Insulin Action and Age

Ele Ferrannini, Silvia Vichi, Henning Beck-Nielsen, Markku Laakso, Giuseppe Paolisso, and Ulf Smith, European Group for the Study of Insulin Resistance (EGIR)

Evidence that age is associated with insulin resistance is discordant. We analyzed euglycemic insulin clamp (1 mU · min^{-1} · kg^{-1}) data collected at 20 centers throughout Europe from 1,146 men and women with normal glucose tolerance, ranging in age from 18 to 85 years. In the whole group, insulin action (as the *M* value) declined slightly with age (at a rate of 0.9 μmol · min^{-1} · kg^{-1} per decade of life, 95% CI = 0.4–1.3, *P* = 0.0002). When adjusted for BMI, this relationship was no longer statistically significant. The same result was obtained whether insulin action was expressed per kilogram of body weight or per kilogram of fat-free mass, expressed as the M:I ratio, or estimated from fasting plasma insulin concentrations. Subgroup analysis showed that a significant BMI-adjusted decrease in insulin action with age was present only in lean (BMI <25 kg/m^2) women (a rate of 1.6 μmol · min^{-1} · kg^{-1} per decade, 95% CI = 0.6–2.5, *P* = 0.001), in whom percentage fat mass also increased with age (by 0.38% body weight per decade, *P* = 0.0007). Insulin action was positively associated with insulin suppression of circulating free fatty acids (FFAs) (+1.5 μmol · min^{-1} · kg^{-1} for each 10% increase in FFA suppression, *P* < 0.0001) in a multivariate model accounting for sex, BMI, age, and fasting FFA levels. Furthermore, insulin suppression of FFAs improved with age in men (2% per decade, *P* < 0.0001) but not in women. In the subgroup of lean women in whom insulin action declined with age, adding FFA suppression to a multiple regression equation canceled the association between age and insulin action. Thus, the small effect of age on insulin action could be adequately explained on the basis of age-related changes in body composition and substrate competition. We conclude that in healthy Europeans, age per se is not a significant cause of insulin resistance of glucose metabolism or lipolysis. *Diabetes* 45:947–953, 1996

From the University of Odense (H.B.-N.), Odense, Denmark; the University of Belfast (P. Bell), Belfast, U.K.; the University of Verona (E. Bonora), Verona; Federico II University (B. Capaldo), Naples; the University of Turin (P. Cavallo-Perin), Turin; the University of Padova (S. Del Prato), Padüva; CNR Institute of Clinical Physiology (E.F.), Pisa; Catholic University (G. Mingrone), Rome; the University of Naples II (G.P.), Napoli, Italy; the University of Heidelberg (D. Fliser), Heidelberg; the University of München (K. Rett), Munich; Kreischa (M. Week); Stadtklinik (S. Jacob), Baden-Baden, Germany; the University of Geneva (A. Golay), Geneva, Switzerland; Lund University (L.C. Groop), Lund; the University of Göteborg (U.S.), Göteberg, Sweden; the University of Kuopio (M.L.), Kuopio; the University of Helsinki (H. Yki-Järvinen), Helsinki, Finland; the University of Belgrade (N. Lalic), Belgrade, Yugoslavia; and the University of Athens (A. Mitrakou), Athens, Greece.

Address correspondence and reprint requests to Dr. E. Ferrannini, CNR Institute of Clinical Physiology, Via Savi, 8, 56126 Pisa, Italy.

Received for publication 2 October 1995 and accepted in revised form 1 February 1995.

FFA, free fatty acid; FFM, fat-free mass; WHR, waist-to-hip ratio.

A negative influence of age on glucose metabolism is unanimously recognized. Glucose tolerance is often reduced in the elderly, and its decline appears to be progressive with advancing age (1). This phenomenon could reflect the delayed expression of timed diabetogenes in predisposed individuals, acting through insulin deficiency, insulin resistance, or both. Alternatively, the biological process of aging could involve a loss in the ability to maintain glucose homeostasis in all individuals, thereby facilitating or accelerating the development of diabetes. In the former model, age is a neutral bystander in the natural history of diabetes; in the latter paradigm, true metabolic senescence partakes of the glucose intolerance of aging.

Deterioration of glucose tolerance can be due to failing insulin secretory capacity or to impaired insulin action (or both). In the pathogenesis of age-related glucose intolerance, both insulin deficiency and insulin resistance have been implicated (2–4) and disclaimed (5–7). With regard to insulin action, the prevailing opinion in medical literature is that aging is accompanied by insulin resistance (8). On close scrutiny, however, the evidence supporting this view is inconclusive. Thus, in large-scale (9) or population-based (10) studies in which fasting plasma insulin concentrations have been used as a surrogate measure of insulin sensitivity, no significant positive association with age has been found. The influence of age has also been investigated by using techniques (euglycemic insulin clamp, minimal model, perfused forearm, combined glucose and insulin infusions) that measure insulin sensitivity directly. While the majority of studies report some decrease in insulin action in the elderly, negative results are well documented (Table 1). Besides methodological differences, the number of subjects in the different age-groups has been relatively small and often biased toward men, the influence of obesity has not always been accounted for, and occasionally, subjects with impaired or borderline glucose tolerance have been included in the older age-group.

Even in experimental animals, conflicting results can be found. Thus, in the hindlimb insulin perfusion studies carried out by Reed et al. (29), reduced glucose uptake was associated with growth and development, whereas Ivy et al. (30) concluded that aging does not result in skeletal muscle insulin resistance.

In this work, we have assessed the relationship between age and insulin action by retrospective analysis of the database of the European Group for the Study of Insulin Resistance (EGIR). This database, collected at 20 European centers, includes data from 1,146 healthy white men and

TABLE 1
Case-control studies of the effect of age on insulin sensitivity

Author	Number (men/women)	Age range (years)	Technique	Effect of age	Notes
Kimmerling et al. (11)	100 (100/0)	22–69	Quadruple infusion	No	IGT in 30% subjects
Kalant et al. (12)	34	22–73	Forearm perfusion hyperglycemic clamp	No	
DeFronzo (13)	84 (47/37)	21–84	Euglycemic clamp hyperglycemic clamp	Yes	
Robert et al. (14)	55 (45/10)	19–86	Glucose kinetics (labeled glucose infusion + insulin)	Yes	Borderline glucose tolerance in the elderly group, body mass not specified
Jackson et al. (15)	32 (32/0)	19–83	Forearm glucose uptake during 100-g OGTT	Yes	
Rowe et al. (16)	27 (27/0)	22–77	Euglycemic clamp	Yes	
Fink et al. (17)	44 (18/26)	20–82	Euglycemic clamp	Yes	Borderline glucose tolerance in the elderly group, three with treated hypertension
Chen et al. (2)	20 (20/0)	18–82	IVGTT minimal model	Yes	
Broughton et al. (18)	23 (23/0)	20–85	Glucose-insulin infusion	No	
Fink et al. (19)	21 (20/1)	21–70	Euglycemic clamp hyperglycemic clamp	Yes	IGT in the elderly group
Pacini et al. (6)	27 (27/0)	23–80	IVGTT minimal model	No	
Broughton et al. (20)	27 (27/0)	25–80	Euglycemic clamp	No	
Khan et al. (21)	25 (25/0)	24–82	IVGTT minimal model	No	Trained elderly subjects
Coon et al. (22)	49 (49/0)	19–73	Euglycemic clamp	No	When accounting for regional fat distribution
O'Shaughnessy et al. (23)	16 (0/16)	Pre- and post-menopausal	Euglycemic clamp	Yes	Lean women only
Fransila-Kallunki et al. (24)	40 (20/20)	21–80	Euglycemic clamp	Yes	Men only
Kohrt et al. (25)	84 (45/39)	21–72	Euglycemic clamp	No	When accounting for abdominal obesity
Boden et al. (26)	12 (12/0)	32–66 (mean)	Euglycemic clamp	No	
Elahi et al. (27)	55	24–90	Hyperglycemic clamp	No	If 2-h plasma glucose >7.8 mmol/l
Bonadonna et al. (28)	14 (8/6)	21–71 (mean)	Euglycemic clamp	Yes	Explained by substrate competition

OGTT, oral glucose tolerance test; IVGTT, intravenous glucose tolerance test; IGT, impaired glucose tolerance.

women ranging in age from 18 to 85 years in whom insulin action was determined by the euglycemic insulin clamp technique. Analysis of this population sample, the largest so far in which insulin action has been measured directly, provided a robust test for the hypothesis that age is a primary insulin-resistant state.

RESEARCH DESIGN AND METHODS

Subjects. Of the 20 participating clinical research centers in Europe (3 in Finland, 1 in Sweden, 1 in U.K., 1 in Denmark, 4 in Germany, 1 in Switzerland, 7 in Italy, 1 in Yugoslavia, and 1 in Greece), each contributed between 21 and 122 cases. These centers agreed to provide all of their available clamp studies (whatever the original purpose of these studies) on the condition that study subjects met the following criteria: *1*) no clinical or laboratory evidence of cardiac, renal, liver, or endocrine disease; *2*) a fasting plasma glucose concentration <6.7 mmol/l and normal glucose tolerance by World Health Organization criteria (31); *3*) normal blood pressure; *4*) no recent change (≥10%) in body weight; and *5*) no current medication. Of the 1,146 subjects in the present series (766 men, 380 women), 425 were recruited in northern Europe (Sweden, Finland, and U.K.), 289 in central Europe (Denmark, Germany, and Switzerland), and 432 in southern Europe (Italy, Serbia, and Greece). At each center, the protocol was reviewed and approved by the local ethics committee, and informed consent was obtained from all subjects before their participation.

Protocol. The minimum information required for each case was age, anthropometric variables, fasting and steady-state (= last 40 min of a 2-h clamp, see below) plasma glucose and insulin measurements. Height was measured to the nearest centimeter, weight to the nearest kilogram. BMI was calculated as the weight divided by the square of height. The waist-to-hip ratio (WHR) was determined (in a subset of 372 men and 157 women) by measuring the waist circumference at the narrowest part of the torso, and the hip circumference was measured in a horizontal plane at the level of the maximal extension of the buttocks.

Insulin action was measured in all subjects by the euglycemic insulin clamp technique (32) using an insulin infusion rate of 1 mU · min^{-1} · kg^{-1} (6 pmol · min^{-1} · kg^{-1}). Briefly, polyethylene cannulas were inserted into an antecubital vein (for the infusion of glucose and insulin) and retrogradely into a wrist vein heated at 60°C in a hot box or a heating pad (for intermittent blood sampling of arterialized venous blood). At time zero, a primed-constant infusion of regular insulin was begun and continued for 120 min. An exogenous glucose infusion was started 4 min into the insulin infusion, and it was adjusted every 5–10 min to maintain plasma glucose within ~10% of its baseline value. Blood samples were obtained at timed intervals in the fasting state and during the clamp for the measurement of plasma glucose, insulin, and free fatty acid (FFA) levels. The latter was measured in a subset of 347 men and 160 women. A further blood sample was obtained in the basal state (591 men, 285 women) for the measurement of serum lipid concentrations.

Analytical procedures. Plasma glucose level was measured by the glucose oxidase method. Plasma insulin concentrations were measured by radioimmunoassay. Serum lipid levels (total cholesterol, triglycerides, HDL cholesterol) were assayed by standard enzymatic assays. LDL cholesterol was calculated from total and HDL cholesterol and triglyceride levels (33). Plasma FFAs were assayed spectrophotometrically.

Data analysis. Insulin action was expressed as the whole-body glucose disposal rate during steady-state euglycemic hyperinsulinemia. With the insulin dose used in the current study, hepatic glucose output has been previously shown to be fully suppressed in elderly as well as young subjects (13,14,19). Therefore, glucose disposal (*M* value) was calculated from the exogenous glucose infusion rate during the last 40 min of the 2-h clamp after correction for changes in glucose concentration in a total distribution volume of 250 ml/kg (34). Additionally, insulin action was expressed as the M:I ratio, i.e., the ratio of *M* to the steady-state plasma insulin concentration, and as the ratio of *M* to the steady-state

TABLE 2
Characteristics of the study group

	Mean ± SD	Range
Age (years)	42 ± 16	18–85
Height (cm)	172 ± 10	140–200
Weight (cm)	77 ± 15	40–151
BMI (kg/m^2)	25.7 ± 4.7	15.4–55.1
Fasting plasma glucose (mmol/l)	5.1 ± 0.5	3.2–6.7
Fasting plasma insulin (pmol/l)	62 ± 40	10–343
Steady-state plasma glucose (mmol/l)	5.0 ± 0.5	3.4–7.1
Steady-state plasma insulin (pmol/l)	492 ± 131	146–1,198
M value (μmol · min^{-1} · kg^{-1} body wt)	34.3 ± 13.1	2.5–102.2
M value (μmol · min^{-1} · kg^{-1} FFM)	49.1 ± 17.7	3.6–127.1

The *M* value is the glucose disposal rate during the final 40 min of the euglycemic insulin clamp, normalized by the subject's body weight or FFM. There were 1,146 participants in this study group (men 67%, women 33%).

plasma glucose concentration (glucose clearance) (35). Whole-body glucose disposal was normalized per kilogram of body weight or per kilogram of fat-free mass (FFM), as calculated by Hume's formula (36). Fat mass was obtained as the difference between body weight and FFM.

BMI and insulin values were log-transformed to normalize their distribution. Data are given as means ± SD. A dummy variable was introduced to account for between-center differences and was included in all regression models. Two-way analysis of variance and simple and multiple regression analysis were carried out by standard techniques. For regression coefficients, 95% CI was calculated.

RESULTS

Characteristics of the study group are given in Table 2. The age range was almost 7 decades (18–85 years). Of the subjects, 47% (49% of men, 42% of women) were obese (i.e., BMI >25 kg/m^2). Women had larger fat mass (34 ± 6 vs. 29 ± 5% of body weight, $P < 0.0001$) and slightly lower *M* values than men (32.6 ± 13.1 vs. 35.1 ± 13.0 μmol · min^{-1} · kg^{-1}, $P = 0.002$). The latter difference disappeared when insulin action was expressed per kilogram of FFM (49.1 ± 18.6 vs. 49.1 ± 17.1 μmol · min^{-1} · kg^{-1}, women vs. men, NS).

In univariate association (Fig. 1), age explained only 1.1% of the variability in insulin action and was associated with a decrement in insulin action of 0.9 μmol · min^{-1} · kg^{-1} per decade of life (CI = 0.4–1.3, $P = 0.0002$). When plotted by sex and decade of age (Fig. 2), insulin action appeared to be marginally higher up to age 35 than during the subsequent decades, particularly in women. Over the same age span, body weight, BMI, percent fat mass, WHR, serum LDL cholesterol concentrations, and fasting plasma glucose levels increased in men as well as women, in a continuous manner (WHR and plasma glucose in men only) or leveling off after

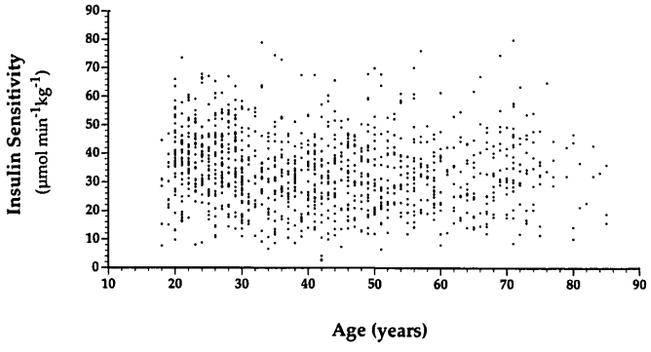

FIG. 1. Scattergram of insulin action versus age in 1,146 healthy subjects.

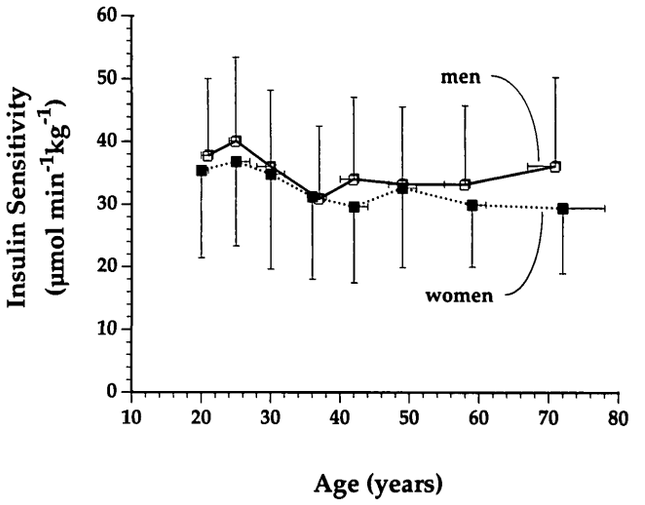

FIG. 2. Insulin action in men and women by decade of age (vertical bars are ± 1 SD).

~50 years of age (body weight, BMI, fat mass, and LDL cholesterol) (Table 3).

The association between insulin action and age was no longer significant ($P = 0.08$) when accounting for BMI. Conversely, body mass was a strong negative correlate of insulin action (5 μmol · min^{-1} · kg^{-1} per 10 kg of body weight, $P < 0.0001$) regardless of age. Segregation by sex and obesity showed a consistent age-related decline in insulin action only in lean women (BMI-adjusted linear rate of 1.6 μmol · min^{-1} · kg^{-1} per decade, CI = 0.6–2.5, $P = 0.001$) (Fig. 3), with no clear evidence of a step decrease in insulin action after age 50 (Fig. 4). In lean women, but not in obese women or in men, percentage fat mass increased with age (by 0.38% of body weight per decade, CI = 0.16–0.60, $P = 0.0003$).

In the subset of subjects in whom fasting FFA levels and suppression of circulating FFA during steady-state euglycemic hyperinsulinemia were measured, the pattern of relationships between age and insulin action was similar to that observed in the whole group (data not shown). In this subgroup, fasting FFAs were consistently higher in women than in men ($P < 0.0001$) and rose significantly ($P < 0.0001$) with age in both (by 0.02 mmol/l per decade, CI = 0.008–0.03, $P < 0.001$, adjusted by BMI) (Fig. 5). Insulin suppression of FFA was significantly better in women than in men up to age 35 ($P < 0.01$) but not in later ages. Consequently, insulin suppression of FFA improved with age in men (2% per decade, CI = 0.9–2.9, $P < 0.0001$) but not in women (Fig. 5). In a multivariate model accounting for sex, BMI, age, and fasting FFA levels, a greater extent of FFA suppression by insulin was independently associated with better insulin action (1.5 μmol · min^{-1} · kg^{-1} per 10% increase in FFA suppression, CI = 0.9–2.3, $P < 0.0001$).

We tested the possibility that the decline in insulin action with age observed in lean women might be due to their reduced ability to inhibit lipolysis. In 92 lean women (aged 18–80 years) in whom FFA data were available, insulin action decreased with age at a rate similar to that of the whole subgroup (1.9 μmol · min^{-1} · kg^{-1} per decade, CI = 0.6–3.2, $P < 0.03$, adjusted by BMI). When added to the multiple regression equation, FFA suppression was independently associated with insulin action ($P < 0.03$) and age was no longer a significant correlate.

TABLE 3
Relevant variables by age intervals

	Age octile								*P* value	
	1	2	3	4	5	6	7	8	Age	Sex
n										
M	75	101	100	76	83	115	112	102	—	—
W	40	55	54	53	53	43	38	44		
Age (years)										
M	21 ± 1	25 ± 1	30 ± 1	37 ± 1	42 ± 2	49 ± 2	58 ± 3	71 ± 4	—	—
W	20 ± 1	25 ± 2	30 ± 2	36 ± 2	42 ± 2	49 ± 2	59 ± 2	72 ± 6		
Weight (kg)										
M	76 ± 13	76 ± 12	81 ± 17	82 ± 15	83 ± 16	81 ± 11	78 ± 11	77 ± 12	<0.001	<0.001
W	71 ± 15	67 ± 14	70 ± 15	74 ± 20	74 ± 20	76 ± 19	72 ± 19	65 ± 10		
BMI (kg/m^2)										
M	24.0 ± 3.4	23.8 ± 3.2	25.3 ± 4.8	27.0 ± 4.8	27.4 ± 5.6	26.4 ± 3.6	25.9 ± 3.3	25.9 ± 3.5	<0.001	NS
W	24.6 ± 5.5	23.2 ± 4.2	24.2 ± 4.4	26.6 ± 4.0	27.7 ± 7.2	28.0 ± 7.2	27.3 ± 4.1	25.2 ± 4.0		
Fat mass (%)										
M	27 ± 5	27 ± 5	29 ± 6	30 ± 6	31 ± 6	30 ± 5	29 ± 4	29 ± 5	<0.001	<0.001
W	33 ± 6	32 ± 5	33 ± 5	35 ± 6	36 ± 6	37 ± 6	36 ± 4	34 ± 4		
Fasting plasma glucose (mmol/l)										
M	5.01 ± 0.48	4.94 ± 0.52	5.02 ± 0.39	5.08 ± 0.43	5.19 ± 0.47	5.13 ± 0.62	5.17 ± 0.49	5.19 ± 0.61	NS	<0.001
W	4.95 ± 0.54	4.95 ± 0.32	4.99 ± 0.44	5.03 ± 0.47	4.95 ± 0.55	5.00 ± 0.54	4.98 ± 0.48	4.95 ± 0.41		
Steady-state plasma glucose (mmol/l)										
M	4.98 ± 0.38	4.98 ± 0.42	5.01 ± 0.37	5.08 ± 0.51	5.12 ± 0.48	5.08 ± 0.50	5.05 ± 0.43	5.14 ± 0.52	<0.05	NS
W	4.98 ± 0.28	5.05 ± 0.56	4.99 ± 0.54	4.93 ± 0.53	4.95 ± 0.50	4.95 ± 0.43	5.02 ± 0.52	5.23 ± 0.46		
Fasting plasma insulin (pmol/l)										
M	55 ± 25	53 ± 30	69 ± 60	76 ± 42	74 ± 57	84 ± 62	75 ± 46	65 ± 29	<0.001	NS
W	62 ± 47	55 ± 31	58 ± 41	81 ± 45	86 ± 58	89 ± 51	79 ± 64	65 ± 28		
Steady-state plasma insulin (pmol/l)										
M	510 ± 126	479 ± 123	462 ± 130	476 ± 150	489 ± 131	529 ± 150	533 ± 160	515 ± 121	<0.001	NS
W	512 ± 160	425 ± 82	450 ± 132	483 ± 184	496 ± 147	502 ± 152	539 ± 128	562 ± 107		
WHR										
M	0.84 ± 0.07 (27)	0.86 ± 0.04 (36)	0.88 ± 0.06 (43)	0.85 ± 0.09 (24)	0.92 ± 0.07 (43)	0.93 ± 0.09 (50)	0.92 ± 0.07 (62)	0.94 ± 0.07 (87)	<0.001	<0.001
W	0.78 ± 0.04 (11)	0.77 ± 0.08 (17)	0.83 ± 0.10 (21)	0.85 ± 0.08 (24)	0.86 ± 0.11 (17)	0.86 ± 0.10 (21)	0.87 ± 0.09 (16)	0.90 ± 0.06 (29)		
LDL cholesterol (mmol/l)										
M	2.9 ± 0.8 (26)	3.0 ± 0.8 (32)	3.6 ± 1.4 (47)	3.5 ± 1.4 (32)	4.2 ± 1.2 (52)	4.6 ± 1.3 (77)	4.5 ± 1.3 (75)	4.4 ± 1.0 (77)	<0.001	NS
W	3.5 ± 1.8 (17)	3.0 ± 1.1 (22)	3.0 ± 0.9 (21)	4.1 ± 1.5 (22)	4.1 ± 1.3 (32)	4.3 ± 1.4 (27)	4.8 ± 1.4 (33)	4.7 ± 0.9 (31)		

Data are means ± SD (*n*). M, men; W, women.

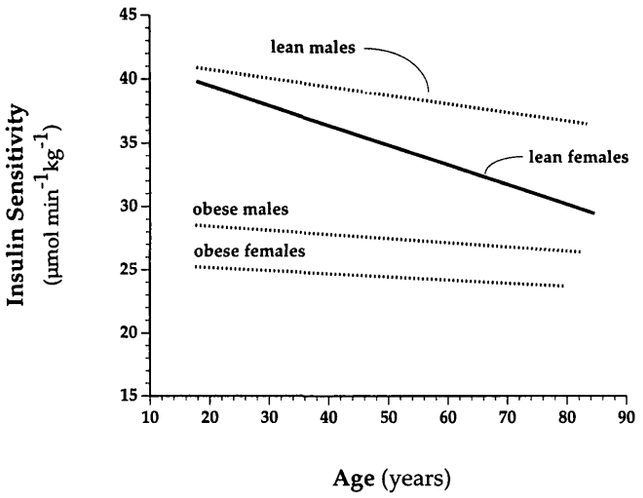

FIG. 3. Regression of insulin action against age in the study population grouped according to sex and obesity (defined as BMI >25 kg/m^2). All regressions are adjusted by BMI and center. Dotted lines indicate regression lines with a slope not significantly different from zero. Lean females, $P < 0.002$.

When the age analysis was carried out by expressing insulin action as whole-body glucose disposal normalized by the FFM (*M*/FFM), the M:I ratio, or the glucose clearance, results were superimposable on those described above. Thus, in the whole group, adjusting by BMI canceled any significant association between *M*/FFM or M:I and age. In lean women, on the other hand, all four indexes of insulin action were negatively related to age, with similar standardized regression coefficients (Table 4).

Using fasting plasma insulin concentration as a surrogate measure of insulin action yielded a weak (1% of explained variance) univariate association with age (an increase of 3 pmol/l per 10 years, CI = 1–5, $P < 0.001$) in the whole group (Table 3). When adjusted by BMI, this association was significant only in lean women (2 pmol/l per decade, CI = 2.0–4.4, $P = 0.03$).

DISCUSSION

The relationship between age and insulin action is confounded by the fact that prevalent diseases, such as diabetes, obesity, and essential hypertension, are all characterized by

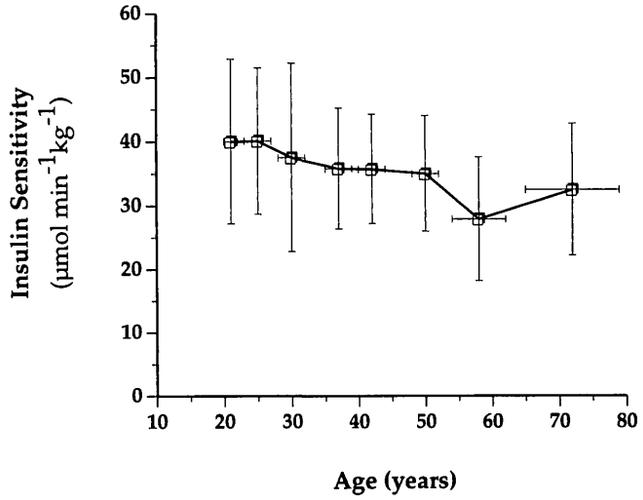

FIG. 4. Insulin action in lean women (n = 219) by octile of age (vertical bars are ± 1 SD).

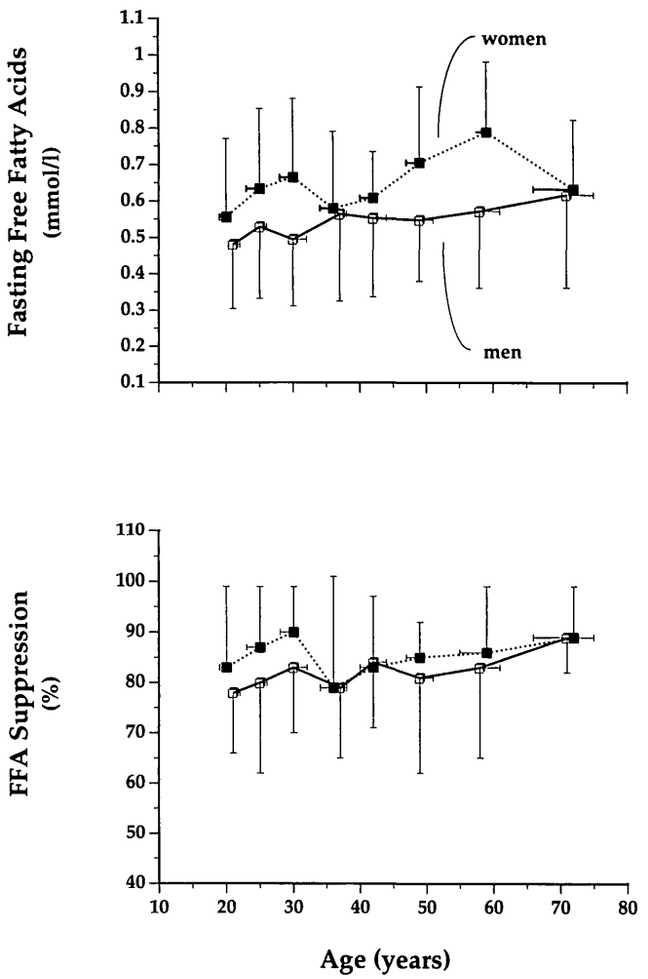

FIG. 5. Fasting FFA concentrations and their percentage suppression by insulin (during the last 40 min of a euglycemic insulin clamp) in men (n = 347) and women (n = 160) by octile of age (vertical bars are ± 1 SD).

age dependence and, at the same time, insulin resistance (37). To further compound the issue, aging is often accompanied by changes in body composition (26), dietary habits (30), and physical activity (38,39), all in a direction that can impair insulin sensitivity. Therefore, to establish whether senescence plays an independent role in the emergence of insulin resistance in the population, studies must have sufficient power to discriminate confounding effects. In our large series of healthy subjects, we found a very weak association between insulin action and age. In the raw data, there was a trend for younger subjects (<35 years) to have higher *M* values than subjects in the older age-groups (Fig. 2). However, when adjusted by a strong determinant of in vivo insulin action, i.e., body mass, the negative association was no longer statistically significant in the whole sample. This was true whether insulin action was expressed in relation to body weight, FFM, or steady-state plasma insulin. The result was also clear when using a common surrogate measure of insulin action, i.e., fasting plasma insulin concentration.

Our study group only included individuals with normal glucose tolerance and arterial blood pressure. Nonetheless, biological parameters characteristically related to age, such as BMI, percentage fat mass, WHR, plasma glucose, and LDL cholesterol levels, did show the expected rise over time (Table 3). In fact, over the age range 25–65 years, the sex-adjusted annual increments in BMI, WHR, and LDL

TABLE 4
Relation of age to indexes of insulin sensitivity in lean women

	M_{bw}	M_{ffm}	M:I	GCR
BMI	−0.103 (ns)	−0.020 (ns)	−0.138 (0.06)	−0.112 (ns)
Age	−0.218 (<0.002)	−0.241 (<0.001)	−0.241 (<0.001)	−0.233 (<0.001)

Data from 219 healthy lean (BMI <25 kg/m^2) women aged 18–80 years. Entries are standardized regression coefficients from a multiple regression model including center. M_{bw}, insulin-stimulated whole-body glucose disposal normalized by kilogram of body weight; M_{ffm}, insulin-stimulated whole-body glucose disposal normalized by kilogram of FFM; M:I, M_{bw} divided by steady-state plasma insulin concentrations; GCR, glucose clearance rate, M_{bw} divided by steady-state plasma glucose.

cholesterol levels were similar to those measured in the San Antonio Heart Study, a population-based survey (0.066 vs. 0.051 kg/m^2 for BMI, 0.002 vs. 0.002 WHR units, and 0.04 vs. 0.02 mmol/l for LDL cholesterol) (10). This confirms that our study group, though not a random sample of the general population, still resembled a general population.

On subanalysis, a definite age-related decline in insulin action was only found in lean women (Fig. 3), in whom fat mass (in proportion to body weight) was higher in older age classes despite a BMI of <25 kg/m^2. In this subgroup, age-related insulin resistance was small (1.6 μmol · min^{-1} · kg^{-1} per decade of life, equivalent to ~3 kg of weight gain) and was not clearly associated with menopause.

It is now well established that body fat distribution plays a role in insulin sensitivity independent of total body adiposity (22,25). In our database, WHR measurements were available in a subgroup of 529 subjects evenly distributed across age-groups (Table 3). Though both the WHR and the waist girth were significantly related to insulin resistance in univariate association as well as after adjustment by age and sex, inclusion of BMI in the regression model canceled the association of both indexes of fat distribution with insulin action. The explanation is suggested in Fig. 6, which shows a very strong association between waist girth and BMI (r = 0.80). Thus, it is likely that WHR and waist circumference carry relatively little information on intra-abdominal fat accumulation over and above what is conveyed by BMI. More precise measures are necessary to bring out an independent effect of abdominal fat on insulin action.

Valuable information was derived from analysis of the FFA data. A prompt and potent action of insulin is to inhibit lipolysis, thereby lowering circulating FFA concentrations. In vivo, the apparent K_m for this effect is substantially lower than that of insulin stimulation of glucose uptake (40). In our whole group, the ability of insulin to suppress plasma FFA was, if anything, better with increasing age, despite rising fasting FFA concentrations (Fig. 5). Thus, insulin action on both lipolysis and glucose uptake is preserved with age.

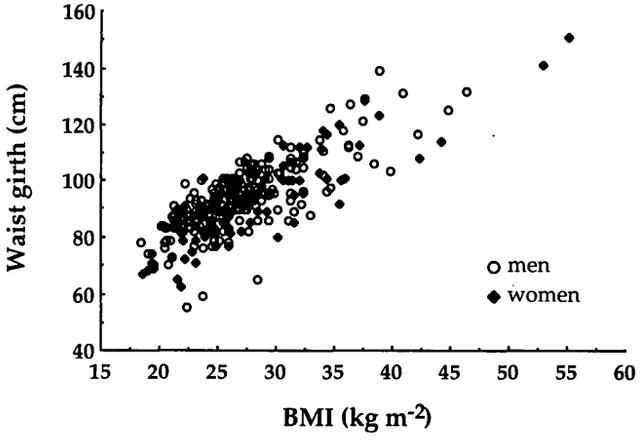

FIG. 6. Scatterplot of waist girth versus BMI in 529 subjects.

In multivariate analysis, insulin suppression of FFA levels made a significant independent contribution to insulin action on glucose uptake. This finding is best explained by the physiological link between the action of insulin on lipolysis and on glucose disposal, i.e., inhibition of lipolysis causing enhanced glucose uptake. In fact, the regression of our data indicated that full suppression of plasma FFA is associated with an increase in glucose uptake of 15 μmol · min^{-1} · kg^{-1}. At the insulin levels achieved in the present clamp studies, 40% of total glucose uptake is glucose oxidation (40). At a circulating concentration of 0.6 mmol/l (i.e., the average fasting level in our group), FFAs are oxidized at a rate of ~2 μmol · min^{-1} · kg^{-1} (28,40). Therefore, under the influence of insulin, roughly 6 μmol · min^{-1} · kg^{-1} (40% of 15) of glucose are oxidized at the cost of sparing 2 μmol · min^{-1} · kg^{-1} of circulating FFA oxidation. This equivalence is strikingly close to the ratio of the caloric equivalent of palmitate (10.0 kJ/μmol) and glucose (2.8 kJ/μmol), implying quantitative replacement of FFA by glucose as fuel for oxidation. Thus, through substrate competition, insulin suppression of plasma FFA contributes significantly to whole-body insulin action at any age and regardless of obesity.

Significantly, in the subgroup of lean women in whom age was independently associated with insulin resistance, accounting for FFA suppression canceled the effect of age on insulin action. This result suggests that the age dependence of insulin action in lean women may be related to impaired inhibition of lipolysis by insulin.

In summary, our data demonstrate that in healthy humans, age per se generally does not carry significant insulin resistance in either glucose metabolism or lipolysis. In all ages, effective inhibition of FFA supply is an important component of insulin action of glucose disposal (Randle's cycle [41]). In lean women, insulin action does decline with advancing age in concomitance with a change in body composition, consisting in the relative accumulation of fat tissue. The likely basis for this sex-specific effect is substrate competition: blunting of insulin inhibition of lipolysis favors FFA over glucose oxidation, thereby decreasing insulin action. Though the current data are cross-sectional, the pattern of age-related changes seen in lean women resonates with the findings from two prospective studies (42,43) showing that in adults insulin resistance predicts low rates of weight gain (42) and that women gain more weight than men under similar circumstances (43). Together, these observations suggest that young insulin-sensitive lean women have enhanced likelihood of developing insulin resistance because of both aging and weight gain.

If the nondiabetic segment of the population maintains

insulin action over time, a rise in the prevalence of insulin resistance with age in the whole population may result, in addition to obesity, from the emergence of glucose intolerance, overt diabetes, and essential hypertension, which are inherently insulin-resistant states with a high incidence rate. This paradigm may be different in ethnic groups other than Europeans, depending on different genetic makeup and lifestyle. With this proviso, we can conclude that, insofar as insulin resistance clusters with cardiovascular risk factors (44) (and may itself be one such factor [45]), age per se does not add to cardiovascular risk via insulin resistance.

ACKNOWLEDGMENTS

On behalf of EGIR, we wish to thank Groupe Lipha in the person of Dr. Christophe Pasik for their generous support of the activities of the group.

REFERENCES

1. Davidson MB: The effect of aging on carbohydrate metabolism: a review of the English literature and a practical approach to the diagnosis of diabetes mellitus in the elderly. *Metabolism* 28:688–705, 1979
2. Chen M, Bergman RN, Pacini G, Porte D: Pathogenesis of age-related glucose intolerance in man: insulin resistance and decreased β-cell function. *J Clin Endocrinol Metab* 60:13–20, 1985
3. Chen M, Bergman RN, Porte D Jr: Insulin resistance and β-cell dysfunction in aging: the importance of dietary carbohydrates. *J Clin Endocrinol Metab* 67:951–957, 1988
4. Jackson RA, Hawa MI, Roshania RD, Sim BM, DiSilvio L, Jaspan LB: Influence of aging on hepatic and peripheral glucose metabolism in humans. *Diabetes* 37:119–129, 1988
5. Palmer JP, Ensinck JW: Acute-phase insulin secretion and glucose tolerance in young and aged normal men and diabetic patients. *J Clin Endocrinol Metab* 41:498–593, 1975
6. Pacini G, Valerio A, Beccaro F, Nosadini R, Cobelli C, Crepaldi G: Insulin sensitivity and β-cell responsivity are not decreased in elderly subjects with normal OGTT. *J Am Geriatr Soc* 36:317–323, 1988
7. Elahi D, Andersen DK, Muller DC, Tobin JD, Brown JC, Andres R: The enteric enhancement of glucose-stimulated insulin release: the role of GIP in aging, obesity, and non-insulin-dependent diabetes mellitus. *Diabetes* 33:950–957, 1984
8. Jackson RA: Mechanisms of age-related glucose intolerance. *Diabetes Care* 13 (Suppl. 2):9–19, 1990
9. Coon PJ, Bleecker ER, Drinkwater DT, Meyers DA, Goldberg AP: Effects of body composition and exercise capacity on glucose tolerance, insulin, and lipoprotein lipids in healthy older men: a cross sectional and longitudinal intervention study. *Metabolism* 38:1201–1209, 1989
10. Ferrannini E, Haffner SM, Mitchell BD, Stern MP: Hyperinsulinemia: a key feature of a cardiovascular and metabolic syndrome. *Diabetologia* 34:416–422, 1991
11. Kimmerling G, Javorski WC, Reaven GM: Aging and insulin resistance in a group of nonobese male volunteers. *J Am Geriatr Soc* 8:349–353, 1977
12. Kalant N, Leibovici D, Leibovici T, Fukushima N: Effect of age on glucose utilization and responsiveness to insulin in forearm muscle. *J Am Geriatr Soc* 7:304–307, 1980
13. DeFronzo RA: Glucose intolerance and aging: evidence for tissue insensitivity to insulin. *Diabetes* 28:1095–1101, 1979
14. Robert JJ, Cummins JC, Wolfe RR, Durkot M, Matthews DE, Zhao XH, Bier DM, Young VR: Quantitative aspects of glucose production and metabolism in healthy elderly subjects. *Diabetes* 31:203–211, 1982
15. Jackson RA, Blix PM, Matthew JA, Hamling JB, Din BM, Brown DC, Belin J, Rubenstein AH, Nabarro JDN: Influence of ageing on glucose homeostasis. *J Clin Endocrinol* 55:840–848, 1982
16. Rowe JW, Minaker KL, Pallotta JA, Flier JS: Characterization of the insulin resistance of aging. *J Clin Invest* 71:1581–1587, 1983
17. Fink RI, Kolterman OG, Griffin J, Olefsky JM: Mechanisms of insulin resistance in aging. *J Clin Invest* 71:1523–1535, 1983
18. Broughton DL, Alberti KGMM, James OFW, Taylor R: Peripheral insulin sensitivity in healthy elderly subjects. *Gerontology* 33:357–362, 1987
19. Fink RI, Wallace P, Olefsky JM: Effects of aging on glucose-mediated glucose disposal and glucose transport. *J Clin Invest* 77:2034–2041, 1986
20. Broughton DL, James OFW, Alberti KGMM, Taylor R: Peripheral and hepatic insulin sensitivity in healthy elderly human subjects. *Eur J Clin Invest* 21:13–21, 1990
21. Khan SE, Larson WG, Schwartz RS, Beard JC, Cain KC, Fellingham GW, Stratton JR, Cerqueira MD, Abrass IB: Excercise training delineates the importance of β-cell disfunction to the glucose intolerance of human aging. *J Clin Endocrinol Metab* 74:1336–1342, 1992
22. Coon PJ, Rogus EM, Drinkwater D, Muller DC, Goldberg AP: Role of body fat distribution in the decline in insulin sensitivity and glucose tolerance with age. *J Clin Endocrinol Metab* 75:1125–1132, 1992
23. O'Shaughnessy IM, Kasdorf GM, Hoffman RG, Kalkhoff RK: Does aging intensify the insulin resistance of human obesity? *J Clin Endocrinol Metab* 74:1075–1081, 1992
24. Franssila-Kallunki A, Schalin-Jäntti C, Groop L: Effect of gender on insulin resistance associated with aging. *Am J Physiol* 263:E780–E785, 1992
25. Kohrt WM, Kirwan JP, Staten MA, Bourey RE, King DS, Holloszy JO: Insulin resistance in aging is related to abdominal obesity. *Diabetes* 42:273–281, 1993
26. Boden G, Chen X, DeSantis RA, Kendrick Z: Effects of age and body fat on insulin resistance in healthy men. *Diabetes Care* 16:728–733, 1993
27. Elhai D, Muller DC, McAloon-Dyke M, Tobin JD, Andres R: The effect of age on insulin response and glucose utilization during four hyperglycemic plateaus. *Exp Gerontol* 28:393–409, 1993
28. Bonadonna RC, Groop LC, Simonson DC, DeFronzo RA: Free fatty acid and glucose metabolism in human aging: evidence for operation of the Randle cycle. *Am J Physiol* 266:E501–E509, 1994
29. Reed MJ, Reaven GM, Mondon CE, Azhar S: Why does insulin resistance develop during maturation? *J Gerontol* 48:B139–B144, 1993
30. Ivy JL, Young JC, Craig BW, Kohrt WM, Holloszy JO: Ageing, exercise and food restriction: effects on skeletal muscle glucose uptake. *Mech Ageing Dev* 61:123–133, 1991
31. World Health Organization: *WHO Expert Committee on Diabetes Mellitus. Second Report.* Geneva, World Health Org., 1980 (Tech. Rep. Ser. no. 646)
32. DeFronzo RA, Tobin JD, Andres R: Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Am J Physiol* 237:E214–E223, 1979
33. De Long DM, De Long ER, Wood PD, Lippel K, Rifkind BM: A comparison of methods for the estimation of low and very low density lipoprotein cholesterol: the Lipid Research Clinic Prevalence study. *JAMA* 256:2372–2377, 1986
34. Ferrannini E, Smith JD, Cobelli C, Toffolo G, Pilo A, DeFronzo RA: Effect of insulin on the distribution and disposal of glucose in man. *J Clin Invest* 76:367–374, 1985
35. Sherwin RS, Kramer KJ, Tobin JD, Insel PA, Liljenquist JE, Berman M, Andres R: A model of the kinetics of insulin in man. *J Clin Invest* 53:1481–1492, 1974
36. Hume R: Prediction of lean body mass from height and weight. *J Clin Pathol* 19:389–391, 1966
37. DeFronzo RA, Ferrannini E: Insulin resistance: a multifaceted syndrome responsible for NIDDM, obesity, hypertension, dyslipidemia, and atherosclerotic cardiovascular disease. *Diabetes Care* 14:173–194, 1991
38. Kohrt WM, Malley MT, Dalsky GP, Holloszy JO: Body composition of healthy sedentary and trained, young and older men and women. *Med Sci Sports Exerc* 24:832–837, 1992
39. Rosenthal M, Haskel WL, Solomon R, Widstrom A, Reaven GM: Demonstration of a relationship between level of physical training and insulin-stimulated glucose utilization in normal humans. *Diabetes* 32:408–411, 1983
40. Bonadonna RC, Groop LC, Zych K, Shank M, DeFronzo RA: Dose-dependent effect of insulin on plasma free fatty acid turnover and oxidation in humans. *Am J Physiol* 259:E736–E750, 1990
41. Randle PJ, Garland PB, Hales CN, Newsholme EA: The glucose fatty acid cycle: its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus. *Lancet* i:785–789, 1963
42. Swinburn BA, Nyomba BL, Saad MF, Zurlo F, Raz I, Knowler WC, Lillioja S, Bogadrus C, Ravussin E: Insulin resistance associated with lower rates of weight gain in Pima Indians. *J Clin Invest* 88:168–173, 1991
43. Valdez R, Mitchell BD, Haffner SM, Hazuda HP, Morales PA, Monterrosa A, Stern MP: Predictors of weight change in a bi-ethnic population: the San Antonio Heart Study. *Int J Obes* 18:85–91, 1994
44. Ferrannini E, Stern MP: Primary insulin resistance: a risk syndrome. In *Diabetes: Clinical Science in Practice.* Leslie RDG, Robbins DC, Eds. Cambridge, Cambridge University Press, 1995, p. 200–220
45. Pyörälä K, Savolainen E, Kaukola S, Haapakoski J: Plasma insulin as coronary heart disease risk factor: relationship to other risk factors and predictive value during 91/2 year follow-up of the Helsinki Policemen Study population. *Acta Med Scand* (Suppl. 1)701:38–52, 1985