

Effect of Aging on Glucose Homeostasis

Accelerated deterioration of β -cell function in individuals with impaired glucose tolerance

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OBJECTIVE — To examine the effect of aging on insulin secretion (first- and second-phase insulin release) and insulin sensitivity in people with normal glucose tolerance (NGT) or impaired glucose tolerance (IGT).

RESEARCH DESIGN AND METHODS — First- and second-phase insulin secretion and insulin sensitivity were assessed in hyperglycemic clamp experiments in 266 individuals with NGT and 130 individuals with IGT, ranging in age from ~20 to ~70 years. Changes in β -cell function were compared using the disposition index to adjust for differences in insulin sensitivity.

RESULTS — As expected, both phases of insulin release and insulin sensitivity were reduced in individuals with IGT (all $P < 0.01$). Insulin sensitivity was not independently correlated with age in either group. In people with NGT, the disposition index for first- and second-phase insulin release decreased similarly at a rate of ~0.7% per year. In people with IGT, the disposition indexes for first- and second-phase insulin release decreased at greater rates (~2.2 and 1.4% per year, $P = 0.002$ and 0.009 , respectively, vs. NGT), with the decrease in first phase being greater than that of second phase ($P = 0.025$).

CONCLUSIONS — Insulin secretion (both first and second phase) normally decreases at a rate of ~0.7% per year with aging; this decrease in β -cell function is accelerated about two-fold in people with impaired glucose tolerance—first phase to a greater extent than second phase. Finally, aging per se has no effect on insulin sensitivity independent of changes in body composition.

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The prevalence of type 2 diabetes and impaired glucose tolerance (IGT) increases with aging (1). About 40% of the U.S. population over 60 years of age now have either type 2 diabetes or IGT (2). With the anticipated further aging of our population, the burden of type 2 dia-

betes and IGT on our health care system will continue to grow.

Type 2 diabetes and IGT result from an imbalance between the body's need for insulin (insulin sensitivity) and its ability to secrete insulin (β -cell function) (3,4). To maintain normal glucose tolerance

(NGT), there is an hyperbolic relationship between insulin secretion and insulin sensitivity such that insulin secretion increases as insulin sensitivity decreases and vice versa (5).

It had been commonly theorized that decreased insulin sensitivity (i.e., insulin resistance) preceded impaired β -cell function and that only after years of increased insulin secretion to compensate for the insulin resistance did β -cell function begin to deteriorate as a result of exhaustion (6–8). This theory implied that β -cell dysfunction was a relatively late and possibly secondary event in the pathogenesis of type 2 diabetes. Numerous recent studies, however, question this concept. For example, people with IGT have been found to have about a 50% reduction in islet cell mass (9), β -cell function has been reported to decrease as fasting plasma glucose levels increase within the normal range (3), and decreased insulin responses to a standardized hyperglycemic stimulus have been found in normoglycemic first-degree relatives of people with type 2 diabetes (10,11).

The above observations thus suggest that decreases in β -cell mass/function may begin early in life and are consistent with numerous studies (12–21) indicating that β -cell function normally decreases with aging. Few studies, however, have assessed the rate of decrease of β -cell function with aging, and virtually all of these have evaluated basal rather than glucose-stimulated insulin secretion.

In the UK Prospective Diabetes Study (UKPDS), at diagnosis of type 2 diabetes, people aged ~53 years had a 50% reduction in basal β -cell function as assessed by homeostasis model assessment (HOMA) modeling (22), and this decreased ~5% per year over the next 6 years (22). In contrast, aging has been reported to decrease basal insulin secretion at a rate of ~0.5% per year in people with NGT (17,20). The rate of decrease in people with IGT has not as yet been investigated.

To date, no studies have compared the effects of aging in first- and second-phase insulin release or assessed the effects of aging on biphasic insulin release

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Abbreviations: HOMA, homeostasis model assessment; IGT, impaired glucose tolerance; NGT, normal glucose tolerance; UKPDS, UK Prospective Diabetes Study.

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Table 1—Clinical characteristics of subjects

	NGT	IGT	P
n	266	130	
Sex (% male)	30.0	40.8	0.025
Age (years)	41.3 ± 0.7 (21–68)	47.4 ± 1.1 (23–69)	<0.001
Family history of diabetes (%)	47	48	0.84
BMI (kg/m ²)	26.4 ± 0.2	27.9 ± 0.4	0.002
Waist-to-hip ratio	0.83 ± 0.01	0.89 ± 0.01	0.001
Fasting plasma glucose (mg/dl)	88.1 ± 0.6	100.2 ± 0.9	<0.001
2-h plasma glucose (mg/dl)	104.7 ± 1.2	160.1 ± 1.5	<0.001
Fasting plasma insulin (mU/l)	8.7 ± 0.4	11.5 ± 0.8	0.001
2-h plasma insulin (mU/l)	49.2 ± 2.2	81.0 ± 4.4	0.001
A1C (%)	5.0 ± 0.02	5.5 ± 0.1	<0.001

Data are means ± SEM, means ± SEM (range), and %.

in people with IGT who are known to have reductions in first- and second-phase insulin release (3). We therefore evaluated our extensive database of individuals who had undergone oral glucose tolerance tests and hyperglycemic clamp studies to test the hypothesis that aging affects first- and second-phase insulin release as well as insulin sensitivity differently in individuals with NGT and those with IGT.

RESEARCH DESIGN AND METHODS

From 1986 to 2005, data were systematically collected from individuals volunteering for research studies. This report includes all subjects with NGT or IGT. Subjects were initially excluded from the study if they were pregnant or lactating; had significant renal or hepatic disease (i.e., patients with serum creatinine >1.6 mg/dl or ALT, AST, total bilirubin, or alkaline phosphatase levels in excess of 2.5 times the upper limit of the normal laboratory range); or any clinically relevant abnormality identified on the screening physical examination or laboratory tests that would preclude safe completion of the study. None of the subjects were taking medications known to affect carbohydrate metabolism. The demographic characteristics of the study population have been previously described (11,23–25). All subjects underwent a standard oral glucose tolerance test as recommended by the American Diabetes Association and a hyperglycemic clamp as previously described (26). All subjects were in good health and had normal physical examinations and routine laboratory tests, and none were taking any medications known to affect glucose metabolism. All subjects gave written informed consent after the

protocol had been approved by the local institutional review boards.

Subjects were divided into two different categories based on their fasting glucose concentrations (mean of two measurements at least 1 week apart) and 2-h plasma glucose concentrations during a standard 75-g oral glucose tolerance test. Normal subjects ($n = 266$) were defined as fasting plasma glucose <100 mg/dl and 2-h postprandial value <140 mg/dl. IGT ($n = 130$) was defined as 2-h postchallenge glucose concentrations ≥ 140 and <200 mg/dl. Data of subjects who had fasting glucose concentrations ≥ 126 mg/dl or 2-h postprandial glucose concentrations ≥ 200 mg/dl were eliminated for the purpose of the present study.

Calculations

HOMA indexes of β -cell function and insulin resistance were calculated from fasting plasma insulin (in microunits per liter) and glucose (in millimoles per liter) concentrations. HOMA of β -cell function was calculated as $20 \times \text{insulin}/(\text{glucose} - 3.5)$ (27). First-phase insulin secretion was considered to be the sum of plasma insulin concentrations at 2.5, 5.0, 7.5, and 10 min of the hyperglycemic clamp. Second-phase insulin release was taken as the average plasma insulin concentration during the last hour of the hyperglycemic clamp. HOMA of insulin sensitivity was calculated as $22.5/(\text{insulin} \times \text{glucose})$ (27) and directly determined by dividing the average glucose infusion rate during the last hour of the hyperglycemic clamp by the average plasma insulin concentration during the same interval, referred to as the insulin sensitivity index. In healthy individuals with normal glucose homeostasis, a decrease in insulin sensitivity

is compensated for by an increase in insulin secretion, so that the product of both of these processes, referred to as the disposition index, remains constant (5). We therefore evaluated the appropriateness of β -cell function in relation to insulin sensitivity by calculating the disposition index of the first- and second-phase insulin response by multiplying the respective insulin responses with the insulin sensitivity index (5).

Statistical analyses

Data are presented as means ± SEM. Nonpaired two-tailed students *t* tests were used to compare data of NGT and IGT subjects. The χ^2 test was used for categorical comparisons. The influence of age on insulin release and insulin sensitivity was assessed using linear regression. To compare the effect of aging on the disposition index for first- and second-phase insulin secretion in the NGT and IGT groups, an ANCOVA was performed using age as the covariate and log-transformed data.

RESULTS— Clinical characteristics of the study participants are presented in Table 1. The age range of subjects with NGT and those with IGT was comparable (~20 to ~70 years). The IGT group was slightly (6 years) older than those with NGT and contained a greater proportion of men (40 vs. 30%, $P = 0.025$). The IGT group was more obese than the NGT group, having significantly greater BMIs (27.9 ± 0.4 vs. 26.4 ± 0.2 kg/m², $P = 0.002$) and waist-to-hip ratios (0.89 ± 0.01 vs. 0.83 ± 0.01 , $P = 0.001$). Fasting and 2-h plasma glucose and insulin levels as well as A1C values in the IGT group were all significantly greater than those in the NGT group.

Assessment of insulin sensitivity

Insulin sensitivity assessed by HOMA and clamp techniques was significantly reduced in the IGT group (0.53 ± 0.03 vs. 0.76 ± 0.04 , $P < 0.001$, and 14.0 ± 0.8 vs. 16.9 ± 0.6 ml · mg · kg⁻¹ · min⁻¹ · μU^{-1} , $P = 0.01$, respectively). HOMA sensitivity was not correlated with age in either group ($P = 0.69$ and 0.08 , respectively). Insulin sensitivity assessed from clamp data showed no correlation with age in the NGT group ($r = 0.013$, $P = 0.83$) but was correlated with age in the IGT group ($r = -0.24$, $P = 0.005$). However, when BMI was included in the regression analysis, no independent effect of age was found ($r = -0.09$, $P = 0.20$).

Table 2—Comparison of β -cell function

	NGT	IGT	P
HOMA			
% β -cell	150 \pm 9	114 \pm 7	0.008
Correlation with age	$r = -0.18$ $P = 0.003$ $Y = -2.1x + 238.6$	$r = -0.05$ $P = 0.53$ $Y = -0.37x + 131.8$	
Decrease/year	1.1%	—	
Hyperglycemic clamp			
First phase (μ U/ml)	186 \pm 7	139 \pm 8	<0.001
Correlation with age	$r = -0.14$ $P = 0.02$ $Y = -1.36x + 241.9$	$r = -0.19$ $P = 0.03$ $Y = -1.48x + 209.3$	
Second phase (μ U/ml)	68 \pm 3	51 \pm 3	0.001
Correlation with age	$r = -0.11$ $P = 0.064$ $Y = -0.54x + 89.8$	$r = -0.054$ $P = 0.54$ $Y = -0.14 + 57.6$	

Data are means \pm SEM.

Assessment of β -cell function

HOMA β -cell and first- and second-phase insulin release were all significantly reduced in the IGT group (Table 2, Fig. 1). HOMA β -cell was inversely correlated with age in the NGT group ($P = 0.003$) such that with each decade it decreased 11% (i.e., \sim 1.1% per year). No significant correlation with age was found in the IGT group ($P = 0.53$). First-phase insulin release was negatively correlated with age in both groups (all $P < 0.05$). Second-phase insulin release did not change with age in either group.

Since the IGT group was less insulin sensitive than the NGT group and since decreases in insulin sensitivity normally

augment insulin secretion (5), mere comparison of plasma insulin responses would underestimate differences in β -cell function between the groups. We therefore analyzed first- and second-phase insulin release in terms of their disposition index (Fig. 1). This index, the product of insulin response and insulin sensitivity, examines the appropriateness of the insulin response for the ambient insulin sensitivity. For first- and second-phase insulin release, an ANCOVA revealed that the regression lines (evaluated for the logarithm of the disposition index) that represented the NGT and IGT groups across the age range of interest were not parallel ($P = 0.0001$ and $P = 0.0083$, respec-

tively). For both phases the slopes for the IGT had a steeper decrease as a function of aging (equations below). Consequently, the data were evaluated to determine where regions of significance could be identified along the age range (28). In the first-phase disposition index analysis, it was found that over 23 years of age the disposition indexes were significantly different in the two groups ($P \leq 0.05$). Since 9 of 266 patients in the normal group (3.38%) and none in the impaired group were <23 years of age, this suggests that we can view the two groups as significantly different along most of the age range of interest. This holds true as well for the second-phase disposition index analysis, where over our entire age range, the log of the second-phase disposition index for NGT and IGT was significantly different ($P \leq 0.05$).

In the NGT group the log of the disposition index for the first-phase ($\log Y_{1st} = 8.0039 - 0.00619Age$) and second-phase ($\log Y_{2nd} = 9.9693 - 0.00607Age$) insulin secretion decreased as a function of age ($P = 0.018$ and $P < 0.001$, respectively) and similarly, as evidenced by inspection of the above slopes and confirmed by the nonsignificant interaction of age by phase in the generalized estimating equation model ($P = 0.97$). Since the form of our regression equation for the disposition index can be expressed as $\log Y_0 = \alpha + \beta x_0$ for a person with age AGE_0 and as $\log Y_1 = \alpha + \beta(x_0 + 1)$ for a person 1 year older ($AGE_0 + 1$) it can be shown that $\beta = \log Y_1 - \log Y_0$ and that $e^\beta = \frac{Y_1}{Y_0}$. Thus, for every extra

year of age, the disposition index of a person is multiplied by the exponential function of the coefficient for age (e^β), and this ratio is 0.993 for both the first and second phase given the very similar slopes. Therefore, for first phase, a person with NGT at age 50 years would have a predicted log of the disposition index of 7.6946 and a person at age 55 years would have a predicted log of the disposition index of 7.6637 or ratio of 0.993^5 . This represents a 0.7% decrease per year for each phase. In the IGT group the log of disposition indexes decreased as a function of age for both first-phase ($\log Y_{1st} = 8.2237 - 0.02225Age$, $P < 0.0001$) and second-phase ($\log Y_{2nd} = 6.8268 - 0.01372Age$, $P < 0.0001$) insulin secretion and to a greater extent than in the NGT subjects. The disposition index for first-phase insulin release decreased to a

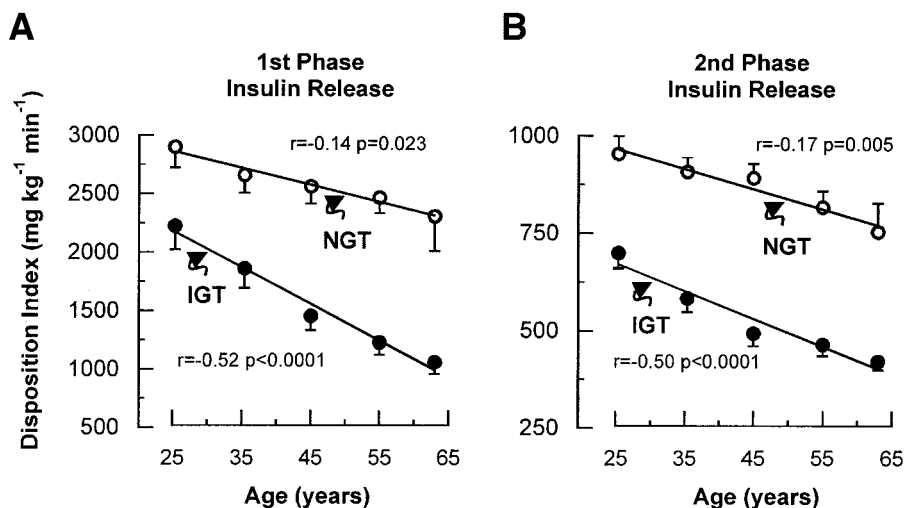


Figure 1—Disposition index of first-phase (A) and second-phase (right) insulin release as a function of age in individuals with NGT and IGT. Equations: first phase, NGT $Y = -14.538x + 3208.2$, IGT $Y = -31.963x + 2983.4$; second phase, NGT $Y = -4.6476x + 1072.7$, IGT $Y = -7.1995x + 849.41$.

greater extent than that for second-phase insulin release in the IGT group ($P = 0.018$). This reflects a ratio of 0.978 for a unit increase in age and a 2.2% decrease per year for the first phase and ratio of 0.986 for a unit age increase and a 1.4% per year decrease for the second phase.

CONCLUSIONS— The present studies demonstrate for the first time that in people with NGT over the age range of ~ 20 to ~ 70 years, glucose-stimulated first- and second-phase insulin release both decrease in a linear fashion at a comparable rate of $\sim 0.7\%$ per year. Previous studies, which only assessed basal insulin release either using HOMA (17) or insulin clearance data (20) found annual decreases of ~ 1.0 and $\sim 0.4\%$, respectively. In the present study, using HOMA we found basal β -cell function to decrease $\sim 1.0\%$ per year in individuals with NGT. Thus, our results are consistent with previous studies.

A second major finding of our study was that people with IGT had greater decreases as a function of age of both first- and second-phase insulin release than people with NGT and that first phase decreased to a greater extent than second phase.

None of the studies performed to date, including our own, can determine to what extent age-related decreases in insulin release are due to changes in β -cell mass and/or changes in function of individual β -cells. However, changes in β -cell mass are most likely involved, since Mædler et al. (29) reported that aging of human pancreatic islets is associated with decreased proliferation and increased sensitivity to hyperglycemia-induced apoptosis. The hyperglycemia associated with IGT may thus have resulted in an accelerated loss of β -cell mass because β -cells from older individuals appear to be more sensitive to adverse effects of glucose-induced apoptosis (29). Moreover, Butler et al. (9) have reported that β -cell mass is reduced $\sim 50\%$ in people with IGT.

Our results have implications on the pathogenesis of type 2 diabetes. In the UKPDS (22), at diagnosis of diabetes (age ~ 55 years) β -cell function (HOMA determined) was reduced $\sim 50\%$. If one assumes a normal starting β -cell function at age 15, a loss of function at a rate of 0.7% per year with aging as found in the present study would result in a 50% reduction in β -cell function at about age 100 years. Thus, normal aging should not

of itself lead to the reduction in β -cell function found at onset of type 2 diabetes.

On the other hand, if one started with a normal β -cell mass/function, one would only have to have a rate of deterioration of 1.25% per year to have a 50% reduction at age ~ 55 —approximately what was found in our IGT group. Thus, one need not postulate a reduced initial β -cell mass to explain the reduction in β -cell function found at diagnosis of type 2 diabetes. It is of interest that once diabetes has developed, decreases in rates of β -cell function (as assessed by HOMA) have ranged from 2 to 6% per annum, as in the UKPDS (22) and ADOPT (30) studies. Whether this increased rate of deterioration merely reflects glucose toxicity (31) and/or an underlying genetic defect is unclear.

In the present study, insulin sensitivity as assessed with HOMA did not change with aging in either group. Using clamp data, insulin sensitivity was found to decrease with aging in the IGT group but not in the NGT group. However, the IGT group was more overweight than the NGT group. When insulin sensitivity was adjusted for BMI, it was no longer correlated with age. Therefore, our results support most previous studies (15–17,19) indicating that insulin sensitivity does not decrease per se with aging and that decreases in insulin sensitivity when observed are most likely secondary to changes in body composition and physical fitness.

The present study has certain limitations. First, it is cross-sectional and not longitudinal. Consequently, our results cannot indicate whether a person's β -cell function's decline with age changes if his/her glucose tolerance deteriorates. Nevertheless, our results suggest that those at greater risk of developing type 2 diabetes (e.g., those with IGT) have a greater rate of β -cell function decline. Second, because individuals who developed type 2 diabetes as they aged were excluded from study, we probably underestimated the effect of aging on β -cell function. We did not exclude those with an increased risk of developing type 2 diabetes based on a positive family history. However, the percentage of subjects with a positive family history in the NGT and IGT groups (47 and 48%, respectively) was similar. Finally, our measures of insulin secretion cannot distinguish between changes due to islet cell mass and islet cell function.

In conclusion, the results of the present study indicate that insulin secretion (both first and second phase) nor-

mally decreases $\sim 0.7\%$ per year and that this deterioration in β -cell function is increased about two-fold in people with impaired glucose tolerance—first phase to a greater extent than second phase. Finally, aging per se has no apparent intrinsic effect on insulin sensitivity.

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