Effect of Aging on Glucose Homeostasis: Accelerated Deterioration of Beta Cell Function in Individuals with Impaired Glucose Tolerance

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ABSTRACT

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 or impaired glucose tolerance.

Research Design and Methods: First and second phase insulin secretion and insulin sensitivity were assessed in hyperglycemic clamp experiments in 266 individuals with normal glucose tolerance (NGT) and 130 individuals with impaired glucose tolerance (IGT), ranging in age from ~20 to ~70 years. Changes in beta 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). In neither group was insulin sensitivity independently correlated with age. 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 Indices 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 about 0.7% per year with aging; this decrease in beta 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.
The prevalence of type 2 diabetes (T2DM) and impaired glucose tolerance (IGT) increases with aging (1). About 40% of the U.S. population over 60 years of age now have either T2DM or IGT (2). With the anticipated further aging of our population, the burden of T2DM and IGT on our health care system will continue to grow.

T2DM 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, 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 T2DM.

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 finally, decreased insulin responses to a standardized hyperglycemic stimulus have been found in normoglycemic first degree relatives of people with T2DM (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 indicating (12-21) 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 United Kingdom Prospective Diabetes Study (UKPDS), at diagnosis of T2DM people aged ~53 yrs had a 50% reduction in basal β cell function as assessed by Homeostasis Model Assessment (HOMA) modeling (22), and this decreased about 5% per year over the next six years (22). In contrast, aging has been reported to decrease basal insulin secretion at a rate of about 0.5% per year in people with normal glucose tolerance (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 nor have they assessed the effects of aging on biphasic insulin release in people with IGT who are known to have reductions in first and second phase insulin release (3). Therefore we 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 normal glucose tolerance and those with IGT.

**RESEARCH DESIGN AND METHODS**

**Subjects.** From 1986 to 2005, data were systematically collected from individuals volunteering for research studies. This report includes all subjects with normal glucose tolerance or impaired glucose
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Subjects were initially excluded from study if they were pregnant or lactating; had significant renal or hepatic disease (i.e., patients with serum creatinine >1.6 mg/dL; ALT, AST, total bilirubin or alkaline phosphatase levels in excess of 2.5 times the upper limit of the normal laboratory range, any clinically relevant abnormality identified on the screening physical examination and laboratory tests which 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 gave informed written consent after the protocol had been approved by the local Institutional Review Boards. All were in good health, and had normal physical examinations and routine laboratory tests. None of the subjects were taking any medications known to affect glucose metabolism.

Subjects were divided into two different categories based on their fasting glucose concentrations (mean of two measurements at least 1 week apart) and 2-hour plasma glucose concentrations during a standard 75-g oral glucose tolerance test. Normal subjects (N=266) were defined as fasting plasma glucose <100 and 2-h postprandial value<140 mg/dl. Impaired glucose tolerance (N=130) was defined as 2-h post-challenge glucose concentrations between ≥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.** Homeostasis model assessment (HOMA) indexes of β-cell function (HOMA-%B) and insulin resistance (HOMA-IR) were calculated from fasting plasma insulin (mU/L) and glucose (mM) concentrations. HOMA-%B was calculated as 20 × insulin / (glucose − 3.5) (27). First phase insulin secretion was considered to be the sum plasma insulin concentrations at 2.5, 5.0, 7.5, and 10 minutes of the hyperglycemic clamp. Second phase insulin release was taken as the average plasma insulin concentration during the last hour of the hyperglycemic clamp. Insulin sensitivity was assessed by using HOMA-%S, calculated as 22.5/(insulin × 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 (ISI). 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 (DI 1st phase) and second phase insulin response (DI 2nd phase) by multiplying the respective insulin responses with the ISI (5).

**Statistical Analyses.** Data are presented as means ± SEMs. Nonpaired two-tailed students t tests were used to compare data of NGT and IGT subjects. Chi square 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 DI 1st phase and DI 2nd phase insulin secretion in the NGT and IGT group an analysis of covariance was performed using age as the covariate and log transformed data.

RESULTS

Clinical Characteristics (Table 1). The age range of subjects with normal glucose tolerance (NGT) and those with impaired glucose tolerance (IGT) was comparable (~20~70 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 body mass indices (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 hr plasma glucose and insulin levels as well as HbA1c values in the IGT group were all significantly greater than those of 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). In neither group was HOMA sensitivity correlated with age (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).

Assessment of Beta Cell Function (Table 2, Figure 1). HOMA beta cell and first and second phase insulin release were all significantly reduced in the IGT group. HOMA beta cell was inversely correlated with age in the NGT group (p=0.003) such that with each decade it decreased 11% (i.e. approximately 1.1% per year). No significant correlation with age was found in the IGT group (p=0.53). First phase insulin release were negatively correlated with age in both groups (all p<0.05). Second phase insulin release did not change with age in either groups.

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 beta cell function between the groups. We therefore analyzed first and second phase insulin release in terms of their disposition index (Figure 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 analysis of covariance revealed that the regression lines (evaluated for the logarithm of the disposition index) that represented the normal and impaired glucose tolerance groups across the age range of interest were not parallel (p=0.0001 and p=0.0083, respectively). For both phases the slopes for the IGT had a steeper decrease as a function of aging (see paragraph below for equations). Consequently, the data were evaluated to determine where along the age range, regions of significance could be identified (28). In the DI 1st phase analysis, it was found that above 23 years of age the disposition indices were significantly different in the 2 groups (p<0.05). Since, 9 out of 266 patients in the normal group (3.38%) and none in the
impaired group were less than 23, this suggests that we can view the 2 groups as significantly different along most of the age range of interest. This holds as well for the DI 2nd phase where over our entire age range, the log of the DI 2nd phase the NGT and IGT were significantly different (p<=0.05).

In the NGT group the log of the disposition index for the first phase (log $Y_{1st} = 8.0039 - 0.00619 Age$) and second phase (log $Y_{2nd} = 9.9693 - 0.00607 Age$) insulin secretion both 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 non-significant 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

$$log Y_i = \alpha + \beta(x_0+1)$$

for a person one year older ($AGE_0+1$) it can be shown that $\beta = log Y_i - log Y_0$ and that

$$e^\beta = \frac{Y_i}{Y_0}.$$ 

Thus, for every extra year of age, the disposition index of a person is multiplied by the exponential function of the coefficient for age ($e^\beta$) and this ratio is .993 for both the first and second phase given the very similar slopes. Therefore, for first phase, a person with normal glucose tolerance at age 50 would have a predicted log of the DI of 7.6946 and a person at age 55 would have a predicted log of DI 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 indices decreased as a function of age for both first phase (log $Y_{1st} = 8.2237 - 0.02225 Age$ p<0.0001) and second phase (log $Y_{2nd} = 6.8268 - 0.01372 Age$ 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 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 normal glucose tolerance over the age range of ~20-70 years, glucose-stimulated first and second phase insulin release both decrease in a linear fashion at a comparable rate of approximately 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 ~0.4% respectively. In the present study, using HOMA we found basal beta cell function to decrease approximately 1.0% per year in individuals with normal glucose tolerance. 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 did 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 beta cell mass and/or changes in function of individual beta cells. However, changes in beta cell mass are probably involved since Maedler 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 beta cell mass because beta 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 beta cell mass is reduced approximately 50% in people with IGT.

Our results have implications on the pathogenesis of type 2 diabetes. In the UKPDS (30), at diagnosis of diabetes (age ~55 yrs) beta cell function (HOMA determined) was reduced about 50%. If one assumes a normal starting beta 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 beta cell function at about age 100. Thus normal aging should not of itself lead to the reduction in beta cell function found at onset of type 2 diabetes.

On the other hand, if one started with a normal beta 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 beta cell mass to explain the reduction in beta cell function found at diagnosis of type 2 diabetes. It is of interest that once diabetes has developed, decreases in rates of beta cell function (as assessed by HOMA) have ranged from 2 to 6% per annum as in the UKPDS (22) and ADOPT studies (31). Whether this increased rate of deterioration merely reflects glucose toxicity (32) 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 body mass index, 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 probably secondary to changes in body composition and physical fitness.

The present study has certain limitations. First of all, it is cross-sectional and not longitudinal. Consequently our results cannot indicate whether a person’s beta cell function’s decline with age changes if his/her glucose tolerance deteriorates. Nevertheless our results suggest that those at greater risk to develop T2DM (e.g. those with IGT) have a greater rate of beta cell function decline. Secondly, because individuals who had developed T2DM as they aged were excluded from study, we probably underestimated the effect of aging on beta cell function. We did not exclude those with an increased risk of developing T2DM based on a positive family history. However, the percentage of subjects with a positive family history in the NGT group and IGT group (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) normally decreases about 0.7% per year
and that this deterioration in beta 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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REFERENCES

### TABLE 1. Clinical Characteristics of Subjects

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<tr>
<th></th>
<th>Normal Glucose Tolerance</th>
<th>Impaired Glucose Tolerance</th>
<th>p</th>
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</thead>
<tbody>
<tr>
<td>N</td>
<td>266</td>
<td>130</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td>30.0% Male</td>
<td>40.8% Male</td>
<td>0.025</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>41.3 ± 0.7 (21-68)</td>
<td>47.4 ± 1.1 (23-69)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Family History of Diabetes</td>
<td>47%</td>
<td>48%</td>
<td>0.84</td>
</tr>
<tr>
<td>Body Mass Index (Kg/M²)</td>
<td>26.4 ± 0.2</td>
<td>27.9 ± 0.4</td>
<td>0.002</td>
</tr>
<tr>
<td>Waist Hip Ratio</td>
<td>0.83 ± 0.01</td>
<td>0.89 ± 0.01</td>
<td>0.001</td>
</tr>
<tr>
<td>Fasting Plasma Glucose (mg/dl)</td>
<td>88.1 ± 0.6</td>
<td>100.2 ± 0.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>2 Hr Plasma Glucose (mg/dl)</td>
<td>104.7 ± 1.2</td>
<td>160.1 ± 1.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fasting Plasma Insulin (mU/L)</td>
<td>8.7 ± 0.4</td>
<td>11.5 ± 0.8</td>
<td>0.001</td>
</tr>
<tr>
<td>2Hr Plasma Insulin (mU/L)</td>
<td>49.2 ± 2.2</td>
<td>81.0 ± 4.4</td>
<td>0.001</td>
</tr>
<tr>
<td>HbA₁c (%)</td>
<td>5.0 ± 0.02</td>
<td>5.5 ± 0.1</td>
<td>&lt;0.001</td>
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TABLE 2. Comparison of Beta Cell Function

<table>
<thead>
<tr>
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<th>Normal Glucose</th>
<th>Impaired Glucose</th>
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<tr>
<td></td>
<td>Tolerance</td>
<td>Tolerance</td>
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<tr>
<td>A. HOMA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Beta Cell</td>
<td>150 ± 9</td>
<td>114 ± 7</td>
<td>0.008</td>
</tr>
<tr>
<td>Correlation with Age</td>
<td>r=-0.18, p=0.003</td>
<td>r=-0.05, p=0.53</td>
<td></td>
</tr>
<tr>
<td></td>
<td>y=-2.1x + 238.6</td>
<td>y=-0.37x + 131.8</td>
<td></td>
</tr>
<tr>
<td>Decrease/yr</td>
<td>1.1%</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>B. Hyperglycemic Clamp</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First Phase (µU/ml)</td>
<td>186 ± 7</td>
<td>139 ± 8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Correlation with Age</td>
<td>r=-0.14, p=0.02</td>
<td>r=-0.19, p=0.03</td>
<td></td>
</tr>
<tr>
<td></td>
<td>y=-1.36x + 241.9</td>
<td>y=-1.48x + 209.3</td>
<td></td>
</tr>
<tr>
<td>Second Phase (µU/ml)</td>
<td>68 ± 3</td>
<td>51 ± 3</td>
<td>0.001</td>
</tr>
<tr>
<td>Correlation with Age</td>
<td>r=-0.11, p=0.064</td>
<td>r=-0.054, p=0.54</td>
<td></td>
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<tr>
<td></td>
<td>y=-0.54x + 89.8</td>
<td>y=-0.14 + 57.6</td>
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</table>
FIGURE LEGEND

**Figure 1** Disposition Index of first phase (left) and second phase (right) insulin release as a function of age in individuals with normal glucose tolerance (NGT) and impaired glucose tolerance (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: $Y = -7.1995X + 849.41$
FIGURE 1

**1st Phase Insulin Release**

- NGT
- IGT

Disposition Index (mg kg⁻¹ min⁻¹)

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**2nd Phase Insulin Release**

- NGT
- IGT

Disposition Index (mg kg⁻¹ min⁻¹)

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- **Correlation Coefficients**:
  - 1st Phase: $r = 0.14, p = 0.023$
  - 2nd Phase: $r = 0.17, p = 0.005$
  - $r = -0.52, p < 0.001$

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**Age (years)**

- 25, 35, 45, 55, 65