Age or Waist as Determinant of Insulin Action?

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Several studies have shown that insulin action deteriorates with age, possibly mediated through accumulation of abdominal fat. We determined peripheral insulin action in elderly and younger men who had participated in a large population study (the Tromsø Study). To 15 elderly participants aged 71 to 77 years, we individually matched 15 younger participants aged 31 to 33 years (Y_1) by body mass index (BMI). A second young group (Y_2) comprised 15 participants also aged 31 to 33 years, but with BMI representative of this age group in the population study. All underwent hyperinsulinemic euglycemic clamps (0.4 mU/kg/min), oral glucose tolerance tests, and determinations of Vo_{2max}. Insulin sensitivity index (ISI = glucose disposal per kg fat-free mass [FFM] divided by steady-state insulin concentration) did not differ between the elderly and Y_1, but was higher in Y_2 (0.10 ± 0.01, 0.12 ± 0.01, and 0.17 ± 0.02, $P = .0011$ by analysis of variance [ANOVA]). Adjustment by waist circumferences (analysis of covariance [ANCOVA]) abolished this difference. In univariate analysis of pooled data, ISI correlated negatively to body fat indices, serum triglycerides, and free fatty acids (FFA), and positively to Vo_{2max}. In multiple regression analysis, waist circumference and triglycerides were the only independent predictors of insulin sensitivity, whereas age had no impact. The results confirm that the decline in insulin action seen in elderly people is related to increased abdominal fat rather than aging per se.

WITH ADVANCING AGE, glucose tolerance declines[1] and the prevalence and incidence of type 2 diabetes increases.[2] Age-related deterioration of the action of insulin on glucose uptake or endogenous glucose output (EGO) could be involved, although a concomitant decrease in insulin secretion is also required for type 2 diabetes to develop.[3] Impairments in other insulin actions could be implicated as well, such as in suppression of adipose tissue lipolysis. This would lead to increased serum levels of free fatty acids (FFA), which inhibit glucose metabolism.[4] Whereas insulin levels of approximately 300 pmol/L are necessary for half-maximal stimulation of glucose uptake,[5] lower concentrations are required to attain half-maximal suppression of EGO (about 150 pmol/L)[5,6] and lipolysis (50 to 100 pmol/L).[6-8]

Initial studies[9-11] using the hyperinsulinemic euglycemic clamp technique[12] showed a reduced rate of peripheral glucose disposal in older as compared to younger persons. This alleged disparity in insulin action was unaltered by adjustment for lean body mass.[10] However, 2 succeeding reports, which also considered total body fat[13,14] and physical fitness,[14] could not confirm this finding. Later work,[15-17] also taking body fat distribution into account, has indicated that the age-related insulin resistance is more closely linked to the increase[18] in abdominal fat seen with aging, than to age per se. Yet, in previous studies, elderly participants were often recruited from sources different from those of the young persons, or the sources were not described. Thus, a variety of confounding factors related to the different origins of recruitment of younger and older participants may have had a major influence on endpoints related to insulin action.

In the present study, we selected elderly and younger subjects who had participated in a large population survey (the Tromsø Study). At insulin infusion rates opted to attain insulin concentrations of approximately ED_{50} (half-maximal dose) for inhibition of EGO and lipolysis, we determined insulin action on peripheral glucose disposal and suppression of EGO and serum FFA in 1 elderly group and 2 groups of younger controls. The first control group consisted of young persons who were individually matched to the elderly by body mass index (BMI), the other comprised young persons with a BMI typical of this age group in the population survey.

MATERIALS AND METHODS

The Tromsø Study

The Tromsø Study[19,20] was started in 1974 as a single-center prospective follow-up study of inhabitants in the municipality of Tromsø, Norway, with the main objective to study cardiovascular risk factors. The fourth survey of the Tromsø Study[21] was started in September 1994 and completed in October 1995. All inhabitants older than 24 years were invited, and 27,161 subjects (77% of the eligible population) participated. The examination included standardized measurements of height, weight, blood pressure, nonfasting serum lipids, serum calcium, γ-glutamyl transferase, hemoglobin and blood counts, and a 20-second electrocardiogram (ECG) of lead I. Two questionnaires covered previous and present diseases and symptoms, use of medications, lifestyle (physical activity, smoking, alcohol intake), dietary habits, and socioeconomic situation.

Subjects and Matching Procedures

The participants of the present study were selected and matched using the database of the fourth survey of the Tromsø Study. All study participants born between 1919 and 1926 or in 1965 were considered, provided they had a BMI within 1 SD of the mean for all male participants irrespective of age, had blood pressure les than 190/100 mm Hg, were not on antihypertensive medication, did not report diabetes mellitus or coronary heart disease, and did not smoke, at the

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time of the population study screening. From these, we excluded 15 elderly persons with other known medical conditions and 2 with a restraint in their consent, and 1 elderly and 3 younger persons who were not of northern European descent. In addition, 1 elderly and 1 young person had died and 3 elderly and 21 young persons had moved to another part of Norway. The remaining persons (elderly and young) received a mailed invitation to attend a screening examination, including a pre-stamped reply form.

Of 89 elderly persons who were invited, 18 did not reply, 18 declined (5 provided medical reasons), and 53 attended the screening examination of the present study. Fifteen elderly persons were consecutively included in the order that they were eligible and volunteered. For the first younger control group (Y_1), we individually matched a person born in 1965 by BMI to each of the elderly subjects. For the other control group (Y_2), we recruited 15 persons also born in 1965. Each had a BMI as close as possible to the mean BMI of all men from this age group who had participated in the population study. These were randomly invited in the order that their BMI departed from the population mean and included in the order that they volunteered and were eligible. Of the 103 young persons who were invited, 41 did not reply, 10 declined, and 52 attended the screening visit.

Screening Visit

The health status of all participants was assessed by medical history, physical examination, automatic blood count, sedimentation rate, serum creatinine, sodium, potassium, magnesium, phosphate, albumin, AST, ALT, free thyroxin, and thyroid-stimulating hormone, urinalysis (dip-stick screening of morning urine for blood, albumin and glucose), and resting ECG. Subjects with a history or evidence of cardiovascular, pulmonary, hepatic, renal, metabolic, or systemic disease were excluded, as were subjects on drugs known to affect glucose or lipid metabolism, and 1 person who smoked. We also excluded subjects with a systolic pressure above 190 mm Hg, a diastolic pressure above 100 mm Hg, an abnormal resting ECG, blood tests, or urinalysis, and subjects unfamiliar with, or having problems interfering with bicycling. Thirty-two young persons were included. Thirty-two elderly and 9 young subjects were excluded. Six elderly and 6 young persons declined to participate for personal reasons. Five young persons declined due to time constraints, and 2 withdrew for personal reasons. The Regional Board of Research Ethics approved the study and all participants gave written informed consent to participate after the nature of the procedure was explained.

Research Design

The participants were tested on 3 separate days, each at least 3 days apart. A bicycle ergometer test was always performed first to determine $V_{O_{2max}}$, followed by an oral glucose tolerance test (OGTT) and a hyperinsulinemic euglycemic clamp (see below), in random order. The investigator was blinded to the results of the OGTT when it was performed prior to the clamp. For each person, all tests were completed within 7 weeks. All participants were instructed to consume a weight-maintaining diet containing at least 250 g of carbohydrates for 3 days prior to the OGTT and clamp. Furthermore, they were instructed to abstain from alcohol and not to engage in heavy physical activity in the same periods.

$V_{O_{2max}}$

$V_{O_{2max}}$ was determined by a metabolic measurement cart (SensorMedics 2900, SensorMedics Corp, Yorba Linda, CA) during exertion on an electronically braked bicycle ergometer (Ergomed 840, Siemens, Erlangen, Germany), using an individualized 1-minute incremental protocol[22] until subjective exhaustion. During testing, the subjects wore a nose-clip and breathed through a non-rebreathing valve separating expired air from room air. V_{O_2} was measured continuously and calculated at 30-second intervals using an automated open-circuit system consisting of an inspired gas meter, a mixing chamber, and electronic O_2 and CO_2 analyzers. The heart rhythm was monitored continuously on an oscilloscope, and blood pressure and a 12-lead ECG registered every minute. A maximal heart rate greater than 90% of age-predicted maximum or a respiratory exchange ratio greater than 1.10 was taken as evidence that $V_{O_{2max}}$ had been attained.

OGTT

The subjects received 1 g dextrose/kg body weight, maximum 75 g. Arterialized venous blood samples were taken at −30, 0, 10, 20, 30, 60, 90, and 120 minutes. Plasma glucose, insulin, C-peptide, and proinsulin responses to a glucose challenge were calculated as the areas under the curve ($AUC_{glucose}$, $AUC_{insulin}$, $AUC_{C\text{-}peptide}$, and $AUC_{proinsulin}$) from 0 to 120 minutes, using the trapezoidal rule. Glucose responses were classified according to the 1999 World Health Organization criteria.[23]

Clamp Visit

All subjects were admitted to the Department of Clinical Research, University Hospital of Northern Norway the evening before the clamp and anthropometric measurements and body composition were determined. After an evening meal, the subjects received only water until the experiment ended in the afternoon the following day.

Anthropometric Measurements

We measured the subjects' weight to the nearest 100 g on an electronic scale and height to the nearest centimeter on a wall-mounted stadiometer and calculated the BMI as the weight divided by the square of the height. Waist circumference was measured as the minimal circumference of the abdomen at the end of a normal expiration, and hip circumference as the circumference of the buttocks at the maximal gluteal protuberance,[24] and the waist-to-hip ratio (WHR) calculated. All measurements were done by the same investigator.

Body Composition

Percent body fat was determined by near-infrared interactance, using a Futrex-5000 Body Composition Analyzer (Futrex, Inc, Gaithersburg, MD) at the biceps site of the dominant arm. The average of 3 measurements was used in the analyses. Body fat mass was calculated by multiplying percent body fat by the body weight and fat-free mass (FFM) by subtracting the fat mass from the body weight.

Blood Pressure

Blood pressure was measured with a mercury sphygmomanometer on the right arm, with the subject supine in a quiet room. After 2 minutes of rest, 3 recordings were taken to the nearest 2 mm Hg at 2-minute intervals. The first and fifth Korotkoff phases were taken as the systolic and diastolic pressure and the mean of the second and third recordings used in the analyses.

Euglycemic Clamp

At 7 AM, the subjects were weighed after voiding. They then reclined in hospital beds for the duration of the procedure. An 18-gauge catheter (Venflon, Ohmeda, Helsingborg, Sweden) was placed in a left antecubital vein. Since study of arteriovenous balances was incorporated in another part of this work, the ipsilateral radial artery was punctured after confirmation of ulnar artery patency with the Allen test.[25] A 20-gauge arterial catheter (arterial catheter with Floswitch, Ohmeda, Swindon, UK) was inserted minimum 90 minutes prior to the start of the clamp and connected to a monitoring kit (Transpac, Abbott Critical Care Systems, Sligo, Ireland) for blood sampling and flushing. The subjects were offered local anesthesia with lidocaine prior to arterial

puncture and topical anesthesia with lidocaine prior to venepuncture. A 20-μCi priming dose of sterile, pyrogen-free 3-^{3}H glucose (Amersham, Buckinghamshire, UK) was given at −150 minutes, followed by a 0.20-μCi/min infusion, lasting 5.5 hours. An insulin infusion (rate 0.4 mU/kg/min) was started at 0 minutes and continued for 3 hours. The insulin was dissolved in 0.9% NaCl to which 2.5 mL of the subjects' own blood serum was added to a total volume of 60 mL. Potassium chloride was infused at a rate of 4 mmol/h to prevent hypokalemia. Plasma glucose concentrations were determined at 5-minute intervals and maintained at 5 mmol/L by a variable infusion of 25% glucose. To minimize rapid dilution of the labeled glucose pool, 0.02 μCi/mL 3-^{3}H glucose was added to the glucose infusion (hot-GINF).[26,27] Blood for glucose specific activity, insulin, C-peptide, and FFA concentrations was drawn at −60, −30, 0, 120, 150, and 180 minutes. Plasma and specimens of the pertinent isotope infusions were stored at −70°C until analysis and scintillation counting.

Analytical Methods

Plasma glucose concentrations were measured bedside on a YSI glucose analyzer (2300 STAT PLUS, Yellow Springs Instrument Co, Yellow Springs, OH). Plasma insulin and C-peptide was measured by radioimmunoassay (RIA).[28] The insulin antibody had less than 0.2% cross-reactivity with proinsulin or its primary circulating split form, Des 31,31 HPI, and the C-peptide antibody less than 4% cross-reactivity with proinsulin. Serum FFA levels were measured spectrophotometrically (kit from Wako Chemicals GmbH, Neuss Germany) and plasma intact proinsulin by an enzyme-linked immunosorbent assay (ELISA) kit (DAKO Diagnostics Ltd, Cambridgeshire, UK). Specific activities in 3-^{3}H glucose tracer infusions and EDTA plasma samples were determined by liquid scintillation counting (Wallac 1411, Wallac Oy, Turku, Finland), in plasma after deproteinization with 7% chilled perchloric acid, drying, and reconstitution. The activity was corrected for recovery using external standards. Serum triglyceride and high-density lipoprotein (HDL)-cholesterol levels were analyzed on a Hitachi 917 Automatic Analyzer (Hitachi, Tokyo, Japan) with reagents from Roche Diagnostics Corp, Mannheim, Germany.

Calculations

The baseline levels of glucose, insulin, C-peptide, and FFA were taken as the mean level from −60 to 0 minutes, the levels during steady state hyperinsulinemia as the mean from 120 to 180 minutes. The glucose infusion rate (GIR) was determined as the mean of the glucose infusion rates at 120, 150, and 180 minutes. Turnover data in the basal state and during steady-state hyperinsulinemia were calculated from the glucose specific activities from −60 to 0 and 120 to 180 minutes. All data were expressed per kilogram FFM. Glucose appearance (R_a) (from exogenous and endogenous sources) and disappearance (R_d) were calculated using Steele's non–steady-state equations,[29] with modifications as described by Hother-Nielsen et al.[27] EGO in the basal state is equal to R_a, whereas EGO during steady-state hyperinsulinemia was determined by subtracting GIR from R_a. When R_a equals GIR, EGO is considered fully suppressed. Although glucose tracer was added to the variable glucose infusion,[26,27] we did encounter negative values for EGO, indicating underestimation of glucose turnover.[27] These values were then assumed to be zero. The insulin sensitivity index (ISI)[12] was calculated as the R_d from 120 to 180 minutes divided by the insulin concentration over the same period.

Statistics

The sample size necessary to identify with 90% power a 20% difference in insulin action between elderly and average young subjects, given a significance level of 0.05, was estimated to 12 in each group.[30] Previously reported insulin action data[14-16] were used to calculate the standardized differences for paired data.[30] The reported SD was used as an approximation of the unknown SD of the difference between elderly and young subjects.

To allow for missing data, we allocated 15 subjects to each group. All subjects completed all tests. Turnover data were inaccessible for 1 subject in Y_2 due to loss of sample and the OGTT results discarded for another subject in Y_2 because of exertion prior to the test. Serum triglyceride measurement was missing for 1 subject in the elderly group. We estimated the power retrospectively to greater than 99% (mean ISI difference between Y_2 and the elderly group: 0.076, with SD of 0.087).[30]

The distributions of triglycerides, HDL-cholesterol, insulin, GIR, R_a, R_d, and ISI were moderately skewed, whereas the distributions of FFA and EGO during hyperinsulinemia were considerably skewed. In the former, differences between groups were assessed with 1-way analysis of variance (ANOVA), in the latter with Kruskal-Wallis tests. Multiple comparisons between the groups were then performed with Bonferroni *t* tests, which are conservative tests. The ISI in the groups were adjusted by analysis of covariance (ANCOVA) with the factor waist circumference. The univariate associations between continuous variables were analyzed with Spearman rank correlation and the multivariate associations between the ISI and independent variables with multiple regression. Study group, waist circumferences, Vo_{2max}, body fat in kilograms, triglyceride levels, levels of FFA in the basal state and during hyperinsulinemia, and systolic blood pressure were chosen as independent variables based on results from previous studies.[15-17,31,32] Waist circumference was chosen rather than the WHR because of its stronger association with insulin action. The variance (r^2) in ISI explained by each of the independent variables was determined when they were entered into a regression model as only variable. The change in total explained variance (Δr^2) induced by each variable was then assessed when they were brought into the multiple model as last variable, by calculating the partial correlation coefficients with the ISI. We checked the model assumptions by analyzing residuals and normal probability plots of errors. Statistical analyses are presented for untransformed data since the results were similar to those for logarithmically transformed data. Data in the text and figures are given as the mean ± SEM and $P < .05$ was considered significant for hypothesis testing. The data were analyzed with the SAS software package (SAS Institute Inc, Cary, NC).

RESULTS

Subject Characteristics

Body fat, expressed in kilograms and as percent of body weight, was highest in the elderly and lowest in Y_2. By ANOVA supplemented with Bonferroni *t* tests, waist circumference was similar in the elderly group and in Y_1 (94.7 ± 1.8 cm and 91.3 ± 1.7 cm), and significantly lower in Y_2 (84.1 ± 1.5 cm, $P = .0002$ for between-group differences). The Vo_{2max} was lower in the elderly (27.1 ± 1.3 mL/kg/min) but did not differ in Y_1 and Y_2 (39.5 ± 1.5 mL/kg/min and 44.4 ± 1.5 mL/kg/min, $P < .0001$ for between-group differences) (Table 1). Three elderly subjects and 4 subjects in Y_1 had first-degree relatives with type 2 diabetes.

OGTT

The results of the OGTT are shown in Table 2. Five elderly subjects had a 2-hour plasma glucose concentration between 8.9 and 12.2 mmol/L. One of these had diabetes mellitus (with fasting plasma glucose 7.0 mmol/L), the remaining had IGT. In addition, 1 elderly subject had a fasting plasma glucose concentration between 6.1 and 7.0 mmol/L together with a 2-hour

Table 1. Clinical Characteristics of Participants According to Study Group (mean ± SEM)

	Elderly (n = 15)	Y_1 (n = 15)	Y_2 (n = 15)	*P* (ANOVA)
Age (yr)	72.5 ± 0.5	32.0 ± 0.1	32.1 ± 0.1	Selection variable
BMI (kg/m^2)	25.1 ± 0.5	25.5 ± 0.6	23.8 ± 0.4	Matching variable
Body weight (kg)	77.2 ± 1.8*	84.8 ± 2.1†	75.1 ± 1.5	.0012
Body fat (%)	18.6 ± 1.4	13.4 ± 1.7	13.0 ± 1.7	.0334
Body fat (kg)	14.6 ± 1.3	11.5 ± 1.5	9.9 ± 1.4	.0661
FFM (kg)	62.6 ± 1.3*	73.3 ± 2.2†	65.2 ± 1.6	.0002
Waist circumference (cm)	94.7 ± 1.8	91.3 ± 1.7†	84.1 ± 1.5‡	.0002
WHR	0.95 ± 0.01*	0.89 ± 0.01	0.86 ± 0.01‡	<.0001
Systolic blood pressure (mm Hg)	139 ± 3*	125 ± 3	125 ± 3‡	.0011
Diastolic blood pressure (mm Hg)	79 ± 3	74 ± 3	77 ± 2	.2481
$\dot{V}o_{2max}$ (mL/kg/min)	27.1 ± 1.3*	39.5 ± 1.5	44.4 ± 1.5‡	<.0001
Serum triglycerides (mmol/L)	1.27 ± 0.11	1.34 ± 0.14†	0.89 ± 0.09	.0223

Abbreviations: Y_1, young persons matched to the elderly according to BMI; Y_2, young persons with BMI representative of all males of this age group in population survey.

Differences significant at the .05 level by Bonferroni *t* tests: *elderly *v* Y_1; †Y_1 *v* Y_2; ‡elderly *v* Y_2.

value below 8.9 mmol/L (impaired fasting glycemia). None of the younger controls had glucose abnormalities.

Euglycemic Clamp

The mean plasma glucose levels during steady state hyperinsulinemia were similar in the groups, with a coefficient of variation (CV) of 3.2% ± 0.4%, 3.0% ± 0.3%, and 3.3% ± 0.3% in the elderly, Y_1, and Y_2 groups ($P = .8728$ by ANOVA). The corresponding CVs for the glucose infusion rates were 18.8% ± 2.9%, 16.3% ± 2.1%, and 12.5% ± 2.1% ($P = .1808$) (data not shown). The plasma insulin levels were higher in the elderly group than in Y_2 ($P = .0031$ by ANOVA for between-group differences), but there were no significant differences between the elderly group and Y_1, or between Y_1 and Y_2 (Table 3). The R_a and R_d during steady-state hyperinsulinemia did not differ between the elderly group and Y_1, whereas both measures were significantly higher in Y_2 than in both the elderly and Y_1 (Table 4). The same pattern was seen for the ISI (Fig 1A). Adjusting the ISI by waist circumferences abolished the differences between the groups (Fig 1B). Adjusting the R_a and R_d by waist circumferences gave the same result (data not shown). When the subjects with glucose abnormalities were excluded from the analysis, ANOVA of unadjusted data showed similar significant differences in R_a, R_d, and ISI between the groups as when these subjects were incorporated (data not shown). The FFA levels and percent FFA suppression (Table 3) and the EGO and percent EGO suppression (Table 4) did not differ between the groups during hyperinsulinemia.

Univariate Correlations

In pooled data, the ISI correlated positively with Vo_{2max} and negatively with waist circumferences (Fig 2A), triglyceride levels (Fig 2B), WHR, BMI, body fat, FFA in the basal state and during hyperinsulinemia, as well as with the systolic and diastolic blood pressures. However, the latter 2 correlations were not significant. Correlation analysis excluding persons with glucose abnormalities gave similar results (data not shown).

Table 2. OGTT Results According to Study Group (mean ± SEM)

	Elderly (n = 15)	Y_1 (n = 15)	Y_2 (n = 14)	*P* (ANOVA)
Plasma glucose				
Fasting (mmol/L)	5.73 ± 0.15	5.27 ± 0.16	5.30 ± 0.08	.0319
2-h (mmol/L)	8.59 ± 0.41*	6.47 ± 0.37	6.02 ± 0.32†	<.0001
AUC (mmol · min/L)	1,086 ± 47*	890 ± 20	905 ± 24†	.0001
Plasma insulin				
Fasting (pmol/L)	43 ± 5	47 ± 6	34 ± 4	.1954
2-h (pmol/L)	277 ± 42	174 ± 27	132 ± 27†	.0104
AUC (pmol · min/L)	35,022 ± 5,081	29,674 ± 3,550	24,766 ± 3,100	.2154
Plasma C-peptide				
Fasting (pmol/L)	875 ± 62	822 ± 50	659 ± 49†	.0224
2-h (pmol/L)	1,734 ± 87*	1,382 ± 94	1,365 ± 100†	.0109
AUC (pmol · min/L)	96,507 ± 5,100	86,467 ± 5,018	84,266 ± 5,090	.2027
Plasma proinsulin				
Fasting (pmol/L)	3.1 ± 0.3	2.1 ± 0.3	1.7 ± 0.3†	.0050
2-hour (pmol/L)	12.9 ± 1.6	8.0 ± 1.2	7.1 ± 1.5†	.0140
AUC (pmol · min/L)	1,052 ± 136	790 ± 111	699 ± 145	.1552

Abbreviations: Y_1, young persons matched to the elderly according to BMI; Y_2, young persons with BMI representative of all males of this age group in population survey; AUC, area under the curve.

Differences significant at the .05 level by Bonferroni *t* tests: *elderly *v* Y_1; †elderly *v* Y_2.

Table 3. Plasma Glucose, Insulin, C-Peptide, and Serum FFA Levels in the Basal State and During Steady-State Hyperinsulinemia According to Study Group (mean ± SEM)

	Elderly (n = 15)	Y_1 (n = 15)	Y_2 (n = 15)	*P*
Basal state				
Plasma glucose (mmol/L)	5.68 ± 0.16	5.47 ± 0.07	5.50 ± 0.07	.3033
Plasma insulin (pmol/L)	39 ± 3	49 ± 6	39 ± 7	.2967
Plasma C-peptide (pmol/L)	805 ± 54	779 ± 53	675 ± 58	.2176
Serum FFA (mmol/L)	0.44 ± 0.03*	0.30 ± 0.03	0.29 ± 0.04†	.0032
Steady-state hyperinsulinemia				
Plasma glucose (mmol/L)	5.08 ± 0.02	5.04 ± 0.03	5.02 ± 0.03	.4108
Plasma insulin (pmol/L)	214 ± 11	179 ± 10	163 ± 9†	.0031
Plasma C-peptide (pmol/L)	390 ± 48	382 ± 46	272 ± 51	.1715
Serum FFA (mmol/L)	0.04 ± 0.01	0.04 ± 0.01	0.02 ± 0.01	.4494‡
FFA suppression (%)	92 ± 2	89 ± 2	93 ± 2	.3935‡

Abbreviations: Y_1, young persons matched to the elderly according to BMI; Y_2, young persons with BMI representative of all males of this age group in population survey.

Differences significant at the .05 level by Bonferroni *t* tests: *elderly *v* Y_1; †elderly *v* Y_2. ‡*P* value by Kruskal-Wallis test, remaining by ANOVA.

Regression Analysis

The regression analyses of the effects of selected independent variables on ISI are shown in Table 5, when entered as only variable (univariate model) and when adjusted for remaining listed variables (multiple model). In a multiple model that explained 61% of the variance in ISI, waist circumferences ($P = .0375$) and triglyceride levels ($P = .0314$) were the only variables that independently predicted ISI. When introduced into this model as the last variable, waist circumferences added 12% and triglyceride levels 13% to the total variance explained by the model. Vo_{2max} added 2%, and study group (which represented age) added less than 1%. Fasting glucose, 2-hour glucose, and $AUC_{glucose}$ were not significant predictors of ISI when each of these variables in turn were brought into the model. Furthermore, their inclusion in the model did not change the significant associations between the ISI and waist circumferences and triglyceride levels.

DISCUSSION

This population-based study, in which the participants were selected according to age and BMI, shows a strong relationship between peripheral insulin action and waist circumference. Its design is unique, since we compared elderly persons with 2 different groups of younger persons. The first consisted of young subjects who each were matched with an elderly subject by BMI, the second of young subjects with a BMI representative of persons in this age group. While our data demonstrate a decline in ISI in elderly men when compared with young men who are leaner, they also demonstrate an identical ISI in elderly and younger men matched by BMI. These latter 2 groups had comparable waist circumferences and adjusting the ISI by waist circumference abolished the differences between all groups.

These findings were substantiated by the results of the regression analysis. Waist circumference and triglyceride levels were the only independent predictors of ISI. Waist circumference explained 47% of the variance in ISI when it was entered in a regression model as the only independent variable. When brought into the multiple model as the last variable, it added as much as 12% to the total variance explained by the model. In contrast, the augments represented by Vo_{2max}, total body fat, FFA levels, blood pressure, and study group (which represented age) were negligible.

At higher physiological insulin concentrations, Kohrt et al[16] also found a strong association between waist circumference and insulin action in older and young persons. At yet higher levels, Coon et al[15] found a strong association between WHR and insulin action. In both studies,[15,16] the independent contributions of age, Vo_{2max}, and total adiposity to insulin action

Table 4. Turnover Data and Glucose Infusion Rate (GIR) According to Study Group (mean ± SEM)

	Elderly (n = 15)	Y_1 (n = 15)	Y_2 (n = 15)	*P*
Basal state				
EGO (μmol/kg_{FFM}/min)	15.3 ± 0.6	14.3 ± 0.7	14.7 ± 0.7	.5679
R_d(μmol/kg_{FFM}/min)	15.3 ± 0.6	14.3 ± 0.7	14.9 ± 0.8	.6109
Steady-state hyperinsulinemia				
GIR (μmol/kg_{FFM}/min)	18.6 ± 1.9	18.6 ± 3.0	27.4 ± 3.2	.0435
EGO (μmol/kg_{FFM}/min)	2.3 ± 0.9	3.7 ± 0.9	1.9 ± 0.7	.3358‡
EGO suppression (%)	89 ± 5	75 ± 6	87 ± 5	.2183‡
R_a(μmol/kg_{FFM}/min)	19.6 ± 0.8	20.5 ± 1.7*	27.2 ± 2.0†	.0028
R_d(μmol/kg_{FFM}/min)	19.6 ± 0.9	20.5 ± 1.7*	26.9 ± 2.1†	.0049

Abbreviations: Y_1, young persons matched to the elderly according to BMI; Y_2, young persons with BMI representative of all males of this age group in population survey; EGO, endogenous glucose output; R_a, glucose appearance; R_d, glucose disappearance; GIR, glucose infusion rate.

Differences significant at the .05 level by Bonferroni *t* tests: *Y_1 *v* Y_2; †elderly *v*. Y_2. ‡*P* value by Kruskal-Wallis test, remaining by ANOVA.

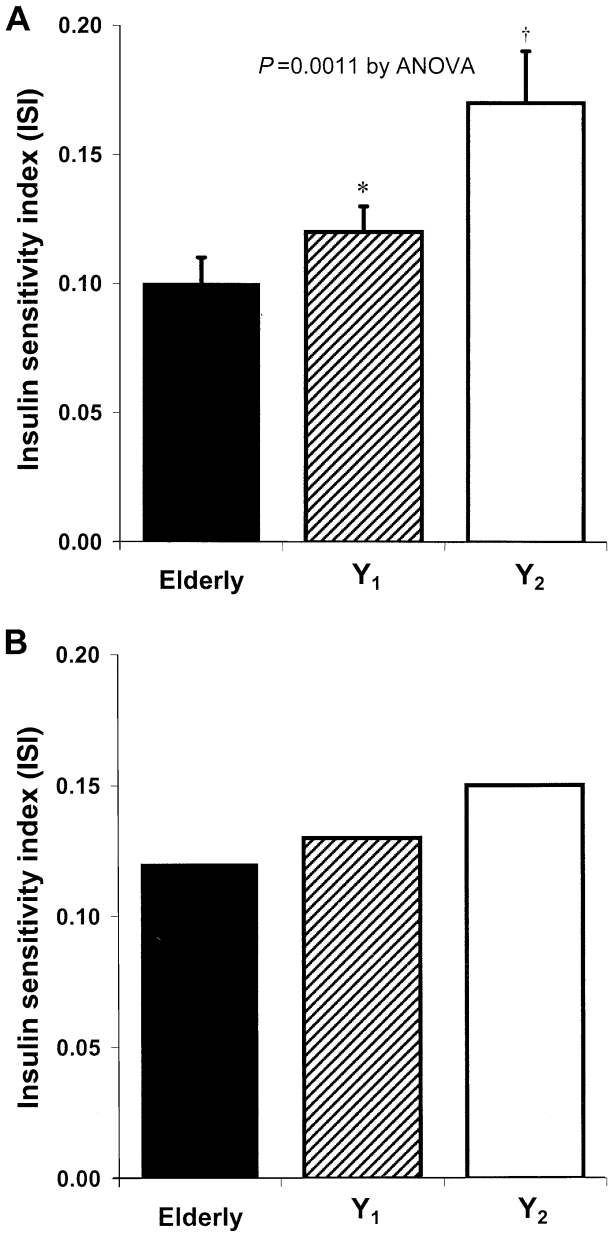

Fig 1. ISI in the 3 groups. Results are (A) unadjusted means ± SEM and (B) means adjusted by waist circumference. Y_1, young persons matched to the elderly according to BMI; Y_2, young persons with BMI representative of males of this age group in population survey. ISI is steady-state R_d (μmol/kg FFM/min) divided by steady-state plasma insulin (pmol/L). Differences significant at the .05 level by Bonferroni *t* tests: *Y_1 *v.* Y_2; †elderly *v* Y_2.

were insignificant. The European Group for the Study of Insulin Resistance (EGIR)[17] analyzed hyperinsulinemic euglycemic clamp data collected at 20 centers throughout Europe from 1,146 men and women aged 18 to 85 years. Glucose disposal rate at insulin levels comparable to those of Kohrt et al[16] declined slightly with age. When adjusted for BMI, however, this relationship with age was no longer statistically significant. Furthermore, in a subgroup of 529 subjects with WHR measurements, both WHR and waist circumference were significantly related to insulin resistance in univariate analysis, as well as after adjustment for age and sex.[17]

As previously reported,[16] we found that insulin action was more strongly related to waist circumference than to WHR. Waist measurements show closer associations than WHR with intra-abdominal fat.[33] This fat has a greater propensity than non-abdominal fat to release FFA,[34] which may induce insulin resistance at several levels of metabolism.[4]

Cefalu et al[35] used magnetic resonance imaging to determine intra-abdominal fat and the minimal model technique to assess insulin sensitivity in men and women aged 23 to 83 years. Intra-abdominal fat mass accounted for the majority of the variance in insulin sensitivity, whereas age, sex, and interactions of age and sex accounted for only 1%.[35]

An assessment of glucose disposal at a single insulin dose as in our study is not a measurement of insulin sensitivity, but of insulin action, as it cannot distinguish between insulin sensitivity (ED_{50}) and responsiveness (V_{max}).[36] However, a euglycemic clamp study with sequential insulin infusions[37] and another study using 4 hyperglycemic plateaus[38] indicated that

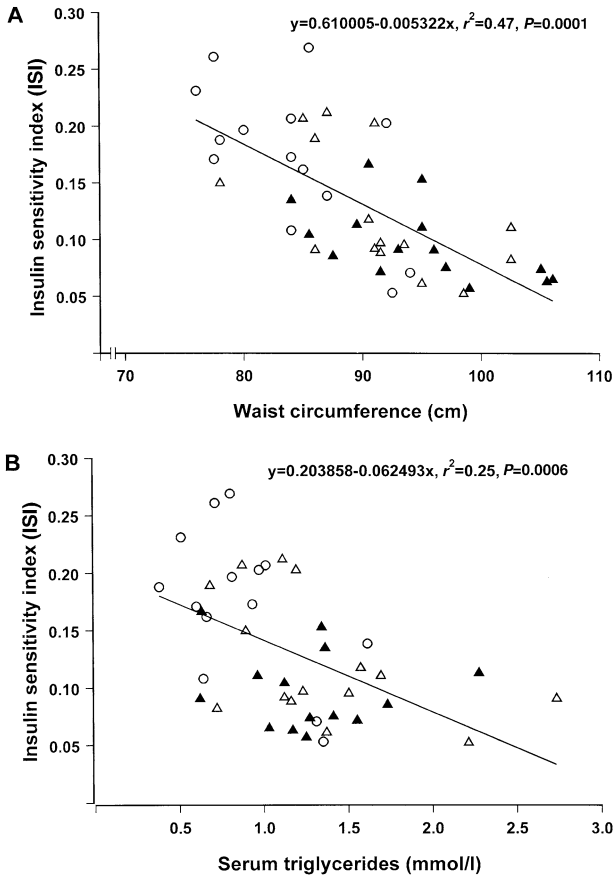

Fig 2. Associations of ISI with (A) waist circumferences and (B) triglyceride levels. Elderly (▲); Y_1 (△); Y_2 (○). Y_1, young persons matched to the elderly according to BMI; Y_2, young persons with BMI representative of males of this age group in population survey. ISI is steady-state R_d (μmol/kg FFM/min) divided by steady-state plasma insulin (pmol/L).

Table 5. Regression Coefficients β, SEM, and Corresponding *P* Values for the Effects on ISI of Independent Variables When Entered as Only Variable (Univariate Model) and When Adjusted for Remaining Listed Variables (Multiple Model): Pooled Data

	ISI							
	Univariate Model				Multiple Model (r^2 = 0.61)			
Independent Variable	β	SEM	*P*	r^2	β	SEM	*P*	Δr^2
Waist circumference (cm)	−0.005322	0.00086429	.0001	0.47	−0.003608	0.00166413	.0375	0.12
Vo_{2max} (ml/kg/min)	0.003976	0.0007954	.0001	0.37	0.001247	0.0016569	.4569	0.02
Body fat (kg)	−0.005781	0.00136222	.0001	0.30	−0.000572	0.00172224	.7421	<0.01
Study group (1,2,3)	0.038264	0.00960217	.0003	0.27	−0.001146	0.01354461	.9331	<0.01
Triglycerides (mmol/L)	−0.062493	0.01672645	.0006	0.25	−0.033857	0.01506661	.0314	0.13
$FFA_{basal\ state}$ (mmol/L)	−0.170794	0.05994735	.0068	0.17	−0.049221	0.06832128	.4763	<0.01
$FFA_{hyperinsulinemia}$ (mmol/L)	−0.697927	0.22772811	.0038	0.18	0.062685	0.24688183	.8011	0.02
Systolic blood pressure (mm Hg)	−0.001102	0.00070471	.1253	0.06	0.000628	0.00063788	.3319	0.03

NOTE. r^2 is variance in ISI explained by each independent variable when entered as only variable and Δr^2 is change in variance induced by variable when brought into model as last variable. ISI is steady-state R_d (μmol/kg_{FFM}/min) divided by steady-state plasma insulin (pmol/L).

insulin sensitivity was decreased in elderly persons with impaired glucose tolerance. Unfortunately, body fat, fat distribution, and level of physical fitness were not considered in these studies.

The variables associated with insulin action are highly intercorrelated and the existing data should be interpreted with some caution. Nevertheless, the present study, taken together with previous studies at higher insulin concentrations,[15-17] strongly suggests that not only insulin action, but also insulin sensitivity is decreased in elderly persons, and that it is caused by increased abdominal fat.

An association of blood triglyceride levels with insulin action, independent of abdominal obesity and FFA levels, was found by the EGIR,[31] as well as in our study. This finding is interesting on the background of recent data indicating that the accumulation of skeletal muscle triglyceride is a contributor to peripheral insulin resistance.[39]

Our study supports previous work,[15,16] demonstrating that Vo_{2max} is not an independent predictor of insulin action when assessed together with a measure of abdominal fat. Our data further illustrate the different impact of waist circumference and Vo_{2max} on insulin action. Although the Vo_{2max} was 30% lower in the elderly compared to the young controls with similar waist circumference, the ISI did not differ. Similarly, even if the Vo_{2max} was similar in the 2 young groups, the ISI was higher in the leaner group. In line with these results, Kohrt et al[16] found that insulin action in older persons with normal glucose tolerance was the same as in young subjects despite the fact that Vo_{2max} was 35% to 40% lower.

We also report that the suppression of EGO and FFA during hyperinsulinemia was similar in the groups, although insulin levels were higher in the elderly. However, the insulin levels that we attained in this study were somewhat higher than the targeted levels (of $\sim ED_{50}$ for inhibition of EGO and lipolysis),[5-8] especially in the elderly group. Thus, in some persons a near-maximal, rather than partial, suppression of FFA and EGO was achieved, which limits the precision of these results.

The methodological issues involved in the study of normal human aging are complex and include such factors as subject selection, higher morbidity rates, and selective mortality[40] By necessity, the elderly participants were a selected group, because they had to be in good health to enter the study. Nevertheless, concurrent disease must be precluded in order to assess the impact of aging per se on glucose metabolism. By including men only, we circumvented the problems imposed by the menopause in the study of aging in women.

We determined body fat by near-infrared interactance, which is a rapid, safe, and noninvasive method. Although it has high reliability,[41] this methodology has been found to systematically underestimate body fat, accentuated with increasing body fatness.[41] A greater underestimation of body fat in the elderly group, due to a redistribution of fat from peripheral to central regions, could have influenced our results. However, methodological problems, attributable to aging, also concern several alternative techniques to determine body composition.[42] This includes the conventional criterion method of hydrostatic weighing and the 2-compartment model used to estimate body fat from body density, as well as various prediction equations for body fat based on these.[42]

As expected, the glucose levels were higher in the elderly group. A justification to include persons with glucose abnormalities in the data analyses is the heterogeneous etiology of glucose abnormalities,[43] wherein both a decreased insulin release and insulin action are involved.[3] Furthermore, the exemption of subjects with glucose values above a certain cut-off level may not be warranted in studies involving different ages. The application of such uniform criteria would then result in a more stringent selection procedure in the elderly,[40] as glucose levels increase with age. Although elevated plasma glucose levels may deteriorate insulin action,[44] this aspect of glucose toxicity was probably not involved with the modest increments in glucose levels observed in this study.[45]

In conclusion, the present population-based study gives support to the view that the decline in insulin action in elderly persons is related to increased abdominal fat rather than to aging per se. This outlook could motivate towards lifestyle modifications that are effective in the prevention and treatment of abdominal obesity and insulin resistance, also in the elderly.[46]

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