

# Effects of Age and Body Fat on Insulin Resistance in Healthy Men

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**OBJECTIVE**— Aging is known to be associated with increasing insulin resistance and declining glucose tolerance. The cause for the insulin resistance, however, remains uncertain. In this study, we examined the hypothesis that at least part of the insulin resistance may be attributable to age-related changes in body composition and muscle blood flow rather than age itself.

**RESEARCH DESIGN AND METHODS**— We studied 6 healthy, elderly ( $66.2 \pm 1.7$  yr) and 6 younger, healthy men ( $31.8 \pm 3.0$  yr) matched for height and weight by determination of their body composition (by underwater weighing), leg blood flow (by mercury strain-gauge plethysmography), rates of glucose uptake (by stable isotope dilution analysis with 6,6  $D_2$ -glucose), and carbohydrate oxidation (by indirect calorimetry) during euglycemic hyperinsulinemic clamping.

**RESULTS**— Body fat (kg fat mass or in percentage of body weight), rates of insulin-stimulated leg blood flow, glucose uptake, oxidation, and storage were all similar in elderly and younger men. Body fat (in percentage of body weight) of both elderly and younger men correlated closely and negatively with glucose uptake ( $r = -0.73$ ,  $P < 0.01$ ), glucose oxidation ( $r = -0.67$ ,  $P < 0.05$ ), and with glucose storage ( $r = -0.65$ ,  $P < 0.05$ ). In contrast, age did not correlate significantly with any parameter of glucose metabolism.

**CONCLUSIONS**— Our data suggested that insulin sensitivity in men until around 60–70 yr of age appears to be determined more by body fat than by age.

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CHO, CARBOHYDRATE; HDL, HIGH-DENSITY LIPOPROTEIN; NGT, NORMAL GLUCOSE TOLERANCE; GTT, GLUCOSE TOLERANCE TEST;  $K_g$ , COEFFICIENT OF GLUCOSE DISAPPEARANCE (%/MIN);  $G_{Ra}$ , RATE OF GLUCOSE APPEARANCE;  $G_{Rd}$ , RATE OF GLUCOSE DISAPPEARANCE; GIR, GLUCOSE INFUSION RATE; HGP, HEPATIC GLUCOSE PRODUCTION; HGO, HEPATIC GLUCOSE OUTPUT; RIA, RADIOIMMUNOASSAY; FFA, FREE FATTY ACID; MANOVA, MULTIPLE ANALYSIS OF VARIANCE; EPI, EPINEPHRINE; NE, NOREPINEPHRINE; BMI, BODY MASS INDEX.

It has been recognized for many years that aging is associated with declining glucose tolerance. This decline begins at ~40 yr of age and progresses throughout adulthood (1). Its cause has been shown to be target tissue unresponsiveness to the action of insulin, rather than secretory insulin deficiency (2–4). The cause for the insulin resistance, however, remains uncertain. It has been suggested that a large part of the insulin resistance may be attributable to age-related changes in body composition and level of physical activity rather than to age itself (5). In support of this notion is the fact that aging is frequently associated with an increase in body fat (6,7) and that obesity correlates strongly with tissue unresponsiveness to insulin (8). The reduction in insulin-stimulated glucose uptake could also be a result of impaired muscle blood flow in the elderly. It has been shown previously, for instance, that the apparent insulin resistance in some diabetic patients could be explained entirely by abnormally low insulin-stimulated muscle blood flow (9). Blood flow was not determined in any of the studies on insulin resistance in the elderly, and thus, reduced blood flow remains a possible explanation for this problem. The question of whether insulin resistance in the elderly is an unavoidable consequence of aging or a preventable result of age-related changes in lifestyle, resulting in changes in body composition is of considerable clinical importance. Insulin resistance and the associated hyperinsulinemia are both significant and independent contributors to morbidity and mortality from coronary artery disease (10,11). In addition, hyperinsulinemia is associated with increased triglyceride and decreased HDL levels, both of which are established risk factors for cardiovascular disease (12). In this study, we have therefore determined body composition (by underwater weighing) and have used the euglycemic hyperinsulinemic clamping, together with indirect calorimetry and stable isotope dilution analysis, to

Table 1—Clinical characteristics of study subjects

	ELDERLY MEN	YOUNG ADULT MEN	P VALUE
n	6	6	
AGE (YR)	66.2 ± 1.7	31.8 ± 3.0	<0.01
WEIGHT (KG)	76.3 ± 5.7	79.9 ± 3.6	NS
HEIGHT (CM)	169.5 ± 4.0	171.3 ± 4.4	NS
TOTAL BODY FAT (KG)	18.2 ± 3.5	13.0 ± 1.6	NS
FAT (% BODY WT)	23.2 ± 3.4	16.6 ± 1.7	NS
LEAN BODY MASS (KG)	58.1 ± 3.6	64.8 ± 2.9	NS

Data are means ± SE.

compare glucose metabolism and strain-gauge plethysmography to compare leg blood flow in healthy, elderly and younger men, matched for height, weight, and physical activity.

## RESEARCH DESIGN AND METHODS

We studied 6 elderly (66.2 ± 1.7 yr) and 6 younger (31.8 ± 3.0 yr) healthy men. Characteristics of the two study groups are listed in Table 1. All subjects were in good health and good physical condition. None were taking medications of any kind. All elderly men led physically active lives but only one participated regularly in sports. All elderly men had NGT as determined by intravenous GTTs (mean  $K_g$ : 2.0, range 1.1–3.6, normal > 1.0). Weights of all subjects had been stable for at least 2 mo and their diets contained a minimum of 250 g/day of CHO for at least 2 days before studies. Informed consent was obtained from all after explanation of the nature, purpose, and potential risks of the study. The study protocol was approved by the Temple University Institutional Review Board for Human Research.

All study subjects were admitted on the evening before the studies to the General Clinical Research Center at Temple University Hospital. After an overnight fast, euglycemic hyperinsulinemic clamps were performed with the subjects reclining in hospital beds. A short polyethylene catheter was inserted into an

antecubital vein for infusion of test substances, a second catheter was inserted into a contralateral forearm vein for blood sampling. This arm was kept at 70°C with a heating blanket to arterialize venous blood.

## Indirect calorimetry

Respiratory gas exchange rates were determined as described previously at 30-min intervals with a metabolic measurement cart (Beckman, Palo Alto, CA) (13). Rates of protein oxidation were estimated from urinary nitrogen excretion after correction for changes in urea nitrogen pool size (14). Rates of protein oxidation were used to determine the nonprotein respiratory quotient.

## Glucose turnover

6,6  $D_2$ -glucose (Tracer Technologies, Somerville, MA) was infused for 6 h (–120 to 240 min) starting with a bolus of 30  $\mu$ mol/kg followed by continuous infusion of 0.3  $\mu$ mol  $\cdot$  kg<sup>–1</sup>  $\cdot$  min<sup>–1</sup>. Plasma glucose was isolated from blood for determination of isotope enrichment with a gas chromatograph mass spectrometer (Model 4610-B, Finnigan-Matt, San Jose, CA) as described previously (15).  $G_{Ra}$  and  $G_{Rd}$  were calculated from the isotope enrichment for a 30-min period before initiation of the clamp (–30 to 0 min) and a 30-min period at the end of the clamp (210–240 min) using Steele's equation (16).  $G_{Ra}$  during hyperinsulinemia was frequently lower than

the GIR, resulting in negative values for HGP. This problem has recently been attributed to errors in the Steele equation (17). To be able to estimate  $G_{Ra}$ , we have assumed that endogenous glucose was completely suppressed whenever the isotopically determined  $G_{Ra}$  was equal to or smaller than GIR. Maintaining stable isotope enrichment by adding 6,6  $D_2$ -glucose to the cold glucose infusion, we recently found that the method used in this study underestimated glucose turnover by ~10%. However, because changes in glucose enrichment were the same in both groups, the error can be expected to be the same for elderly and younger men.

## Euglycemic hyperinsulinemic clamp

Regular human insulin (Humulin R, Lilly, Indianapolis, IN) was infused intravenously at a rate of 6 pmol  $\cdot$  kg<sup>–1</sup>  $\cdot$  min<sup>–1</sup> for 4 h starting at 0 min. Glucose concentrations were clamped at ~4.7 mM by a feedback controlled infusion of 20% dextrose. Blood glucose concentrations were determined every 5–10 min with a Beckman glucose analyzer, and glucose infusions were adjusted accordingly.

## Body composition

Body fat mass was determined by underwater weighing in a water tank using standard methods (18). Residual lung volume was estimated after immersion in a sitting position by means of a closed circuit O<sub>2</sub> dilution method (18).

## Leg blood flow

Leg blood flow was determined every 30 min by venous occlusion plethysmography with a mercury strain-gauge apparatus (Model EC-5R, Hokanson, Issaquah, WA) (19). Two minutes before blood flow determination, circulation to the foot was interrupted by inflating a cuff around the ankle to a suprasystolic pressure.

### Analytical procedures

Plasma glucose was measured with a Beckman glucose analyzer, serum insulin by RIA (20), and plasma catecholamines were measured radioenzymatically (21). Plasma FFA were measured according to Lorch and Gey (22) after extraction according to Dole and Meinertz (23); plasma urea nitrogen was measured colorimetrically (24). Urinary nitrogen was measured by the method of Kjeldahl (25).

### Statistical analysis

All data are expressed as the means  $\pm$  SE. Statistical significances were assessed with MANOVA and two-tailed Student's *t* test.

## RESULTS

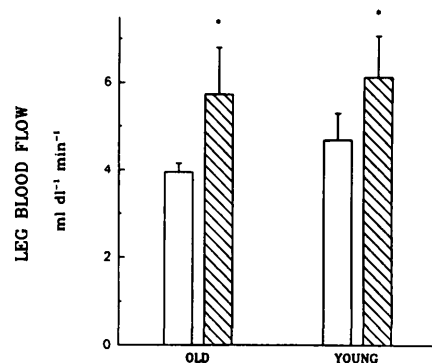
### Euglycemic hyperinsulinemic clamp

Basal serum insulin concentrations were  $47 \pm 10$  and  $59 \pm 13$  pM (NS) in elderly and younger men, respectively, and rose to a mean of  $365 \pm 38$  and  $382 \pm 76$  pM, respectively (NS), during the last hour of the insulin infusion. Plasma glucose concentrations were clamped at  $\sim 4.7 \pm 0.06$  mM in both groups. The GIR rose from 0 to  $39 \pm 9$   $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  in the elderly and from 0 to  $43 \pm 5$   $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  in the younger men (NS).

### FFA, catecholamines, and metabolic rates

Basal plasma FFA concentrations were  $677 \pm 49$  and  $556 \pm 60$   $\mu\text{M}$  (NS) in elderly and younger men, respectively. FFA decreased similarly during insulin infusion to  $\sim 90$   $\mu\text{M}$  in both groups during the last hour of the study.

Preclamp plasma EPI and NE concentrations were  $191 \pm 35$  pM and  $1.73 \pm 0.26$  nM, respectively, in the elderly men and  $172 \pm 39$  pM and  $1.15 \pm 0.26$  nM, respectively, in the younger men. At the end of the insulin infusions, EPI and NE were  $240 \pm 65$  pM and  $1.64 \pm 0.17$  nM, respectively, in the elderly men and  $173 \pm 57$  pM and



**Figure 1**—Effects of insulin ( $6 \text{ pmol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) on rates of leg blood flow (in  $\text{ml} \cdot 100 \text{ ml}$  of leg tissue<sup>-1</sup>  $\cdot \text{min}^{-1}$ ) in elderly and younger men. ( $\square$ ), Preclamp values ( $n = 6$ ); ( $\text{hatched}$ ), values at the end of a 4-h euglycemic hyperinsulinemic clamp ( $n = 6$ ). Data are means  $\pm$  SE. \* $P < 0.05$ .

$1.19 \pm 0.17$  nM in the younger men. Neither age nor insulin infusion had statistically significant effects on catecholamine concentrations.

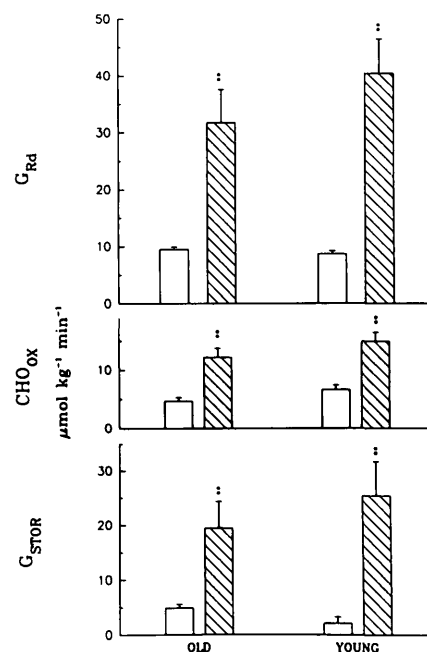
Preclamp resting metabolic rates were  $13 \pm 2$  and  $11 \pm 2$   $\text{cal} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  in elderly and younger subjects, respectively (NS), and rose to  $15 \pm 2$  and  $15 \pm 1$   $\text{cal} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ , respectively, during insulin infusions.

### Leg blood flow

Leg blood flow increased in response to insulin from  $4.0 \pm 0.2$  to  $5.7 \pm 1.1$   $\text{ml} \cdot \text{dl}^{-1} \cdot \text{min}^{-1}$  ( $P < 0.05$ ) in the elderly and from  $4.7 \pm 0.6$  to  $6.1 \pm 0.9$   $\text{ml} \cdot \text{dl}^{-1} \cdot \text{min}^{-1}$  ( $P < 0.05$ ) in the younger men (Fig. 1). The differences between elderly and younger men were not statistically significant.

### Glucose metabolism

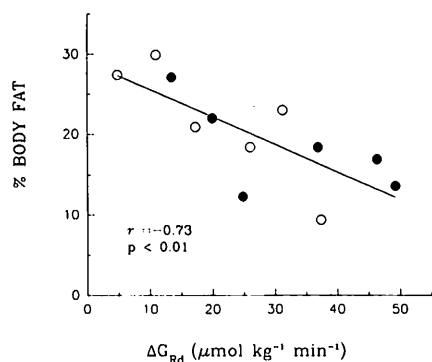
Hyperinsulinemia increased  $G_{\text{Rd}}$  from  $9.5 \pm 0.4$  to  $31.7 \pm 5.8$   $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  in elderly men ( $P < 0.01$ ) and from  $8.7 \pm 0.6$  to  $40.3 \pm 6$   $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  in younger men ( $P < 0.01$ ) (Fig. 2). Rates of glucose oxidation rose from  $4.7 \pm 0.6$  to  $12.2 \pm 1.6$   $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  in elderly men ( $P < 0.01$ )



**Figure 2**—Effects of insulin on  $G_{\text{Rd}}$  and rates of glucose oxidation ( $\text{CHO}_{\text{OX}}$ ) and storage ( $G_{\text{STOR}}$ ) in elderly and younger men. ( $\square$ ), Preclamp values ( $n = 6$ ); ( $\text{hatched}$ ), values at the end of a 4-h euglycemic hyperinsulinemic clamp ( $n = 6$ ). Data are means  $\pm$  SE. \*\* $P < 0.01$ .

and from  $6.6 \pm 0.8$  to  $14.9 \pm 1.6$   $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  in younger men ( $P < 0.01$ ). Rates of glucose storage rose from  $4.8 \pm 0.7$  to  $19.4 \pm 4.9$   $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  in elderly men and from  $2.1 \pm 1.2$  to  $25.4 \pm 6.3$   $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  in younger men ( $P < 0.01$ ). The differences in basal or insulin-stimulated rates of glucose uptake, oxidation, or storage between elderly and younger men were not statistically significant.

A highly significant negative correlation ( $r = -0.73$ ,  $P < 0.01$ ) was observed between percentage of body fat and the increase in insulin-stimulated glucose uptake ( $\Delta G_{\text{Rd}}$ ) in elderly and younger men (Fig. 3). In addition, the percentage of body fat correlated negatively with  $\text{CHO}_{\text{OX}}$  ( $r = -0.67$ ,  $P < 0.05$ ) and with glucose storage ( $r = -0.65$ ,  $P < 0.05$ ). Insulin-stimulated glucose uptake correlated much



**Figure 3**—Correlation between the percentage of body fat and change in glucose uptake produced by insulin ( $\Delta G_{Rd} = G_{Rd}$  at 240 min  $- G_{Rd}$  at 0 min). (○), Elderly men; (●), younger men.

better with body fat than with lean body mass ( $r = -0.73$  vs.  $r = -0.52$ ) and did not correlate significantly with age ( $r = -0.48$ , NS).

**CONCLUSIONS**— The purpose of this study was to determine whether aging per se or age-related changes in body composition or blood flow affected insulin sensitivity. To this end, we compared CHO metabolism in elderly (mean age 66.2 yr) and younger men (mean age 31.8 yr) matched for height and weight and as closely as possible for degree of physical activity. The elderly men were recruited through advertisements in local newspapers. All individuals in the study were healthy and not grossly obese (>30% overweight). Both groups had similar body composition, although the elderly men tended to have slightly more body fat (23.2 vs. 16.6%, NS) and less lean body mass (58.1 vs. 64.8%, NS). We reasoned that if unresponsiveness to insulin was an unavoidable consequence of age, it would be demonstrable despite the similarity of both groups with respect to body composition and physical activity.

Because glucose uptake, the most widely used parameter of insulin sensitivity, is the product of blood flow and fractional glucose uptake, it also was im-

portant to demonstrate that elderly and younger subjects had comparable muscle blood flow. Muscle is the most important tissue in this respect because it accounts for 75–85% of glucose uptake under the euglycemic hyperinsulinemic conditions (26). Assuming that leg blood flow was representative of blood flow to all muscle, we found that insulin-stimulated muscle blood flow was not impaired in the elderly.

Whole-body sensitivity to insulin was not significantly different in elderly compared with younger men, although elderly men tended to have rates of glucose uptake, oxidation, and storage that were slightly lower than those of the younger men. Of particular interest was that all parameters of glucose metabolism correlated well with body fat (glucose uptake,  $r = -0.73$ ,  $P < 0.01$ ; glucose oxidation,  $r = -0.67$ ,  $P < 0.05$ ; glucose storage  $r = -0.65$ ,  $P < 0.05$ ) less well with lean body mass ( $r = 0.5$ ,  $P = 0.05$ ) but not with age ( $P = -0.48$ , NS). It is quite possible that the small number of subjects in our study prevented differences in CHO metabolism between elderly and younger men from reaching statistical significance. In this case, however, the differences would probably have been related to differences in body fat, which correlated closely with CHO metabolism, and not to differences in age, as age did not correlate with any parameters of insulin action on CHO metabolism. Moreover, it could be argued that insulin doses smaller than those used might have produced differences between elderly and younger men. We consider this unlikely because the insulin concentrations attained ( $\sim 350$  pM) were close to the concentration, producing half-maximal stimulation of glucose uptake (27) and thus, was at the most sensitive part of the dose response curve.

Although our findings seem to be in disagreement with several previous studies, the differences were more apparent than real. For instance, Fink et al. (3) using euglycemic hyperinsulinemia sim-

ilar to that used in our study, found a 39% decline in the rate of peripheral glucose uptake in a group of elderly men and women. The elderly men in their study, however, had significantly reduced lean body mass (52.6 vs. 58.2 kg,  $P < 0.01$ ) and a nonsignificant increase in BMI (24 vs. 22.9 kg/m<sup>2</sup>) and thus must have had an increased body fat mass, which could have been responsible for the decrease in glucose uptake. DeFronzo (2) found that the rate of glucose infusion needed to maintain euglycemia during infusion of insulin (42.6 mU/m<sup>2</sup>) was 24% less in elderly (60  $\pm$  1 yr) compared with young (25  $\pm$  1 yr) healthy men and women. Interpretation of the meaning of these relatively small differences was complicated by the fact that body composition was not determined and glucose infusion rates needed to maintain euglycemia and not glucose uptake were measured. The two are equal only if HGO is completely suppressed. Smaller degrees of suppression in elderly than young subjects, as observed by Fink et al. (3), could result in overestimation of insulin resistance in the elderly. The same was true for a report by Rowe et al. (4), who studied elderly men with NGT who were slightly more obese (19.2 vs. 16.9% body fat) than control subjects and found a 30% decrease in the rate of glucose infusion needed to maintain euglycemia in the elderly at insulin concentrations of 50–80  $\mu$ U/ml. A previous analysis of 743 participants in the Baltimore Longitudinal Study of Aging found that obesity and lack of fitness could account for the difference in glucose tolerance between young adults (17–39 yr) and middle-aged (40–59 yr) men and women, but that a further decline in old subjects (60–92 yr) also was influenced by age (28). This study is difficult to compare with our study because the 60–70 yr age-group (5 of our 6 men were between 62 and 69 yr) was not analyzed separately. The possibility that age itself may adversely affect CHO metabolism in individuals >70 yr is certainly compatible with our results.

The association between insulin resistance and obesity has been well established (8). The concept that body fat is a major determinant for insulin sensitivity in the elderly also is supported by animal data showing that a decline in glucose tolerance associated with aging can be avoided if rats are prevented from becoming obese (29,30). In addition, it is supported by observations in humans showing that the age-related decrease in glucose tolerance correlated significantly with the degree in obesity in Italian factory workers (31).

Our findings may be questioned because 1) we only studied 6 elderly men and 2) these men were not representative of the elderly male population, which tends to be more obese and less active. We believe that if age per se had major adverse effects on CHO metabolism, we should have detected it in our 6 elderly men. It appears extremely unlikely that by chance, we studied 6 individuals with exceptional metabolic capabilities. The fact that they were slimmer and fitter than the average population does not detract at all from our conclusion that lifestyle and body composition appeared to have a greater impact on CHO metabolism than age.

In summary, our results suggest that the effect of aging per se on CHO metabolism (at least through 70 yr of age) is probably minor, and the deterioration of glucose tolerance seen in many elderly individuals may be mostly attributable to changes in lifestyle, i.e., a decrease in physical activity and an increase in body fat, and thus may be preventable.

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