

# Mechanisms of the Age-Associated Deterioration in Glucose Tolerance

## Contribution of Alterations in Insulin Secretion, Action, and Clearance

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Glucose tolerance decreases with age. For determining the cause of this decrease, 67 elderly and 21 young ( $70.1 \pm 0.7$  vs.  $23.7 \pm 0.8$  years) participants ingested a mixed meal and received an intravenous injection of glucose. Fasting glucose and the glycemic response above basal were higher in the elderly than in the young participants after either meal ingestion ( $P < 0.001$ ) or glucose injection ( $P < 0.01$ ). Insulin action (Si), measured with the meal and intravenous glucose tolerance test models, was highly correlated ( $r = 0.72$ ;  $P < 0.001$ ) and lower ( $P \leq 0.002$ ) in the elderly than in the young participants. However, when adjusted for differences in percentage body fat and visceral fat, Si no longer differed between groups. When considered in light of the degree of insulin resistance, all indexes of insulin secretion were lower ( $P < 0.01$ ) in the elderly participants, indicating impaired  $\beta$ -cell function. Hepatic insulin clearance was increased ( $P < 0.002$ ), whereas total insulin clearance was decreased ( $P < 0.002$ ) in the elderly subjects. Multivariate analysis ( $r = 0.70$ ;  $P < 0.001$ ) indicated that indexes of insulin action (Si) and secretion ( $\Phi_{i_{total}}$ ) but not age, peak oxygen uptake, fasting glucose, degree of fatness, or hepatic insulin clearance predicted the postprandial glycemic response. We conclude that the deterioration in glucose tolerance that occurs in healthy elderly subjects is due to a decrease in both insulin secretion and action with the severity of the defect in insulin action being explained by the degree of fatness rather than age per se. *Diabetes* 52:1738–1748, 2003

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Bio T, bioavailable testosterone; IVGTT, intravenous glucose tolerance test;  $V_{O_{2max}}$ , peak oxygen uptake.

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**B**oth diabetes and glucose intolerance are common in the elderly. The pathogenesis of carbohydrate intolerance in the elderly has been an area of active investigation. Many (1–6) but not all (7–11) studies have reported that older individuals are more insulin resistant than are younger individuals. The effect of aging on insulin secretion also has been a source of debate. Insulin secretion during a hyperglycemic clamp has been reported not to differ in elderly and young subjects (3,12,13). Conversely, insulin secretion in response to an intravenous glucose injection has been reported to be abnormal in elderly subjects in most (1,7,14) but not all (8,11) studies. In addition, there seems to be disagreement as to the cause of this abnormality. Studies have variably reported decreased early insulin secretion (14), normal early insulin secretion (8,11), or normal first-phase insulin secretion but decreased second-phase insulin secretion (1,7). Similarly, insulin concentrations after glucose ingestion have been reported to be either higher (2,15,16) or no different (4,10) in elderly and young subjects.

Many of these discrepancies may be more apparent than real. Insulin concentrations commonly have been used to assess insulin secretion (1–4,8,10,12,16,17). This introduces uncertainty because hepatic insulin extraction has been reported to change with age (7,14). Insulin secretion (18,19) and perhaps insulin action (20) are modulated by incretin hormones after glucose ingestion but not intravenous glucose injection. A variety of factors may influence insulin action and secretion, including the degree and type of obesity (21), level of fitness (22,23), and the prevailing counter insulin (e.g., glucagon, growth hormone, cortisol) hormone concentrations (24–26). In addition, the appropriateness of insulin secretion needs to be interpreted in light of the degree of insulin resistance (27–29). No difference in insulin secretion in the presence of a decrease in insulin action denotes relative  $\beta$ -cell failure. A decrease in insulin secretion after intravenous injection of glucose that is not observed after ingestion of glucose suggests a compensatory effect of the gastrointestinal incretin hormones.

Androgen concentrations fall with age (30,31). It is not known whether this fall impairs carbohydrate tolerance or whether replacement of either gonadal or adrenal andro-

gens restores glucose metabolism to normal. The present data were derived from the baseline studies of a prospective trial seeking to address this question. These data afforded us the opportunity to assess comprehensively insulin action, insulin secretion, and insulin clearance in a large number of carefully characterized healthy elderly individuals whose plasma dehydroepiandrosterone (DHEA; men and women) and testosterone (men) concentrations were in the lower range of normal relative to young individuals, reflecting the hormonal milieu that commonly occurs with “normal” aging. Results were compared with those observed in healthy young participants who were studied in an identical manner.

## RESEARCH DESIGN AND METHODS

**Participants.** After approval from the Mayo Institutional Review Board, 67 healthy elderly participants (37 elderly men and 30 elderly women) and 21 healthy young participants (11 men and 9 women) gave informed written consent to participate in the study.

**Experimental design.** Studies were conducted at the Mayo General Clinical Research Center. Subjects consumed a weight maintenance diet (55% carbohydrate, 15% protein, and 30% fat) provided by the General Clinical Research Center kitchen for 3 days preceding study. All subjects were admitted at 1600 on the afternoon before study (day 1) and given a standard 10 kcal/kg meal (55% carbohydrate, 15% protein, and 30% fat), which was consumed between 1700 and 1730. No additional food was eaten until the next morning.

**Mixed-meal study.** At ~0600 on the morning of day 2, an 18-G cannula was inserted in a retrograde manner into a dorsal hand vein. The hand was then placed in a heated plexibox (~55°C) to obtain arterialized venous blood samples. Another 18-G cannula was inserted in the opposite forearm for infusion of tracers to measure glucose and palmitate turnover as a separate part of the overall project. At 0900 (0 time), a mixed meal (10 kcal/kg, 45% carbohydrate, 15% protein, 40% fat) consisting of three scrambled eggs, Canadian bacon, and Jell-O (containing 1.2 g/kg body wt of dextrose) was consumed within 15 min. Blood was sampled from the arterialized venous site at -120, -30, -20, -10, 0, 5, 10, 15, 20, 30, 40, 50, 60, 75, 90, 120, 150, 180, 210, 240, 260, 280, 300, 360, and 420 min. Intravenous lines were then removed, and the participant ate lunch immediately after completion of the study (~1600–1700) and a supper at ~2000–2030.

**Intravenous glucose tolerance test.** At ~0600 on the morning of day 3, an intravenous 18-G cannula was inserted into each arm. One was used for infusion of glucose, insulin, and tracers, and the other was used for withdrawal of blood. At 0900 (0 time), glucose (0.3 g/kg total body wt) was injected intravenously over 2 min followed by infusion of insulin (0.02 units/kg total body wt, begun at 20 min) given as a square wave over 5 min. Blood was sampled at -120, -30, -20, -10, 0, 2, 4, 6, 8, 10, 15, 20, 22, 25, 26, 28, 31, 35, 45, 60, 75, 90, 120, 180, and 240 min.

**Analytical techniques.** Plasma samples were placed on ice, centrifuged at 4°C, separated, and stored at -20°C until assay. Plasma glucose concentration was measured using a glucose oxidase method (YSI, Yellow Springs, OH). Plasma insulin, cortisol, and growth hormone concentrations were measured using a chemiluminescence assay with reagents obtained from Beckman (Access Assay; Beckman, Chaska, MN). Plasma glucagon and C-peptide concentrations were measured by radioimmunoassay (Linco Research, St. Louis, MO). DHEA was measured by a competitive chemiluminescent immunoassay on the Immulite automated immunoassay system (Diagnostic Products, Los Angeles, CA). Testosterone and bioavailable testosterone (Bio T) were measured in men only. Testosterone was measured using a competitive chemiluminescent immunoassay using an ACS-180 automated immunoassay system (Bayer Diagnostics, Tarrytown, NY). Bioavailable testosterone was measured by differential precipitation of sex hormone-binding globulin by ammonium sulfate after equilibration of the serum sample with tracer amounts of tritium-labeled testosterone. Palmitate concentrations were measured using high-performance liquid chromatography (32).

Body composition was measured using dual energy X-ray absorptiometry (DPX scanner; Lunar, Madison, WI). Visceral fat was measured by a single-slice computed tomographic scan at the level of L2/L3 as previously described by Jensen et al. (33). Peak oxygen uptake ( $V_{O_{2max}}$ ) was measured using a standard treadmill stress test (34). Knee extensor strength was measured by having each participant lift a progressively higher weight using a bilateral leg press machine (Cybex, Medway, MA) until the one-repetition maximum was reached. Consecutive attempts were separated by 1 min of rest (35). Partici-

pants were familiarized with the equipment and test procedures before data collection.

**Insulin action.** The index of insulin action after mixed-meal ingestion,  $SI_{Meal}$ , was calculated using the “oral” minimal model (36,37). Model identification requires insertion of values for glucose effectiveness (Sg), glucose volume of distribution (V), the rate constant of insulin action ( $p_2$ ), and the fraction of ingested glucose that appears in the systemic circulation (f) (36). Sg was assumed to equal 0.014/min, V to equal 1.7 dl/kg,  $p_2$  to equal 0.03/min, and f to equal 0.87 (38). Indexes of insulin action ( $SI_{IVGTT}$ ) and glucose effectiveness ( $Sg_{IVGTT}$ ) were calculated after intravenous glucose injection as previously described (39).

**Insulin secretion.** Indexes of insulin secretion after mixed-meal ingestion and intravenous glucose injection were calculated as previously described by using the minimal model of C-peptide secretion and kinetics during oral (37) and intravenous glucose tests (40,41) incorporating age-associated changes in C-peptide kinetics as measured by Van Cauter et al. (42). The oral minimal model enabled calculation of  $\Phi_{dynamic}$  (an index of insulin secretion in response to a change in glucose concentration),  $\Phi_{static}$  (an index of insulin secretion in response to a given glucose concentration), and  $\Phi_{total-Meal}$  (a global sensitivity-to-glucose index of postprandial insulin secretion). Similarly, the intravenous glucose minimal model enabled calculation of  $\Phi_1$  (an index of first-phase insulin secretion),  $\Phi_2$  (an index of second-phase insulin secretion), and  $\Phi_{total-IVGTT}$  (a global sensitivity-to-glucose index of insulin secretion in response to an intravenous glucose injection).  $\Phi_{total-IVGTT}$  was calculated from model indexes  $\Phi_1$  and  $\Phi_2$  by using a formula similar to the one developed for  $\Phi_{total-Meal}$  (37).

**Disposition index.** To determine whether insulin secretion was appropriate for the degree of insulin resistance, we calculated disposition indexes by multiplying the various indexes of insulin secretion by insulin sensitivity, in analogy with Bergman et al. (43). Thus, three different disposition indexes were calculated after mixed-meal ingestion, by multiplying  $\Phi_{dynamic}$ ,  $\Phi_{static}$ , and  $\Phi_{total-Meal}$  by  $SI_{Meal}$ . Similarly, three different disposition indexes were calculated after intravenous glucose injection, by multiplying  $\Phi_1$ ,  $\Phi_2$ , and  $\Phi_{total-IVGTT}$  by  $SI_{IVGTT}$ . Disposition index  $\Phi_1 \times SI_{IVGTT}$  is analogous to that proposed previously as the product of the acute insulin response by  $SI_{IVGTT}$  (27–29), because  $\Phi_1$  is a model-derived index of insulin secretion in response to the glucose increase observed immediately after the intravenous glucose administration.

**Insulin clearance and hepatic insulin extraction.** Exogenous insulin administration during the intravenous glucose injection allowed calculation of total body insulin clearance by using the minimal model of posthepatic insulin secretion and insulin kinetics (44). The use of this model, in conjunction with the minimal model of C-peptide secretion and kinetics, enabled calculation of the fraction of secreted insulin that is extracted by the liver after the intravenous glucose injection (45).

**Calculations.** Values from -30 to 0 min were averaged and considered as basal. Area above basal was calculated using the trapezoidal rule. Parameters of all models were estimated by using the SAAMII software (46). Measurement error of C-peptide concentration has been assumed to be independent and gaussian, with zero mean and with either a constant coefficient of variation equal to 1.5% (glucose data) or a constant but unknown variance (C-peptide and insulin data). Details of model identification have been previously described (37,39–41,44).

**Statistical analysis.** Data are presented as mean  $\pm$  SE. Two sample comparisons between the elderly and young participants were made using *t* tests or rank-sum tests for data that were not normally distributed.  $\Phi_{total-IVGTT}$  and human growth hormone were transformed to the natural log scale because of skewness. Pearson's or Spearman's *r* was used to evaluate univariate correlations. All predictors were considered in a forward stepwise model selection process using multiple linear regression to determine significant multivariate predictors. Model *r*'s are reported as an indicator of model fit. Partial *r*'s are reported as an indicator of the contribution of single variables to the overall model. Age was used as an indicator of elder or young status.  $P < 0.05$  was considered to be statistically significant.

## RESULTS

**Patient characteristics.** By design, the elderly participants were older than the young participants ( $70.1 \pm 0.7$  vs.  $23.7 \pm 0.8$  years). Weight ( $79.6 \pm 1.9$  vs.  $73.4 \pm 3.0$  kg), lean body mass ( $48.5 \pm 1.4$  vs.  $48.5 \pm 2.5$  kg), and serum creatinine ( $1.1 \pm 0.01$  vs.  $1.1 \pm 0.0$ ) did not differ in the elderly and young participants. However, the elderly participants had a greater BMI ( $27.5 \pm 0.5$  vs.  $25.0 \pm 0.6$  kg/m<sup>2</sup>;  $P < 0.01$ ), percentage body fat ( $33.6 \pm 1.1$  vs.  $28.9 \pm$

TABLE 1  
Participant characteristics

	Elderly	Young
Age (years)	70.1 ± 0.7	23.7 ± 0.8
Weight (kg)	79.6 ± 1.9	73.4 ± 3.0
LBM (kg)	48.5 ± 1.4	48.5 ± 2.5
Total testosterone (ng/dl)*	365 ± 20.4§	570 ± 63.4
Bio T (ng/dl)*	64.4 ± 3.7§	202 ± 25.1
DHEA s (μg/ml)	0.61 ± 0.05§	2.31 ± 0.31
BMI (kg/m <sup>2</sup> )	27.5 ± 0.5‡	25 ± 0.6
Body fat (%)	33.6 ± 1.1†	28.9 ± 1.7
Visceral fat (cm <sup>2</sup> )	172 ± 12§	62 ± 12
VO <sub>2max</sub> (ml/kg)	24 ± 0.8§	42.5 ± 2.6
Double knee extensions (lb)	85.3 ± 3.7§	138.8 ± 10.8

Data are means ± SD. \*Men only. † $P < 0.05$ , ‡ $P < 0.01$ , and § $P < 0.001$  vs. young. LBM, lean body mass.

1.7%;  $P < 0.05$ ), and visceral fat ( $172 \pm 12$  vs.  $62 \pm 12$  cm<sup>2</sup>;  $P < 0.001$ ) than did the younger participants. VO<sub>2max</sub> ( $24.0 \pm 0.8$  vs.  $42.5 \pm 2.6$  ml · kg<sup>-1</sup> · min<sup>-1</sup>) and double knee extension ( $85.3 \pm 3.7$  vs.  $138.8 \pm 10.8$  lb) were lower ( $P < 0.001$ ) in the elderly than in the young participants (Table 1).

**Plasma glucose, insulin, and C-peptide concentrations in response to ingestion of a mixed meal.** Plasma glucose concentrations were higher ( $P < 0.001$ ) in the elderly than in the young participants before meal ingestion ( $5.2 \pm 0.04$  vs.  $4.8 \pm 0.16$  mmol/l) and increased to a higher ( $P < 0.001$ ) peak ( $11.0 \pm 0.2$  vs.  $9.5 \pm 0.2$  mmol/l) after meal ingestion (Fig. 1A). This resulted in a greater

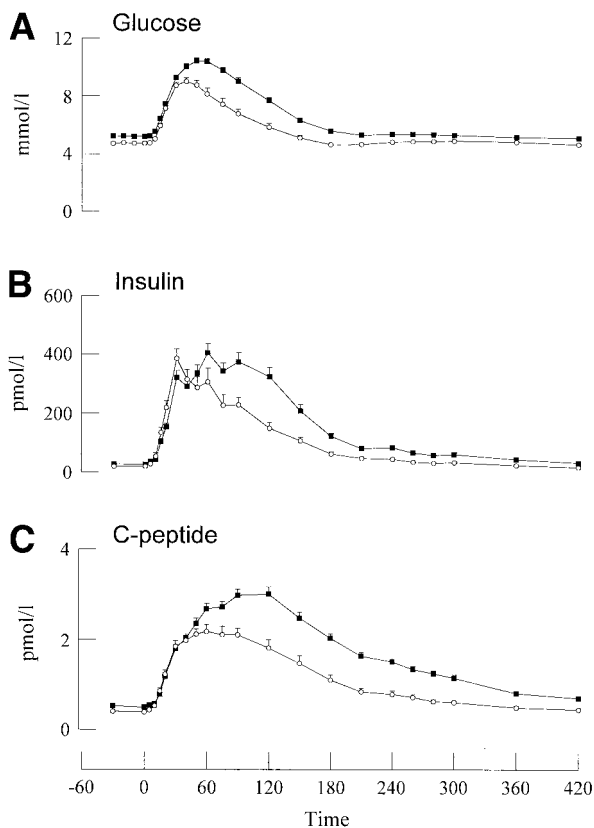


FIG. 1. Glucose (A), insulin (B), and C-peptide (C) concentrations observed in the elderly (■) and the young (○) participants. A mixed meal was ingested at 0 min.

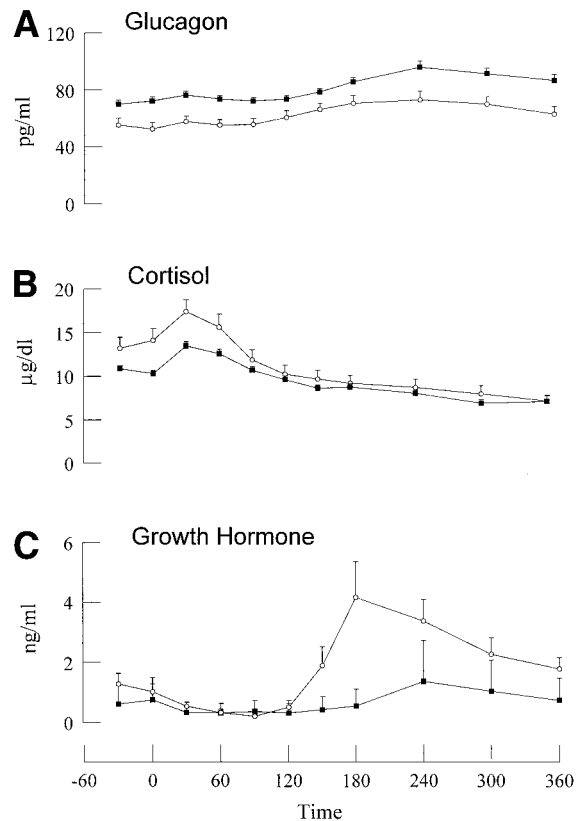


FIG. 2. Glucagon (A), cortisol (B), and growth hormone (C) concentrations observed in the elderly (■) and the young (○) participants. A mixed meal was ingested at 0 min.

( $P < 0.001$ ) integrated response above basal in the elderly than in the young participants ( $521 \pm 26$  vs.  $341 \pm 34$  mmol · l<sup>-1</sup> · 7 h<sup>-1</sup>).

Neither fasting ( $28 \pm 2$  vs.  $21 \pm 1$  pmol/l) nor peak postprandial ( $510 \pm 36$  vs.  $440 \pm 38$  pmol/l) plasma insulin concentration differed in the elderly and young participants (Fig. 1B). However, the integrated response above basal was greater ( $P < 0.01$ ) in the elderly than in the young participants ( $51.8 \pm 3.8$  vs.  $33.4 \pm 3.3$  nmol · l<sup>-1</sup> · 7 h<sup>-1</sup>). Of note, although the overall response was greater, the increase in plasma insulin above basal immediately after meal ingestion was lower ( $P < 0.01$ ) in the elderly than in the young participants during the first 20 min ( $773 \pm 84$  vs.  $1,287 \pm 186$  pmol · l<sup>-1</sup> · 20 min<sup>-1</sup>).

Despite no differences in fasting insulin concentrations, fasting C-peptide concentrations were higher ( $P < 0.01$ ) in the elderly than in the young participants ( $0.51 \pm 0.02$  vs.  $0.38 \pm 0.02$  nmol/l) (Fig. 1C). Peak postprandial ( $3.4 \pm 0.16$  vs.  $2.6 \pm 0.15$  nmol/l) and the integrated C-peptide response above basal also were greater ( $P < 0.01$ ) in the elderly than in the young participants ( $489 \pm 23$  vs.  $278 \pm 19$  nmol · l<sup>-1</sup> · 7 h<sup>-1</sup>). As with insulin, the increase in plasma C-peptide above basal immediately after meal ingestion was lower ( $P < 0.02$ ) in the elderly than in the young participants during the first 20 min after meal ingestion ( $3.4 \pm 0.4$  vs.  $5.2 \pm 0.8$  nmol/l).

**Plasma glucagon, cortisol, growth hormone, and palmitate concentrations in response to ingestion of a mixed meal.** Plasma glucagon concentrations (Fig. 2A) were higher ( $P < 0.002$ ) in the elderly than in the young

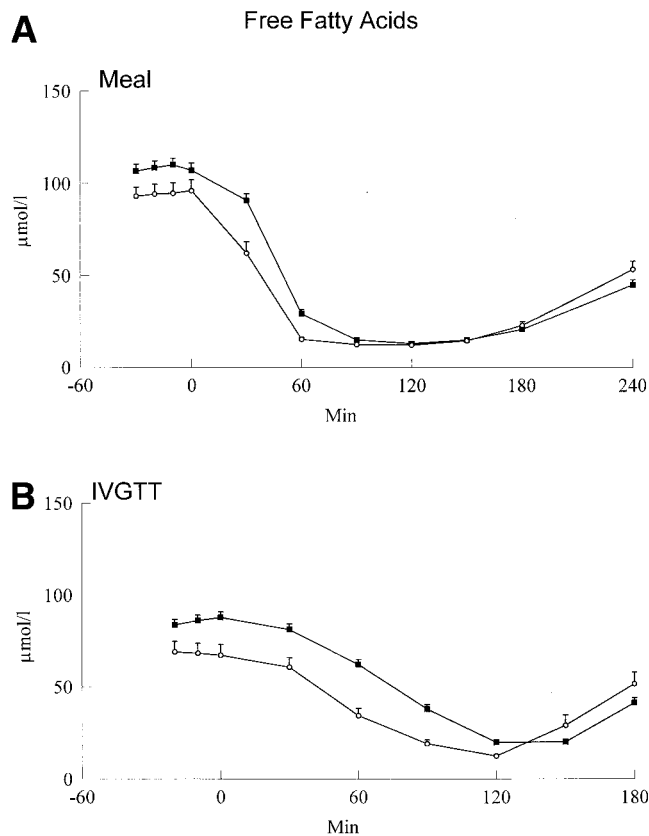


FIG. 3. Plasma palmitate concentrations observed in the elderly (■) and the young (○) participants after either ingestion of a mixed meal (A) or intravenous injection of glucose (B).

participants before meal ingestion ( $71 \pm 3$  vs.  $54 \pm 4$  pg/ml) and increased to a higher peak ( $P < 0.01$ ) after meal ingestion ( $105 \pm 4$  vs.  $82 \pm 6$  pg/ml). In contrast, plasma cortisol concentrations (Fig. 2B) were lower ( $P < 0.01$ ) in the elderly than in the young participants before meal ingestion ( $10.6 \pm 0.3$  vs.  $13.7 \pm 1.2$   $\mu\text{g/dl}$ ) and increased to a lower peak ( $P < 0.05$ ) after meal ingestion ( $16.0 \pm 0.5$  vs.  $19.1 \pm 1.4$   $\mu\text{g/dl}$ ). Plasma growth hormone concentrations did not differ in the elderly and young participants before meal ingestion ( $0.7 \pm 0.1$  vs.  $1.2 \pm 0.3$  ng/ml) and fell comparably immediately after meal ingestion (Fig. 2C). Plasma growth hormone concentrations subsequently rose as glucose concentrations fell toward basal levels in both groups; however, the postprandial peak was lower ( $P < 0.001$ ) in the elderly than in the young participants ( $2.2 \pm 0.2$  vs.  $6.2 \pm 1.0$  ng/ml).

Plasma palmitate concentrations were higher ( $P < 0.05$ ) in the elderly than in the young participants before meal ingestion ( $108 \pm 4$  vs.  $95 \pm 5$   $\mu\text{mol/l}$ ). Plasma palmitate fell to the same nadir in both groups after meal ingestion (Fig. 3A). This resulted in a greater ( $P < 0.01$ ) suppression below basal in the elderly than in the young participants ( $-17.0 \pm 0.7$  vs.  $-14.6 \pm 1.1$   $\text{mmol} \cdot \text{l}^{-1} \cdot 4 \text{ h}^{-1}$ ).

**Plasma glucose, insulin, and C-peptide and palmitate concentrations in response to intravenous injection of glucose.** Plasma glucose concentrations (Fig. 4A) were higher ( $P < 0.001$ ) in the elderly than in the young participants before intravenous glucose injection ( $5.2 \pm 0.0$  vs.  $4.8 \pm 0.1$  mmol/l) and increased to a higher ( $P < 0.01$ ) peak after intravenous glucose injection ( $19.0 \pm 0.4$

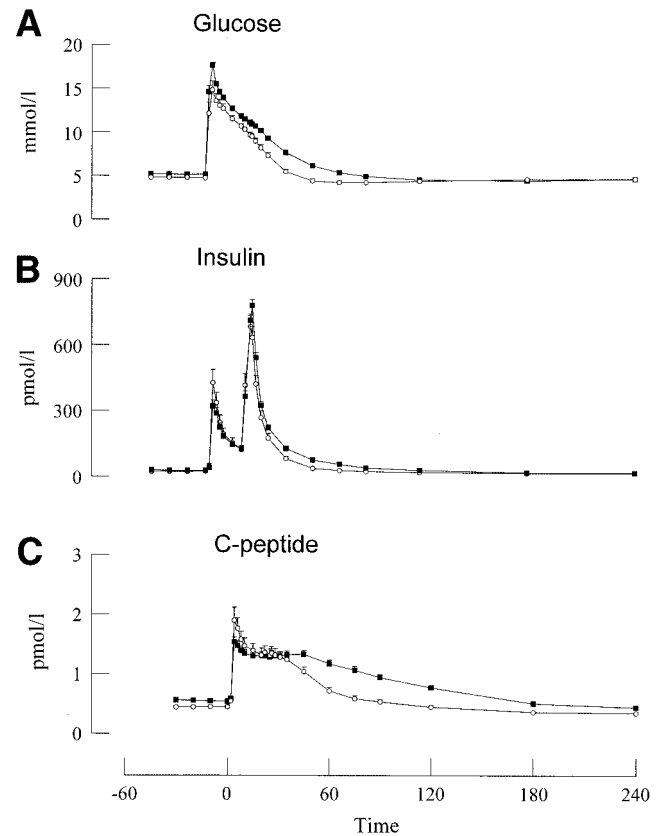


FIG. 4. Glucose (A), insulin (B), and C-peptide (C) concentrations observed in the elderly (■) and the young (○) participants. Intravenous glucose was injected at 0 min, and exogenous insulin was injected at 20 min.

vs.  $16.1 \pm 0.8$  mmol/l). This resulted in a greater increase ( $P < 0.01$ ) in plasma glucose concentration above basal ( $236.7 \pm 10.5$  vs.  $179.7 \pm 14.6$   $\text{mmol} \cdot \text{l}^{-1} \cdot 4 \text{ h}^{-1}$ ). The increase in plasma glucose above basal during the first 20 min after glucose injection (i.e., before injection of exogenous insulin) also was greater ( $P < 0.05$ ) in the elderly than in the young participants ( $168.2 \pm 3.1$  vs.  $145.0 \pm 9.5$   $\text{mmol} \cdot \text{l}^{-1} \cdot 20 \text{ min}^{-1}$ ).

Plasma insulin concentrations did not differ in the elderly and the young participants before glucose injection ( $27.7 \pm 1.8$  vs.  $23.9 \pm 1.6$  pmol/l). Insulin concentrations increased in the elderly and the young participants immediately after glucose injection. The peak ( $346 \pm 26$  vs.  $437 \pm 59$  pmol/l) and area above basal during the first 20 min after intravenous glucose injection ( $2.9 \pm 0.2$  vs.  $3.6 \pm 0.5$  nmol/l) was slightly but not significantly lower in the elderly than in the young participants (Fig. 3B). The increase in plasma insulin after injection of exogenous insulin at 20 min did not differ between groups.

Despite no differences in fasting insulin concentrations, plasma C-peptide concentrations were higher ( $P < 0.02$ ) in the elderly than in the young participants ( $0.55 \pm 0.02$  vs.  $0.45 \pm 0.02$  nmol/l) before glucose injection (Fig. 4C). Plasma C-peptide concentrations promptly rose in both groups after glucose injection. Peak C-peptide concentrations ( $1.72 \pm 0.07$  vs.  $2.00 \pm 0.2$  nmol/l) and the area above basal during the first 20 min after glucose injection ( $14.4 \pm 0.8$  vs.  $18.4 \pm 2.2$  nmol/l) were slightly but not significantly lower in the elderly than in the young participants. Con-

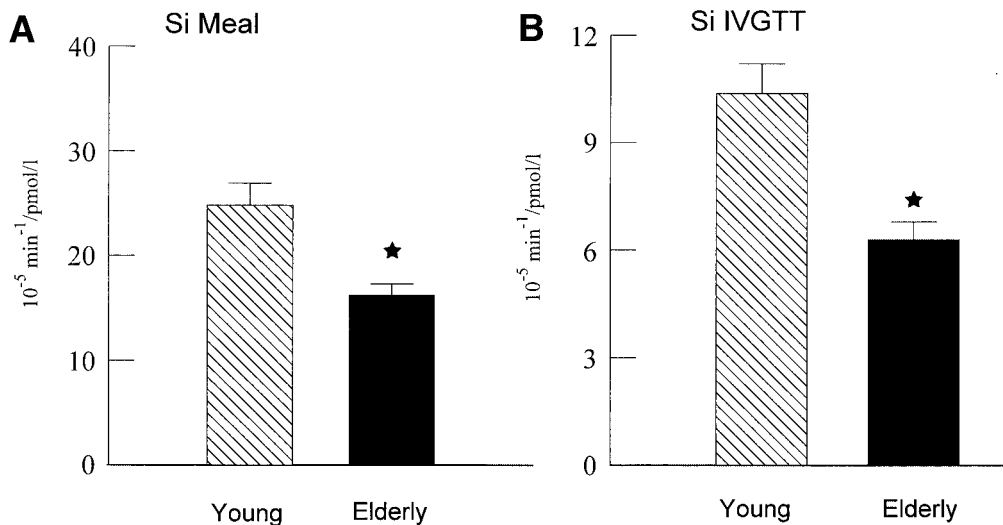


FIG. 5. Insulin action (Si) measured in the elderly and the young participants with the meal (A) and intravenous glucose (B) minimal models. \* $P < 0.002$ .

versely, plasma C-peptide concentrations were higher in the elderly than in the young participants from 20 min onward, resulting in a greater ( $P < 0.01$ ) overall integrated response ( $70.9 \pm 4.9$  vs.  $42.8 \pm 6.0$   $\text{nmol} \cdot \text{l}^{-1} \cdot 4 \text{ h}^{-1}$ ).

Plasma palmitate concentrations were higher ( $P < 0.01$ ) in the elderly than in the young participants ( $86 \pm 3$  vs.  $66 \pm 3$   $\mu\text{mol/l}$ ) before intravenous glucose injection (Fig. 3B). Plasma palmitate concentrations fell to the same nadir in both groups after glucose injection (Fig. 3B). This resulted in greater ( $P < 0.01$ ) suppression below basal in the elderly than in the young subjects ( $-6.7 \pm 0.5$  vs.  $3.9 \pm 1.0$   $\text{mmol} \cdot \text{l}^{-1} \cdot 3 \text{ h}^{-1}$ ).

**Indexes of insulin action and secretion.** Insulin action (Si) can be measured with both the meal and intravenous glucose minimal models (Fig. 5). Si was lower in the elderly than in the young participants after both meal ingestion ( $16.2 \pm 1.1$  vs.  $24.8 \pm 2.1$   $\text{dl} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  per  $\text{pmol/l}$ ;  $P < 0.002$ ) and glucose injection ( $6.3 \pm 0.5$  vs.  $10.4 \pm 0.8$   $10^{-5} \text{ min}^{-1}$  per  $\text{pmol/l}$ ;  $P < 0.001$ ).  $S_g$  (an index of glucose effectiveness), measured after intravenous glucose injection, did not differ in the elderly and the young participants ( $1.8 \pm 0.0$  vs.  $1.9 \pm 0.1$   $10^{-2}/\text{min}$ ).

Insulin secretion indexes after meal ingestion ( $\text{Phi}_{\text{dynamic}}$ ,  $\text{Phi}_{\text{static}}$ , and  $\text{Phi}_{\text{total-Meal}}$ ) all tended to be lower in the elderly than in the young participants (Fig. 6); however, only  $\text{Phi}_{\text{total-Meal}}$  was statistically significant ( $P < 0.05$ ). First-phase insulin secretion index after glucose injection,  $\text{Phi}_1$ , and the natural log of  $\text{Phi}_{\text{total-IVGTT}}$  were lower ( $P < 0.05$ ) in the elderly than in the young participants.  $\text{Phi}_2$  did not differ between groups.

All disposition indexes, which adjust insulin secretion for insulin action, were lower ( $P < 0.01$ ) in the elderly than in the young participants after both meal ingestion and glucose injection (Fig. 7). This was true regardless of whether the disposition index was calculated as  $\text{Phi}_{\text{dynamic}}$ ,  $\text{Phi}_{\text{static}}$ , or  $\text{Phi}_{\text{total-Meal}}$  times  $\text{Si}_{\text{Meal}}$  using the meal minimal model or  $\text{Phi}_1$ ,  $\text{Phi}_2$ , or  $\text{Phi}_{\text{total-IVGTT}}$  times  $\text{Si}_{\text{IVGTT}}$  using the intravenous minimal model.

**Insulin clearance and hepatic insulin extraction.** Total body insulin clearance was lower ( $P < 0.002$ ) in the elderly than in the young participants (Fig. 8). In contrast, hepatic insulin extraction was greater in the elderly than in the young participants whether measured as basal, i.e.,

before glucose injection ( $P < 0.001$ ), or after glucose injection ( $P < 0.002$ ).

**Multivariate analyses of meal and intravenous minimal model indexes.** Although the meal and intravenous glucose minimal models both assess insulin secretion, they do so in response to different stimuli administered by different routes. It therefore was of interest that Si measured with the meal minimal model (Fig. 9) was correlated with Si measured with the intravenous glucose minimal model ( $r = 0.72$ ;  $P < 0.001$ ). In addition,  $\text{Phi}_{\text{dynamic}}$ ,  $\text{Phi}_{\text{static}}$ , and  $\text{Phi}_{\text{total}}$  measured with the meal minimal model were correlated respectively with  $\text{Phi}_1$  ( $r = 0.45$ ;  $P < 0.001$ ),  $\text{Phi}_2$  ( $r = 0.67$ ;  $P < 0.001$ ), and  $\text{Phi}_{\text{total}}$  ( $r = 0.67$ ;  $P < 0.001$ ) measured with the intravenous glucose model (Fig. 10).

Univariate analyses indicated that insulin action measured with the meal minimal model (i.e.,  $\text{Si}_{\text{Meal}}$ ) was significantly correlated with percentage body fat ( $r = -0.60$ ;  $P < 0.001$ ), visceral fat ( $r = -0.35$ ;  $P < 0.001$ ), double knee extension ( $r = 0.34$ ;  $P < 0.01$ ),  $\text{VO}_{2\text{max}}$  ( $r = 0.36$ ;  $P < 0.001$ ), and fasting glucose ( $r = -0.31$ ;  $P < 0.01$ ). However, when these factors, as well as age and sex, were included in a multivariate model ( $r = 0.68$ ;  $P < 0.0001$ ), only percentage body fat (partial  $r = 0.58$ ;  $P < 0.0001$ ) and visceral fat (partial  $r = 0.28$ ;  $P < 0.01$ ) remained significant, suggesting that the degree of fatness rather than age per se, leg strength, or aerobic fitness was the primary determinant of insulin action. Similarly, univariate analyses indicated that insulin action measured with the intravenous minimal model (i.e.,  $\text{Si}_{\text{IVGTT}}$ ) was significantly correlated with percentage body fat ( $r = -0.44$ ;  $P < 0.001$ ), visceral fat ( $r = -0.49$ ;  $P < 0.001$ ), fasting glucose ( $r = -0.35$ ;  $P < 0.001$ ), and total insulin clearance ( $r = 0.34$ ;  $P < 0.01$ ). However, when these factors, as well as age and sex, were included in a multivariate model ( $r = 0.63$ ;  $P < 0.001$ ), only percentage body fat (partial  $r = 0.42$ ;  $P < 0.001$ ) and visceral fat (partial  $r = 0.41$ ;  $P < 0.001$ ) remained significant, again suggesting that degree of fatness rather than age per se was the primary determinant of insulin action.

Postprandial glucose tolerance for a given individual is determined by multiple factors, including his or her ability to secrete and respond to insulin. Univariate analysis

## Insulin Secretion Indexes

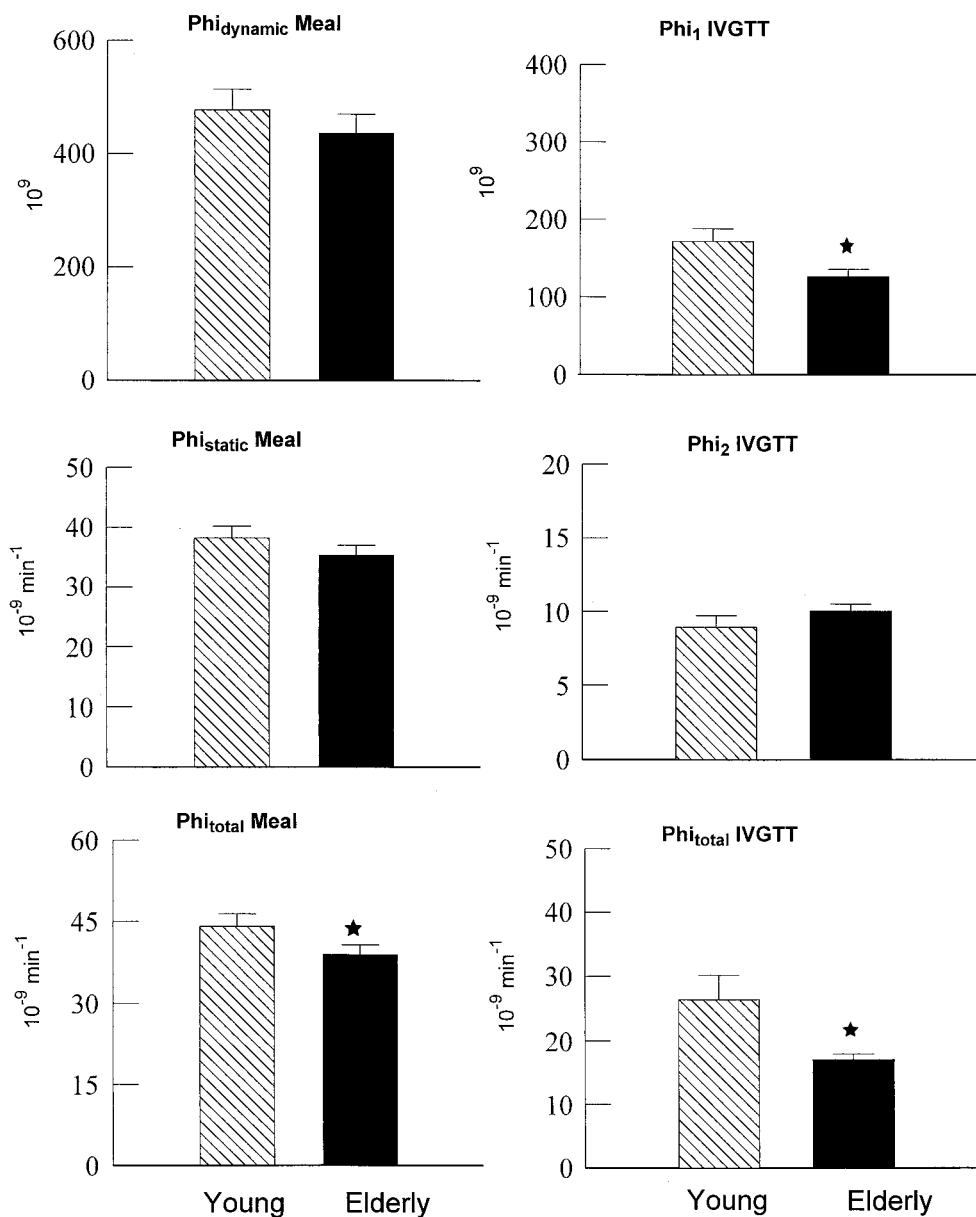


FIG. 6. Insulin secretion indexes obtained with the meal (left panels) and intravenous glucose (right panels) minimal models. \* $P < 0.05$ .

indicated that  $\Phi_{total-Meal}$  ( $r = -0.30$ ;  $P < 0.01$ ),  $\Phi_{static}$  ( $r = -0.25$ ;  $P < 0.05$ ),  $Si_{Meal}$  ( $r = -0.49$ ;  $P < 0.001$ ),  $VO_{2max}$  ( $r = -0.36$ ;  $P < 0.001$ ), and double knee extension ( $r = -0.39$ ;  $P < 0.001$ ) were significantly correlated with the meal glycemic response above basal.

When these factors, as well as age, were included in a multivariate analysis ( $r = 0.68$ ;  $P < 0.001$ ), only  $Si_{Meal}$  (partial  $r = 0.51$ ;  $P < 0.0001$ ) and  $\Phi_{total-Meal}$  (partial  $r = 0.45$ ;  $P < 0.0001$ ) remained significant. Multivariate analysis ( $r = 0.53$ ;  $P < 0.001$ ) indicated that indexes of insulin secretion ( $\Phi_{total-IVGTT}$ ) and action ( $Si_{IVGTT}$ ) derived during the intravenous glucose tolerance tests also correlated with the meal glycemic response above basal (partial  $r = 0.29$ ,  $P < 0.01$ ; and partial  $r = 0.26$ ,  $P < 0.05$ , respectively). However, neither of these parameters remained significant

when the corresponding meal indexes were included in the same multivariate model.

#### DISCUSSION

Glucose tolerance decreases with age. The present data indicate that defects in both insulin secretion and action contribute to this decline. Insulin action, measured with either the meal or the intravenous glucose minimal models, was lower in the elderly than in the young participants. Insulin secretion was also impaired in the elderly individuals. When considered in light of the degree of insulin resistance, all indexes of insulin secretion were decreased in the elderly participants, indicating decreased  $\beta$ -cell secretory reserve. Although total body insulin clearance was lower in the elderly than in the young subjects,

## Disposition Indexes

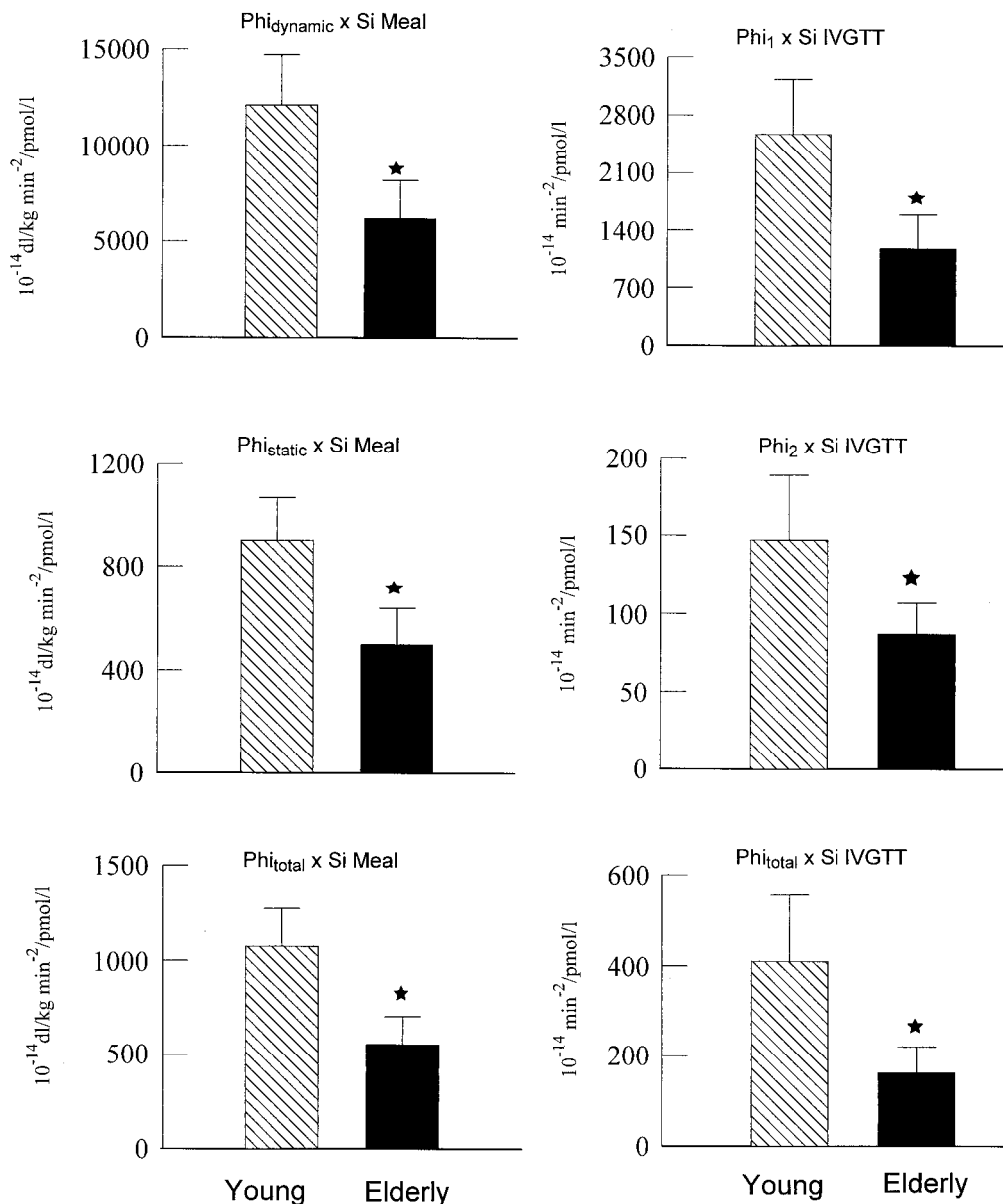


FIG. 7. Disposition indexes in the elderly and the young participants calculated by multiplying insulin secretion indexes and insulin action indexes obtained from the meal (left panels) and intravenous glucose (right panels) minimal models. \* $P < 0.01$ .

hepatic insulin extraction was higher, thereby limiting the amount of insulin that reached extrahepatic tissues. Cortisol and growth hormone did not seem to contribute to the age-associated decline in postprandial insulin action because concentrations of these hormones were lower in the elderly than in the young participants after meal ingestion. However, both glucagon concentrations and body fat (visceral as well as total) were higher in the elderly than in the young participants, whereas  $VO_{2max}$  (an index of aerobic fitness) was lower. However, addition of these factors to a model that already included indexes of insulin secretion and action did not further improve the ability of the model to predict the postprandial glycemic response.

Many (1–6) but not all (7–11) previous studies have

reported that elderly subjects are insulin resistant. The current study extends these findings by showing that insulin action is lower in elderly participants after both mixed-meal ingestion and glucose injection. Because insulin action with these two approaches was measured on different days and calculated using entirely different datasets, concordant and comparable decreases in elderly participants with both tests strongly supports the conclusion that elderly individuals are more insulin resistant than younger individuals. Of perhaps greater interest, not only was the magnitude of the decrease comparable with the two methods, but also insulin action measured with the meal minimal model correlated with that determined in the same individual with the IVGTT minimal model. This supports the contention that insulin action assessed using

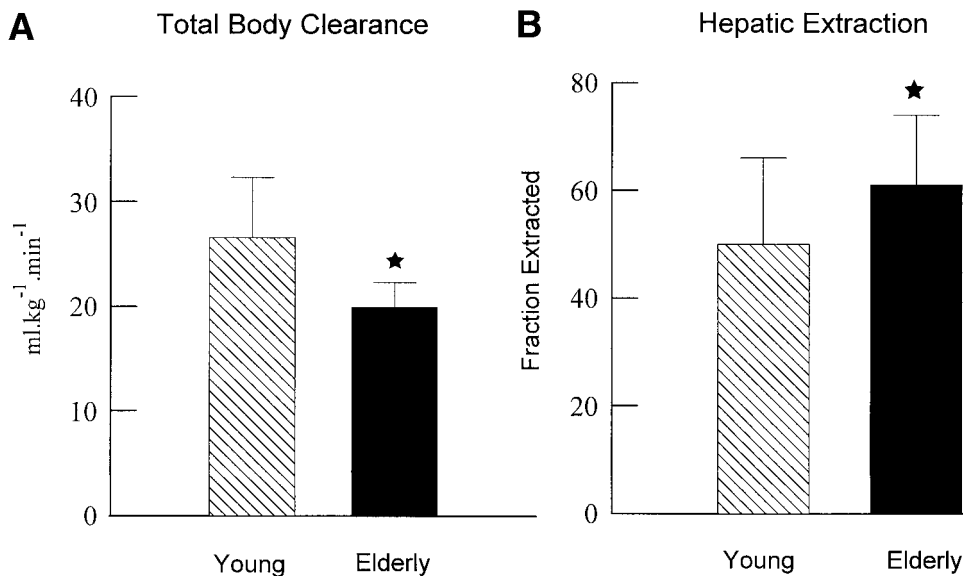


FIG. 8. Total body insulin clearance (A) and hepatic insulin extraction (B) calculated in the elderly and the young participants using the intravenous glucose minimal model. \* $P < 0.01$ .

the “cold” minimal model during an intravenous glucose tolerance test reflects insulin action present under more physiologic conditions such as occurs after eating a meal. The strength of the correlation also argues against an effect of factors uniquely present after meal ingestion (e.g., incretins) on insulin action.

The cause of insulin resistance in the elderly has been a matter of active debate. Consistent with previous reports (2,6,22,47), the elderly individuals in the present study had a greater percentage body fat, more visceral fat, a lower level of aerobic fitness as measured by a  $VO_{2max}$ , and less leg strength as measured by the double knee extension than did the younger comparison group. After adjustment for these factors using multivariate analysis, insulin action no longer differed in the elderly and the young groups. This observation supports previous reports (2,6) that insulin action measured with either an IVGTT or a euglycemic-hyperinsulinemic clamp did not differ in elderly and young subjects when adjusted for BMI or waist-to-hip ratio,

respectively. It is interesting that the strongest determinant of insulin action in the present study seemed to be body fat. Although  $VO_{2max}$  and double knee extension also predicted insulin action, these effects became nonsignificant when adjusted for percentage body fat and visceral fat. However, correlations do not prove causality. They therefore do not rule out an independent effect of age per

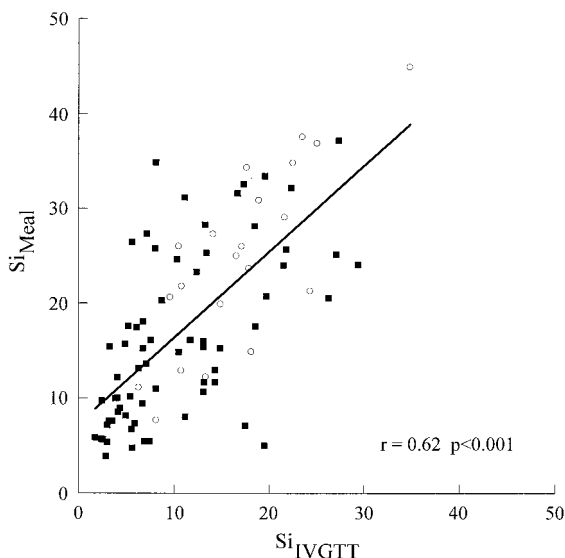


FIG. 9. Correlations between insulin action indexes obtained with the meal ( $Si_{Meal}$ ) and intravenous ( $Si_{IVGTT}$ ) minimal models measured in the elderly (■) and the young (○) participants.

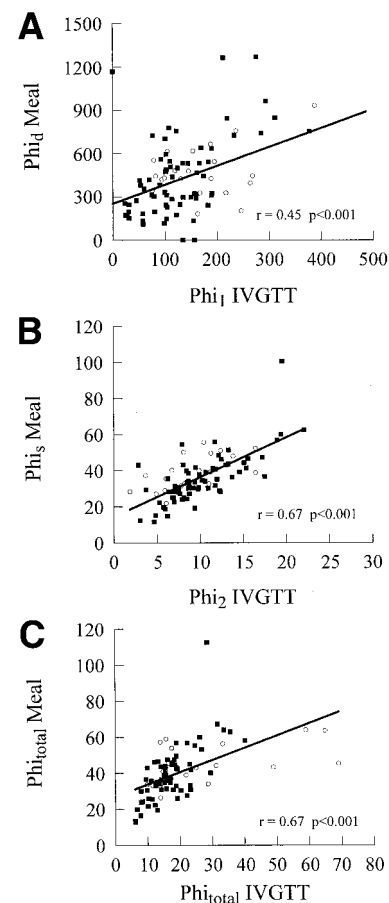


FIG. 10. A–C: Correlations between insulin secretion indexes obtained with the meal ( $\Phi_{Meal}$ ) and intravenous ( $\Phi_{IVGTT}$ ) minimal models measured in the elderly (■) and the young (○) participants.



se or a possible effect of relative androgen deficiency because the elderly volunteers were selected for testosterone and DHEA levels in the lower range of normal. They also do not contradict the well-established beneficial effects of exercise undertaken by previously sedentary individuals on insulin action (8,11,22,47) because the present studies assessed the relationship between insulin action and the level of fitness in the untrained state rather than the response to aerobic training. Nevertheless, the present data add further support to the concept that the degree of fatness is an important determinant of insulin action in the elderly.

The effects of age on  $\beta$ -cell function has been a matter of debate with previous investigators reporting an increase (2,15,16), decrease (1,7,8,11,14), or no change (3,4,10,12, 13) in insulin secretion in the elderly. These discordant results likely have been due in large part to the differences in methods used to assess insulin secretion. In the present studies, fasting insulin concentrations did not differ in the elderly and the young participants on either the meal or the intravenous glucose study days. However, fasting C-peptide concentration were significantly higher in the elderly than in the young individuals on both study days, indicating that an increase in insulin secretion was offset by a concomitant increase in hepatic insulin extraction. This was confirmed by the intravenous minimal model that showed that hepatic insulin extraction was higher in the elderly than in the young participants both before and after glucose injection.

Although decreased C-peptide clearance as a result of impaired renal function potentially could have influenced the fasting insulin-to-C-peptide ratio, we believe this to be unlikely. Plasma creatinine concentrations in these healthy elderly participants did not differ from those observed in the young participants, indicating at most a minimal decrease in renal function. Furthermore, the C-peptide model used to calculate insulin secretion and hepatic insulin clearance during the IVGTT minimal model uses the C-peptide kinetic data of Van Cauter et al. (42) that explicitly takes into account age-associated changes in C-peptide clearance. Decreased total body insulin clearance and increased hepatic insulin extraction have been reported by other investigators, albeit often assessed using different methods in different individuals (7,48,49). The opposite changes in total body and hepatic insulin extraction observed in the elderly individuals in the present study are intriguing because they suggest compensatory changes and indicate that hepatic and extrahepatic insulin metabolism are differentially regulated. The latter may be due to differences in liver and vascular insulin proteases and/or insulin receptor binding. The present data also emphasize the need to use C-peptide in conjunction with validated models to measure insulin secretion rather than merely measure peripheral insulin concentrations.

Multiple facets of insulin secretion were abnormal in the elderly participants. The increase in plasma C-peptide concentrations immediately after either meal ingestion or intravenous glucose injection tended to be lower in the elderly than in the young participants. C-peptide concentrations subsequently became higher in the elderly presumably as a result of the higher prevailing glucose concentrations. Calculation of the various indexes of

insulin secretion indicated that  $\text{Phi}_{\text{total-Meal}}$ ,  $\text{Phi}_1$ ,  $\text{Phi}_{1\text{-IVGTT}}$  and  $\text{Phi}_{\text{total-IVGTT}}$  were lower in the elderly participants. However, when considered in light of the degree of insulin resistance as reflected by the disposition index, all indexes of insulin secretion were lower in the elderly participants, indicating a global defect in insulin secretion. This conclusion is consistent with the demonstration by Meneilly et al. (50) that normal aging is associated with a reduction in both mass and amplitude of the rapid insulin pulses that occur during intravenous glucose infusion. First-phase insulin secretion after intravenous glucose injection is believed to be primarily determined by the rate of exocytosis of previously docked insulin granules (51,52). In contrast, second-phase insulin secretion is believed to be determined by multiple factors, including the rate of new insulin synthesis, insulin granule translocation, and membrane fusion (51,52). Experimental reduction of  $\beta$ -cell mass decreases both early and late insulin secretion (53). However, although an age-related decrease in  $\beta$ -cell mass could account for the alterations in insulin secretion, it is unlikely to explain the increase in hepatic insulin extraction observed in the present study because partial pancreatectomy results in a decrease (rather than an increase) in hepatic insulin extraction (54).

Both fasting and postprandial glucose concentrations were higher in the elderly than in the young participants. Multivariate analysis indicated that  $\sim 45\%$  of the postprandial glycemic response could be explained by the indexes of insulin action and secretion, indicating that the variables being assessed by both the meal and the IVGTT minimal models are physiologically relevant. However, not surprising, the strength of the prediction was greater with the meal as evident by the fact that the IVGTT indexes were no longer significant when the meal indexes were included in the model. These data indicate that although the IVGTT minimal model is useful in predicting the glycemic response to a mixed meal in the same individual, the meal minimal model is better. Essentially the same relationship was observed when peak postprandial glucose concentration rather than the postprandial glycemic response (i.e., area above basal) was used as the dependent variable (data not shown). Addition of pre- or postprandial glucagon, cortisol, or growth hormone concentrations to the model did not improve the ability of the model to predict the postprandial glycemic response. This observation argues against a role for these hormones in the glucose intolerance of the elderly.

In summary, the present studies provide a comprehensive assessment of glucose tolerance in the elderly. They indicate that both fasting and postprandial glucose concentrations are higher in elderly than in young subjects. These alterations in glucose tolerance are associated with defects in insulin action, secretion, and clearance. The severity of the reduction in insulin action is in large part explained by percentage body fat and visceral fat indicating that both obesity and the site of the fat seem to be the major determinants of insulin action in elderly as well as young individuals. Comparable decreases in insulin secretion are evident after both meal ingestion and glucose injection, suggesting an intrinsic alteration in  $\beta$ -cell function rather than an age-related alteration in the response to nutrients or incretins. Hepatic insulin extraction is greater

in the elderly than in the young participants, suggesting an alteration in hepatic insulin action and/or metabolism. Conversely, total body insulin clearance is lower, suggesting a concomitant and perhaps compensatory alteration in extrahepatic insulin metabolism.

The elderly participants in the present studies were less fit, weaker, and more obese than the younger control group. Therefore, the present study did not assess the effects of aging per se but rather the effects of the physical changes associated with aging. Perhaps more important, the elderly participants were specifically selected to have low normal DHEA (in the men and women) and testosterone (in the men) concentrations, reflecting the hormonal milieu present in a large number of otherwise healthy older individuals. It will be of considerable interest to determine the extent to which replacement of these hormones ameliorates or reverses these defects in carbohydrate tolerance.

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