Relationships Among Age, Proinsulin Conversion, and β-Cell Function in Nondiabetic Humans

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The aim of the present study was to examine the relationships among β-cell function, proinsulin conversion to insulin, and age. We studied insulin and proinsulin secretion in nondiabetic subjects during an oral glucose tolerance test (OGTT) using published indexes of β-cell function (n = 379, age 16–68 years) and a modified hyperglycemic clamp (10 mmol/l, additional glucagon-like peptide [GLP-1] infusion, final arginine bolus; n = 50, age 19–68 years). Proinsulin conversion to insulin was assessed using proinsulin/insulin (PI/I) ratios immediately after an acute stimulus (OGTT, 30 min; hyperglycemic clamp, 2.5–5.0 min after glucose and arginine). There was a negative correlation between age and β-cell function (adjusted for insulin sensitivity, BMI, and fasting glucose) in the OGTT (r = −0.21, P < 0.001) and first phase of the hyperglycemic clamp (r = −0.30, P = 0.03), but not second phase (r = −0.08, P = 0.6) or arginine-induced insulin secretion (r = 0.06, P = 0.7). There was a positive correlation between age and the PI/I ratio in the OGTT (r = 0.24, P < 0.001). Analogously, there was also a positive correlation between age and the PI/I ratio during first phase (r = 0.37, P = 0.009) and arginine stimulation (r = 0.33, P = 0.01) of the hyperglycemic clamp. First-phase insulin secretion of the hyperglycemic clamp was inversely correlated with the PI/I ratio (r = −0.60, P < 0.001). Interestingly, adjusting first-phase secretion rate for the PI/I ratio abolished the linear relationship with age (r = −0.06, P = 0.7). In conclusion, aging is associated with deteriorating β-cell function and deteriorating proinsulin conversion to insulin. The age effect on insulin secretion appears to be attributable at least in part to an impairment of proinsulin conversion to insulin. *Diabetes* 51 (Suppl. 1):S234–S239, 2002

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AUC_I/AUC_G, ratio of the insulin over glucose area under the curve; GLP-1, glucagon-like peptide; HOMA, homeostatic model assessment; IGT, impaired glucose tolerant; ISR, insulin secretion rate; IVGTT, intravenous glucose tolerance test; NGT, normal glucose tolerant; OGTT, oral glucose tolerance test; PI/I, proportion of proinsulin to insulin.

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A deterioration in β-cell function is an independent predictor for the development of type 2 diabetes (1). A negative impact on β-cell function by aging was shown in many (2–8) though not all studies (9,10). This impact was similar in diabetic and nondiabetic subjects. The mechanism underlying this age-related deterioration of β-cell function is not known.

One important aspect of β-cell function is the conversion of proinsulin to insulin. The relative proportion of proinsulin to insulin (PI/I) in the secretory granule represents an estimate for the efficiency of the conversion of proinsulin to insulin (proinsulin processing). In vivo, the ratio of circulating PI/I has been used to make inferences on proinsulin processing. However, the clearance rates of proinsulin and insulin are substantially different (11). Therefore, the PI/I ratio in plasma provides an accurate estimate for the PI/I ratio in the secretory granule only after acute stimulation of insulin secretion (12–16), when differences in elimination kinetics have negligible influence on concentrations. An elevated PI/I ratio has been observed in conditions with impaired β-cell function, such as type 2 diabetes (13,17,18) and impaired glucose tolerance (15,19,20).

Only two studies have assessed age effects on proinsulin processing (6,7). In these, no effect of aging was observed, but proinsulin and PI/I ratios were not assessed following an acute stimulus. The purpose of the present studies was to assess the influence of age on β-cell function and proinsulin processing. We therefore examined the relationships between insulin and proinsulin secretion in 389 nondiabetic subjects (age 16–68 years) during an oral glucose tolerance test (OGTT). β-Cell function was estimated using published indexes (21) and the PI/I ratio at 30 min (i.e., time point of most acute secretory response) was considered to provide the best estimate for proinsulin conversion to insulin during the OGTT. In addition, a subset of 50 subjects (age 19–68 years) underwent a square-wave hyperglycemic clamp. β-Cell function was directly measured as first and second phases of insulin secretion and maximal stimulation after glucagon-like peptide (GLP-1) and arginine. Proinsulin conversion to insulin was assessed as PI/I ratio immediately (2.5–5.0 min) after the glucose and arginine bolus of the hyperglycemic clamp.

Glucose, insulin, proinsulin, and the PI/I ratio during the OGTT are shown Fig. 1. In the 389 subjects undergoing the OGTT, age was negatively correlated with β-cell function

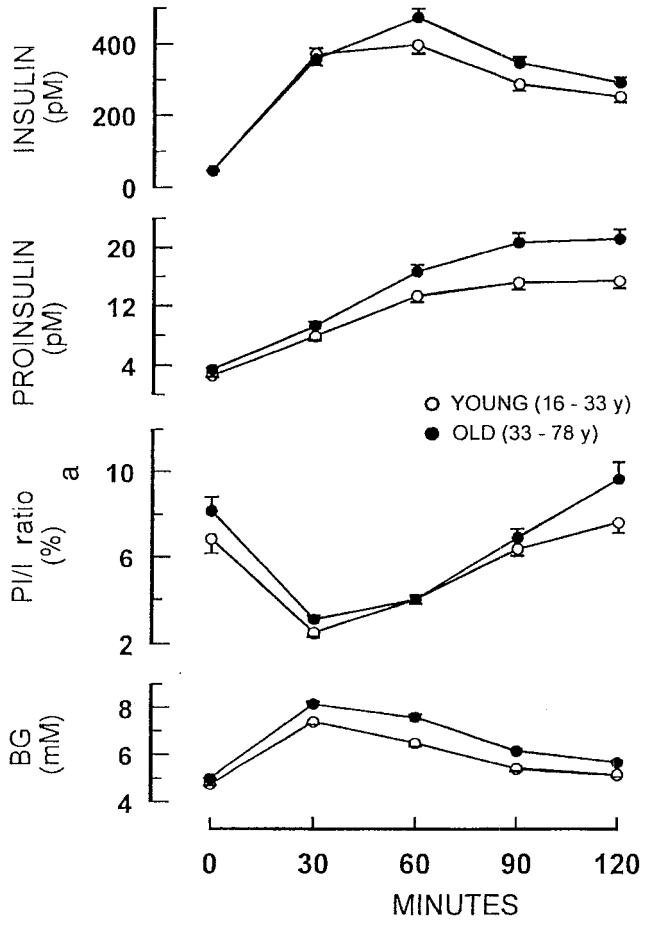

FIG. 1. Insulin and proinsulin concentrations, PI/I ratios in plasma and blood glucose (BG) concentrations during a 75-g oral glucose tolerance test in young and old subjects.

after adjusting for insulin sensitivity. Age was positively correlated with the PI/I ratio at 30 min, indicating a progressive derioration of proinsulin processing with aging (Fig. 2).

This suggestive observation from the OGTT, a rather crude test for β-cell function, was reproduced in the modified hyperglycemic clamp studies in 50 subjects. Figure 3 shows insulin secretion rates, plasma insulin and proinsulin concentrations, the plasma PI/I ratio, and blood glucose concentration during the hyperglycemic clamp. The sharp decline of the PI/I ratio following the glucose prime and the arginine bolus reflects the acuity of these stimuli, supporting the concept that the circulating PI/I ratio at these time points represents the best estimate for the granular PI/I ratio and thus proinsulin conversion to insulin. Similar considerations, albeit less clear-cut, hold true for the 30-min time point of the OGTT. In analogy to the OGTT, age was negatively correlated with first-phase insulin secretion and positively with the PI/I ratio at 2.5–5.0 min (Fig. 4*A* and *B*). Age was also correlated with the PI/I ratio ($r = 0.33$, $P = 0.01$) but not insulin secretion following arginine ($r = 0.06$, $P = 0.67$).

Figure 4*C* shows a strong hyperbolic relationship between the first-phase insulin secretion and the acute PI/I ratio. Interestingly, adjusting the first phase for the influence of the PI/I ratio along this hyperbola resulted in disappearance of the age dependency of the first phase (Fig. 4*D*). Figure 5 illustrates the situation for the two arbitrarily generated age groups. This mathematical relationship suggests that the age-dependent deterioration of proinsulin processing is, at least in part ($r^2 = 0.36$; i.e., 36%), responsible for the age effect on β-cell function.

Age was negatively correlated with insulin sensitivity (ISI, estimated from the OGTT, $r = -0.16$, $P = 0.001$) and positively with glucose tolerance (plasma glucose at 120 min of the OGTT). Upon stepwise multiple linear regression, however, ISI and age were independently correlated with glucose tolerance (multiple $r = 0.47$), while inclusion of a β-cell function parameter (estimated first phase) as an independent variable eliminated age as a determinant of glucose tolerance (multiple $r = 0.52$). This indicates that the age effect on glucose tolerance is related to its effect on insulin secretion, but not on insulin sensitivity.

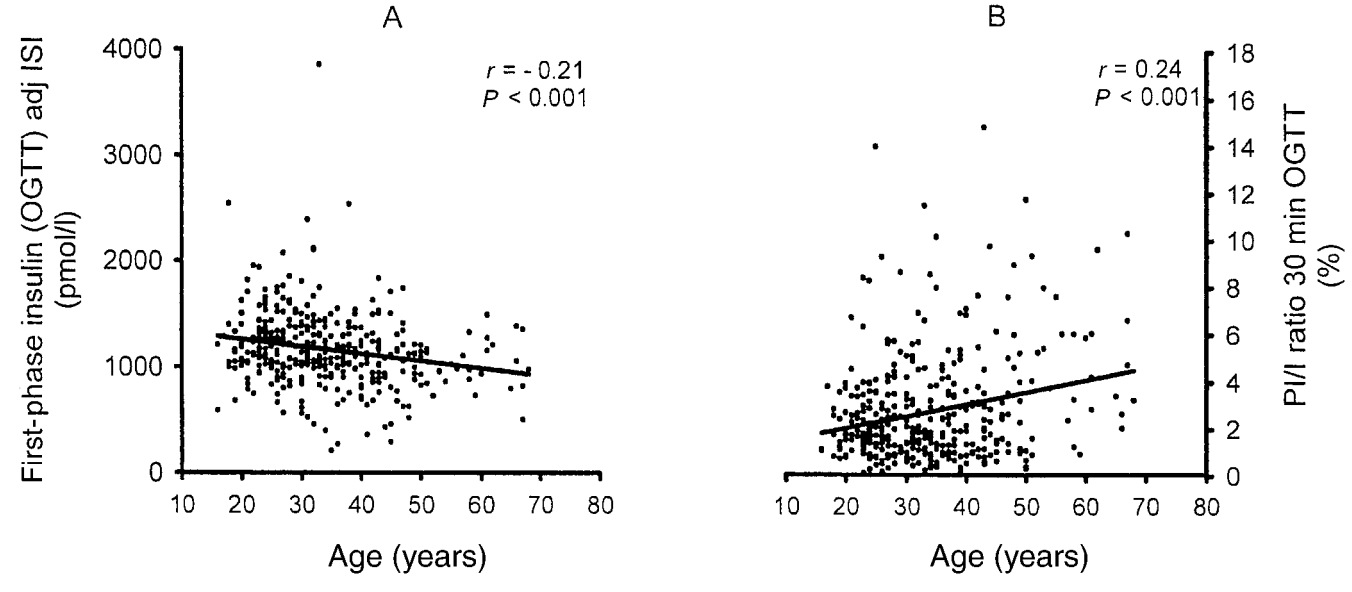

FIG. 2. Age dependency of first-phase insulin secretion (estimated from the OGTT [22]) and PI/I ratio (determined at 30 min of the OGTT) in 379 subjects with normal glucose tolerance. First-phase insulin secretion was adjusted for insulin sensitivity, BMI, and fasting blood glucose concentration.

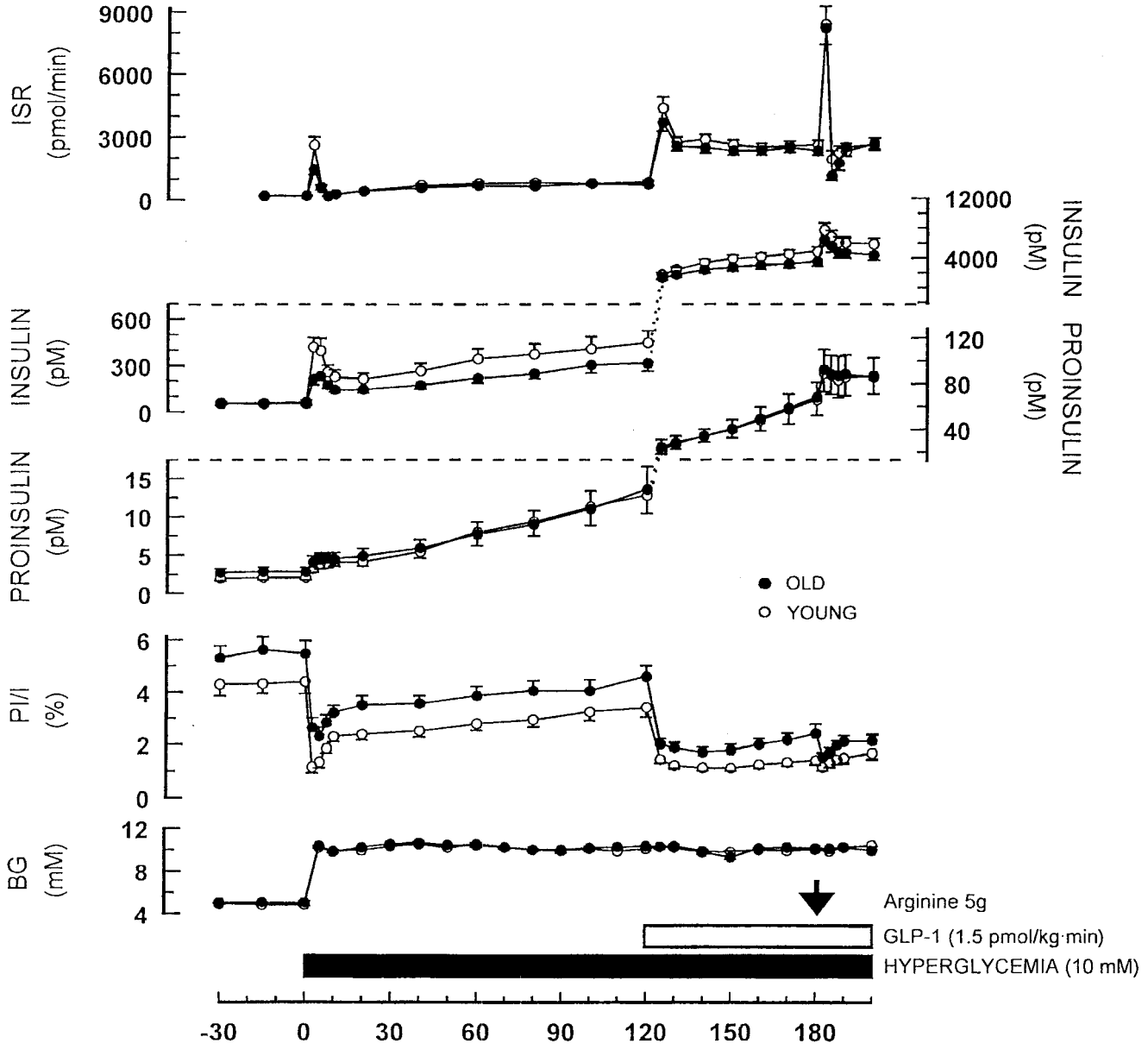

FIG. 3. Insulin secretion rate, insulin and proinsulin concentrations, proinsulin/insulin (PI/I) ratio in plasma, and blood glucose (BG) concentration during the hyperglycemic clamp (10 mmol/l) in old versus young nondiabetic subjects.

Our results from the OGTT and the hyperglycemic clamp confirm previous reports from studies using homeostatic model assessment (HOMA) and intravenous glucose tolerance test (IVGTT) (2–8), showing that age results in a deterioration of β-cell function independent of possible confounders, such as insulin sensitivity, fasting glucose, or obesity. While overall the evidence from the literature is overwhelmingly uniform regarding the effect of age on β-cell function, analogous data regarding insulin sensitivity are less consistent. The most powerful study (European Group of Insulin Resistance [EGIR]) found no effect of age on insulin sensitivity after adjustment for BMI in 1,146 subjects (22). Smaller studies that reported an age-dependent decline in insulin sensitivity had not corrected for obesity or fat distribution. It thus appears that age effects on insulin sensitivity, in contrast to β-cell function, are not independent but mediated by age effects on body composition.

An increased PI/I ratio similar to ours was previously reported in a small group of elderly subjects undergoing an OGTT (7). Basal PI/I ratios on the other hand were not found to be different in older compared with younger glucose-tolerant subjects (6), suggesting no age effect on proinsulin processing. We only found a difference in basal PI/I ratios in the larger OGTT cohort, but not in the hyperglycemic clamp cohort. However, as mentioned above, basal PI/I ratios are not as meaningful as post-stimulus PI/I ratios with respect to making inferences on intravesicular ratios. Therefore, our data are the first to describe an age effect on PI/I ratio following an acute stimulus and thus proinsulin processing. The disappearance of the linear relationship between age and first-phase insulin secretion upon adjusting for the PI/I ratio provides preliminary evidence that the well-established age-dependent deterioration of β-cell function may, at least in part, be secondary to impaired proinsulin processing.

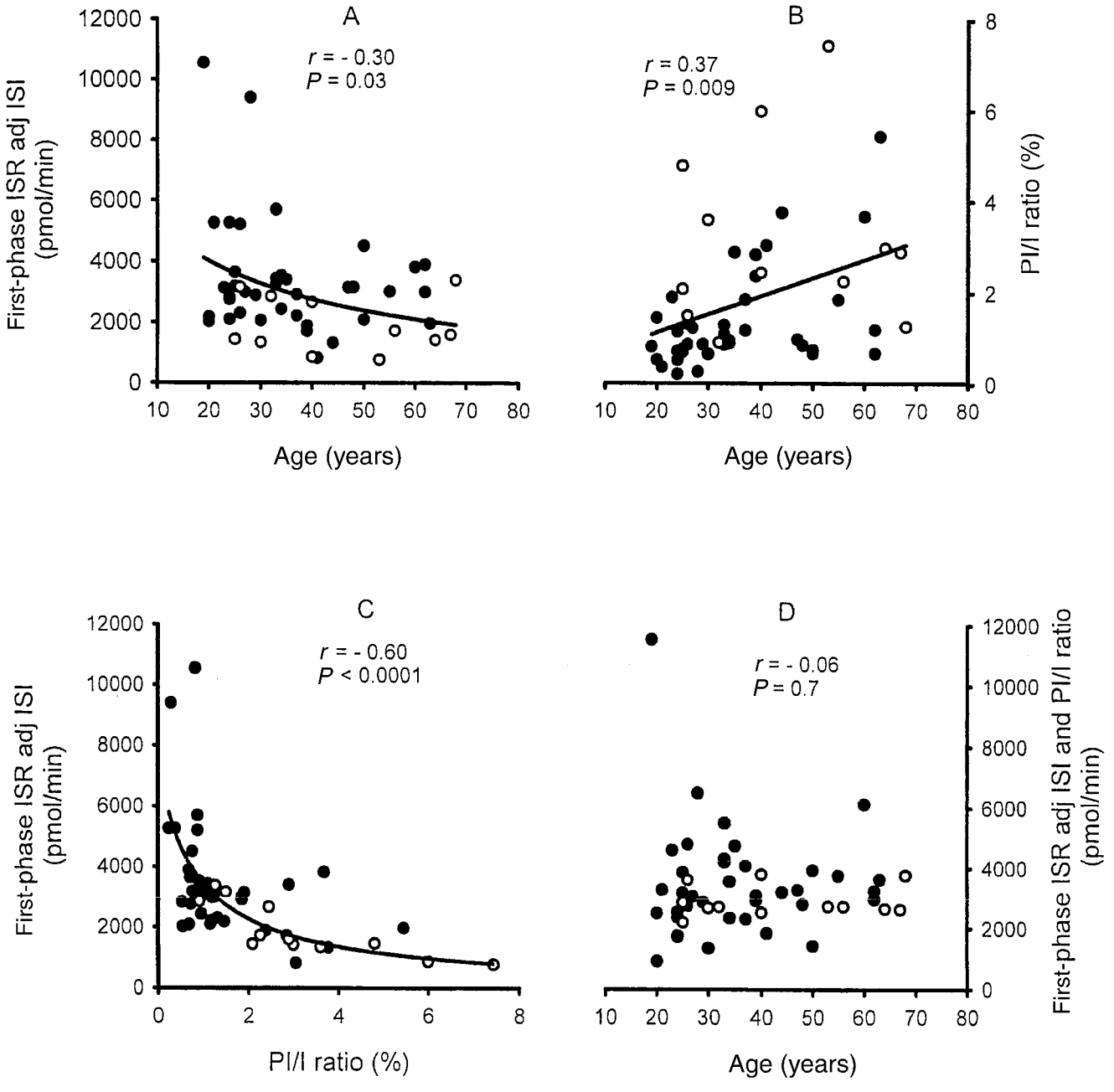

FIG. 4. Relationships between age, insulin secretion, and proinsulin processing from the hyperglycemic clamp. *A*: Age-dependency of first-phase insulin secretion rate (ISR). *B*: Age-dependency of PI/I ratio (determined acutely [2.5–5.0 min] after the glucose prime). *C*: Hyperbolic relationship between insulin secretion and proinsulin processing (PI/I ratio). *D*: Loss of the age dependency of first-phase insulin secretion (compare panel *A*) upon adjusting for PI/I ratio. ○, IGT; ●, NGT.

In conclusion, aging is associated with deteriorating β-cell function and deteriorating proinsulin conversion to insulin. The age of effect on insulin secretion appears to be highly attributable to an impairment of proinsulin conversion to insulin. This does not preclude an age effect on other aspects of β-cell function.

EXPERIMENTAL DESIGN AND METHODS

Subjects. The subjects were recruited from an ongoing family study (characteristics listed in Table 1). The protocol was approved by the ethics committee of the University of Tübingen. Before the study, informed written consent was obtained from the participants.

OGTT. A 75-g OGTT was performed with determination of glucose, insulin, and proinsulin at 0, 30, 60, 90, and 120 min. Subjects were classified as normal glucose tolerant (NGT) or impaired glucose tolerant (IGT) according to World Health Organization criteria (23).

Hyperglycemic clamp. After an overnight fast, at around 8.00 A.M., a hand vein was cannulated retrogradely and kept in a thermoregulated box at 55°C to obtain arterialized blood samples. At the same time, an antecubital vein was cannulated for infusions. After baseline samples had been obtained, a hyperglycemic clamp was performed for 120 min. An intravenous bolus of 20% glucose over 1 min was given to instantaneously raise blood glucose to 10 mmol/l [bolus dose (mg) = body weight (kg) × desired increase in blood glucose (mg/dl) × 1.5]. Subsequently, a glucose infusion was adjusted to maintain blood glucose at 10 mmol/l according to the glucose determined every 5 min. Samples for C-peptide (Byk-Sangtec, Dietzenbach, Germany), insulin (Microparticle Enzyme Immunoassay; Abbott, Laboratories, Tokyo, Japan; CV 2.5–6%), and proinsulin (enzyme immunoassay; IBL, Hamburg, Germany) determination were taken at 30, −15, 0, 2.5, 5, 7.5, 10, 20, 40, 60, 80, 100, and 120 min. The proinsulin assay has 0% cross-reactivity with human

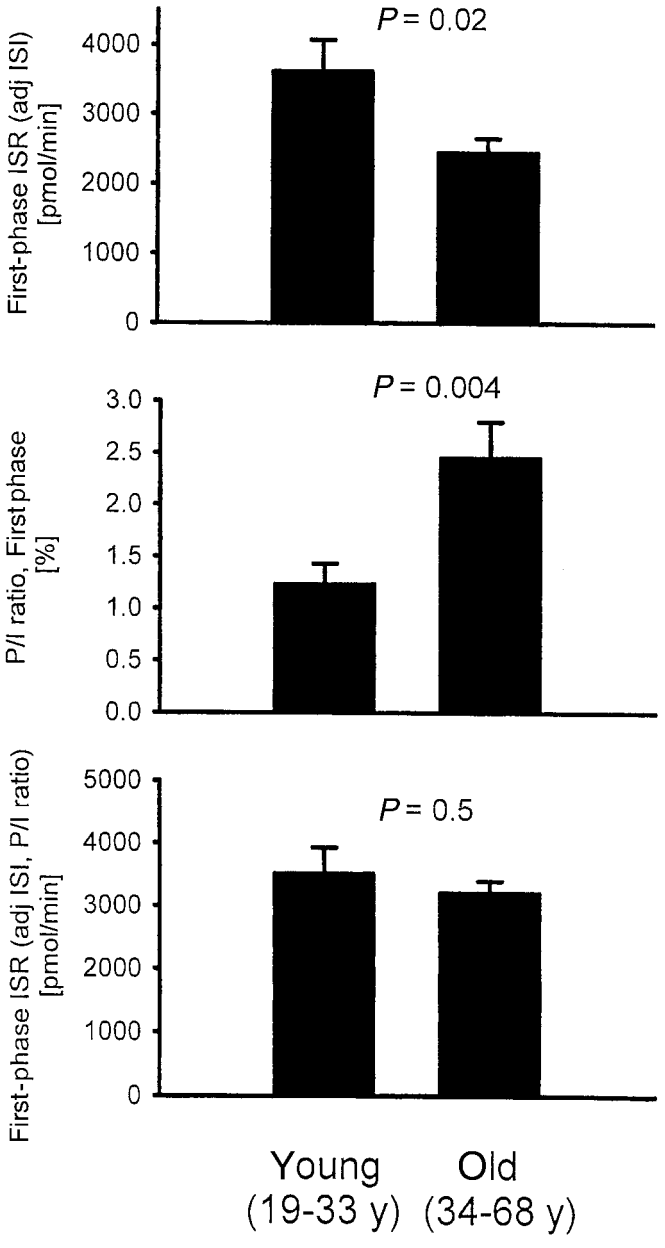

FIG. 5. Direct comparison of insulin secretion and proinsulin processing (PI/I ratio) in young versus old subjects. Note that the difference in first-phase insulin secretion disappears upon adjusting for proinsulin processing (PI/I ratio).

insulin and C-peptide. The insulin assay has 0% cross-reactivity with proinsulin.

Calculations. An estimate for first-phase insulin secretion calculated as $1,283 + 1.829 \cdot Ins_{30} - 138.7 \cdot Gluc_{30} + 3.772 \cdot Ins_0$ and the ratio of the insulin over glucose area under the curve (AUC_I/AUC_G) was used to assess β-cell function from the OGTT (21). The PI/I ratio was calculated at 30 min.

During the hyperglycemic clamp, insulin secretion rates (ISRs) were calculated by deconvolution from C-peptide concentrations using standard kinetic parameters from the literature adjusted for age, sex, BMI, and body surface area as previously described (24,25). Phases of insulin secretion were calculated as previously described (26). The PI/I ratio was calculated as the mean proinsulin concentration divided by the mean insulin concentration at 2.5–5.0 min. Insulin sensitivity was assessed as an insulin sensitivity index, calculated by dividing the average glucose infusion rate during the last 40 min of the hyperglycemic clamp by the average plasma insulin concentration during the same interval (27). Insulin sensitivity from the OGTT was estimated using the index proposed by Matsuda et al. (28).

Statistical analysis. Unless otherwise specified, data are given as means ± SE. Linear or nonlinear correlations were calculated by least-square regression analysis. For direct comparison, subjects were abitrarily divided into a "young" and an "old" group by the median (33 years). Insulin secretion parameters estimated from the OGTT were adjusted for insulin sensitivity, BMI, and fasting blood glucose concentration. For statistical comparison between two groups, a two-tailed Student's *t* test or Wilcoxon test was used as appropriate. A P value of <0.05 was considered to be statistically significant.

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TABLE 1
Subjects characteristics

	Hyperglycemic clamp			OGTT		
	Young	Old	*P*	Young	Old	*P*
Male/female (*n*)	15/10	13/12	0.6*	85/105	62/127	0.02*
NGT/IGT (*n*)	20/5	16/9	0.2*	190/0	189/0	1.0
Age (years)†	26 ± 4	49 ± 11	<0.001	26 ± 4	42 ± 8	<0.001
BMI (kg/m^2)†	25.2 ± 7.4	26.0 ± 3.9	0.8	24.8 ± 5.7	26.3 ± 5.2	0.007
Fasting glucose (mmol/l)	4.8 ± 0.1	5.1 ± 0.1	0.3	4.7 ± 0.04	5.0 ± 0.04	<0.001
Glucose 120 min (OGTT)	5.7 ± 0.4	6.9 ± 0.4	0.05	5.1 ± 0.1	5.6 ± 0.1	<0.001
Fasting insulin (pmol/l)	54 ± 9	50 ± 6	0.7	46 ± 2	47 ± 2	0.06
Fasting proinsulin (pmol/l)	2.0 ± 0.3	2.8 ± 0.6	0.3	2.5 ± 0.2	3.4 ± 0.002	0.006
Fasting PI/I ratio (%)	4.3 ± 0.4	5.5 ± 0.5	0.1	6.9 ± 0.6	8.2 ± 0.7	0.2
ISI ($\mu mol \cdot kg^{-1} \cdot min^{-1} \cdot pmol/l^{-1}$)	0.16 ± 0.2	0.15 ± 0.2	0.7	24 ± 1‡	20 ± 1‡	0.001

*By χ^2 test; †mean ± SD; ‡estimated from glucose and insulin values during the OGTT using the Matsuda index (28).

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