the presence of 100 ng/ml insulin.
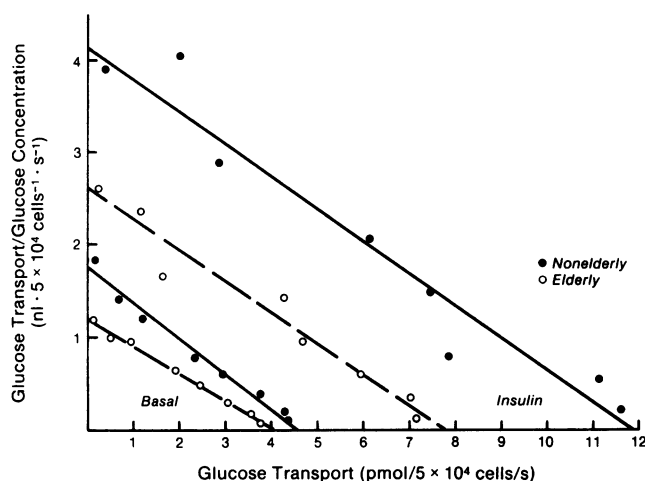
Figure 4. Kinetic characteristics of 3-*O*-methyl glucose transport in adipocytes from nonelderly (•) and elderly (○) subjects. Eadie-Hofstee plot of the data in Fig. 3 in the basal state and in the presence of 100 ng/ml insulin.

curve for insulin for a single glucose concentration (4). Overall glucose disposal encompasses the processes of glucose transport and metabolism and the effects of insulin on these processes. The presence of insulin resistance at various insulin concentrations at a single glucose concentration does not, however, distinguish between the effects of insulin on the glucose metabolism processes and a defect in the glucose metabolism processes themselves. One way to approach this problem is to study insulin stimulation of glucose disposal across a range of plasma glucose concentrations at a fixed insulin concentration. Increasing the plasma glucose concentration leads to increased rates of glucose disposal due to the mass action effectiveness of glucose in increasing flux through the glucose transport system. Therefore, by studying insulin-stimulated glucose disposal across a range of glucose concentrations, one can distinguish between abnormalities of the glucose transport/glucose utilization system per se and insulin action on this system.

The results of our study indicate that the affinity of glucose utilization, as measured by the percent increment in glucose disposal rate per increment in plasma glucose concentration (slope), is the same in both age groups, implying that the similar reduction in glucose disposal observed in the elderly at all plasma glucose concentrations is not due to an impaired affinity of the glucose transport/metabolism units for glucose, but rather due to a reduction in the number of functioning units available. This

conclusion is supported by the in vivo data, which demonstrate a 30–35% decrease in glucose disposal rates in the elderly at all levels of glycemia, ranging from 100–350 mg/dl. However, despite the presence of insulin resistance, the incremental change in the percent of maximal glucose disposal rate per incremental change in glucose concentration, i.e., the slope, (Fig. 2) was similar in both age groups, as was the EC_{50}, indicating that the affinity of the glucose uptake system for glucose is unaltered with aging. These in vivo findings are supported by the in vitro data, which indicate that the affinity of glucose transport as measured by the K_m is equal in both age groups. Since the functional integrity of the glucose transport system as measured by its K_m and the functional integrity of the glucose utilization system as measured by incremental glucose uptake is similar in both age groups, it follows that the affinity of glucose transport for total body glucose utilization must be normal.

Our results further indicate that although the affinity of in vivo glucose utilization is normal with age, there is a reduction in the maximal capacity of glucose utilization as demonstrated by the 30–35% reduction in glucose disposal rates at all glucose concentrations studied. This reduction in maximal capacity indicates that a decrease in the number of glucose transport/metabolic units exists. Once again, the in vitro data support this conclusion, since the maximal capacity of glucose transport, as reflected in the V_{max}, was reduced by 34% in the elderly group. Since insulin's ability to increase V_{max} has been shown to be due to translocation of glucose transporters from low density intracellular microsomes to the plasma membrane (21, 22), a decrease in V_{max} could be due to either a smaller absolute number of transporters or to decreased translocation or availability of transporters, either of which results in a decreased in vivo glucose disposal at all glucose concentrations.

Chen and her colleagues (23) recently evaluated the mechanisms of age-related glucose intolerance using the minimal model approach. They also showed that older subjects were insulin-resistant but that so-called glucose effectiveness, i.e., the increase in fractional disappearance of glucose per unit increase in glucose, was unchanged with aging. Our results are in agreement with these data, and indicate that the effect of increasing glucose concentrations to increase glucose uptake is not decreased in the glucose-intolerant aged population both at basal serum insulin levels (23) and at serum insulin levels of about 100 $\mu U/ml$.

Documenting the effects of aging per se to influence insulin action and glucose metabolism is fraught with the difficulties of separating out age-related variables such as obesity, physical activity, and caloric intake, each known to independently alter insulin action. It is unlikely, however, that the differences between the nonelderly subjects and elderly subjects could be entirely accounted for by these factors. Firstly, LBM was not significantly different between the two groups, and when the data were analyzed on a per kilogram LBM basis, similar differences in glucose disposal rates between the two age groups could be seen. Secondly, fat cell size, another independent marker of obesity, was not significantly different between the nonelderly and elderly groups and, therefore, the differences in the glucose transport data could not be solely attributed to increased adiposity in the elderly. Thirdly, within age groups, and considering all glucose infusion levels, there were no statistically significant relationships between steady state glucose disposal rates and percent body fat (nonelderly, $r = -0.29$, P = NS; elderly, $r = +0.24$, P = NS). Although no measurements of the degree of

Table IV. Kinetic Characteristics of Glucose Transport

	Nonelderly	Elderly
K_m (*mM*)		
Basal	3.1±0.2	3.6±0.3
Insulin	3.2±0.2	3.8±0.5
V_{max} (*pmol/5 × 10^4 cells per s*)		
Basal	5.0±0.6	4.1±0.5
Insulin	12.6±1.5	8.3±1.3 P < 0.05

physical conditioning of the subjects were made in our study, all subjects selected were fully active and leading nonsedentary lifestyles, and none were involved in any vigorous exercise program that may have unduly affected our results. Although dietary carbohydrate intake is often reduced with aging and could result in impairment in measurements of insulin action, it is unlikely to have accounted for any significant differences in our study, since all subjects were maintained on at least 150 g carbohydrate in the week before studies and on a weight-maintenance isocaloric diet during the study.

All the glucose clamp studies were done with insulin infusion rates of 40 mU/M^2 per min and in the presence of SRIF. Even with steady state plasma glucose levels of 350 mg/dl, SRIF was still effective in suppressing endogenous insulin release as assessed by serum C peptide concentrations. The inhibition of insulin secretion was 40% compared with 92% at steady state plasma glucose levels of 150 mg/dl, but importantly, no breakthrough of endogenous insulin secretion occurred under these hyperglycemic conditions. Serum insulin levels were therefore comparable in both age groups at all glucose concentrations with the exception of a small 14% increased steady state insulin concentration in the elderly group studied at a plasma glucose concentration of 350 mg/dl. This did not alter the results of the studies, since glucose disposal rates were still decreased by 30–35% in the elderly at all plasma glucose concentrations, including the studies done at 350 mg/dl where the serum insulin levels were, in fact, higher.

Each of the clamp studies was done on a separate day and in order to compare results both between and within groups it was essential that serum insulin levels be closely matched. 40 mU/M^2 per min insulin infusions were thus chosen, since we and others (3–5) have previously demonstrated that serum insulin concentrations of 100 μU/ml were attained in both younger and older subjects. A lower insulin infusion rate would have resulted in considerably higher serum insulin levels in the elderly because of the decrease in the metabolic clearance rate of insulin, which is more evident at lower insulin concentrations (24) and has been shown in prior studies (24–26).

Recently, it has been suggested that SRIF may have an independent effect in increasing peripheral glucose utilization (27). These results were demonstrated in dogs, and although there has been no confirmation of this observation in humans, it is unlikely that this would have significantly influenced our data. We have used a 600 μg/h dose of SRIF to ensure inhibition of insulin secretion at the higher plasma glucose levels. Even this dose, however, is only 15–20% (on a per kilogram basis) of the dose used in dog studies, which demonstrated the independent glucose lowering effect of SRIF (27). In addition, we used the same dose of SRIF for all studies regardless of the steady state plasma glucose and, therefore, valid comparisons of glucose disposal rates can be made within and between the two age groups. Finally, the magnitude of the SRIF effect would likely be quite small in relation to the total glucose disposal rates measured at hyperinsulinemia and hyperglycemia. The previously reported SRIF effect was demonstrated during basal insulin replacement (27).

SRIF inhibits not only insulin release but also growth hormone secretion and glucagon release. The deficiencies of both these hormones would have very little impact on the results of our studies for the following reasons. Glucagon has previously been shown to have no effect on peripheral glucose uptake (28) and prolonged infusions (longer than 6 h) of growth hormone

are necessary to demonstrate an effect on glucose uptake (29). The duration of our infusions was considerably less (3–4 h) and therefore it is unlikely that we were overestimating the glucose disposal rates as a result of growth hormone deficiency.

A finding of considerable interest in our study was the abrupt saturation of glucose uptake that occurred in both age groups between glucose concentrations of 250 and 350 mg/dl. Studies done at plasma glucose concentrations of 350 mg/dl resulted in an insignificant 3.7% increase in glucose disposal rate compared with studies done at glucose levels of 250 mg/dl in both age groups. The plateauing of glucose uptake that occurs at these high plasma glucose levels could be due either to saturation of glucose transport or to the emergence of a more distal postglucose transport step as the rate-limiting process for overall metabolism. If the rate-limiting step for overall glucose utilization resides at an intracellular step when the glucose concentration is raised from 250 to 350 mg/dl, then this would indicate that with aging the decrease in the V_{max} of glucose transport accounts for the decrease in glucose disposal at plasma glucose concentrations between 100 and 250 mg/dl, but that further intracellular defects in glucose metabolism also exist which impair overall glucose utilization when the plasma glucose level is increased above 250 mg/dl. This conclusion is based on the fact that at a plasma glucose level of 350 mg/dl overall glucose disposal rates were 31% lower in the elderly group. If intracellular glucose metabolism is rate-limiting at this rate of glucose flux, then the defect in aging must reside at an intracellular locus with a resultant decreased capacity of glucose metabolism. On the other hand, if glucose transport is still rate-limiting at a glucose concentration of 350 mg/dl, then the plateauing of in vivo glucose uptake is consistent with saturation of the transport system. The decrease in glucose disposal at all glucose levels would then be due to a decrease in maximal capacity of glucose transport and a normal affinity of the glucose transport system with aging. Further studies will be necessary to distinguish between these possibilities.

It has previously been suggested by Gottesman et al. that in vivo glucose uptake could be analyzed by Michaelis-Menten kinetics and that insulin increases the V_{max} of glucose uptake without a change in the K_m (20). Glucose uptake was measured at several insulin concentrations, but at a maximal glucose concentration of only 160 mg/dl, a point on the glucose dose-response curve where glucose uptake is still linear. Therefore, it was not demonstrated in that study that saturation of glucose uptake occurred, and one can question the assumption that Michaelis-Menten kinetics can be used to assess total body in vivo glucose uptake. Our data tend to cast doubt on the accuracy of this kind of analysis. Thus, when our data were analyzed using this assumption, Eadie-Hofstee plots generated straight lines with a K_m of ~300 mg/dl in both groups and a V_{max} of 29 and 21 mg/kg per min in the nonelderly and elderly groups, respectively. The K_m values are clearly much higher than the actual EC$_{50}$ values calculated from the glucose dose-response curves (~100 mg/dl in both age groups). Additionally, the calculated V_{max} is a full twofold greater than the actual maximally measured glucose uptake at a plasma glucose concentration of 350 mg/dl. There are many reasons why whole-body glucose uptake may not follow Michaelis-Menten kinetics. Firstly, there is now recent evidence to indicate that not all tissues in the body respond equally to insulin in increasing glucose uptake (30, 31). For example, the maximal responsiveness of rat diaphragm is fivefold greater than that of rat lung tissue (31). Secondly, differences exist even in similar tissues, such that insulin-mediated glucose uptake in

skeletal muscle consisting primarily of oxidative fibers is substantially different from glucose uptake in glycolytic muscle (32). Therefore, differences in muscle composition between one individual and another may play a role in determining total-body glucose uptake and these different types of insulin-responsive tissue may also have different kinetics of glucose uptake. Thirdly, glucose uptake is not a homogeneous process but consists of both insulin-mediated glucose uptake and noninsulin-mediated glucose uptake (NIMGU) (33). NIMGU largely involves central nervous system glucose metabolism, and it is likely that the kinetics of glucose uptake into the central nervous system differ substantially from that in peripheral tissues; this further weakens the analysis of whole-body glucose uptake by simple Michaelis-Menten kinetics. Therefore, it appears that overall glucose disposal rates are composed of several different uptake rates in different tissues and on aggregate they do not conform to Michaelis-Menten kinetics. It is probable, though, that in each individual tissue one could use this analysis to define the glucose uptake characteristics.

It has previously been shown that glucose uptake is not independent of the plasma glucose concentration and that the metabolic clearance rate of glucose (MCR) decreases with increases in glucose concentrations (34–36). Our data are consistent with this view and indicate that even when glucose utilization is linear over a range of glucose concentrations, the MCR is still falling. In the nonelderly group the MCR decreased by 26% with increasing glucose concentrations from 100 to 250 mg/dl, while it fell by a similar 24% in the elderly group. The reduction in MCR occurred in the presence of insulin levels of about 100 μU/ml, a level at which the effectiveness of increasing glucose concentration to decrease the MCR has been shown to be minimal (37). Thus, even in the presence of hyperinsulinemia, it is clear that the saturation kinetics of glucose uptake coupled with the smaller relative contribution of NIMGU to total glucose uptake result in a decreasing MCR of glucose with increasing glucose concentrations.

In conclusion, we have evaluated the effectiveness of glucose in increasing glucose utilization in the presence of physiological hyperinsulinemia in a group of younger and older subjects. Glucose uptake is linear in both groups up to a plasma glucose of about 250 mg/dl; thereafter total glucose disposal is virtually completely saturated. Glucose increases glucose uptake to a similar degree in both age groups, but at a lower total glucose disposal rate in the elderly, indicating their insulin resistance. The decrease in maximal glucose uptake with normal affinity of glucose uptake that occurs with aging is reflected in a decreased V_{max} of glucose transport activity with a normal K_m in the elderly group. These data are consistent with the possibility that a decrease in glucose transporter numbers or a lower rate of translocation of transporters, rather than an altered function of a glucose transporter unit, underlies the insulin resistance of aging. In addition, a decrease in the maximal capacity of intracellular glucose metabolism may also exist with aging. These data, when analyzed together with previous studies (4, 5, 9) indicate that there are two major components to the insulin resistance of aging. First, there is a decrease in the total capacity of glucose utilization, although each of the transport/metabolic units functions normally. Second, evidence exists that insulin action is abnormal in aging. The evidence for abnormal insulin action is that basal glucose transport is normal but that the effectiveness of insulin in stimulating transport (fold increase) is decreased. In addition, the in vivo insulin dose-response curves are shifted

right-ward in the face of normal insulin-binding, indicating a postbinding defect in the insulin action system.

Acknowledgments

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