# Glucose and Insulin Measurements from the Oral Glucose Tolerance Test and Mortality Prediction

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**OBJECTIVE** — To verify what information from oral glucose tolerance tests (OGTTs) independently predicts mortality.

**RESEARCH DESIGN AND METHODS** — A total of 1,401 initially nondiabetic participants from the Baltimore Longitudinal Study of Aging aged 17–95 years underwent one or more OGTTs (median 2, range 1–8), with insulin and glucose measurements taken every 20 min over the course of 2 h included in this study. Proportional hazards using the longitudinally collected data and Bayesian model averaging were used to examine the association of OGTT measurements individually and grouped with mortality, adjusting for covariates.

**RESULTS** — Participants were followed for a median 20.3 years (range 0.5–40). The first-hour OGTT glucose and insulin levels increased only modestly with age, whereas levels during the second hour increased 4% per decade. Individually, 100- and 120-min glucose measures and fasting and 100-min insulin levels were all independent predictors of mortality. When all measures were considered together, only higher 120-min glucose was a significant independent risk factor for mortality.

**CONCLUSION** — The steeper rise with age of the OGTT 2-h glucose values and the prognostic primacy of the 120-min glucose value for mortality is consistent with previous reports and suggests the value of using the OGTT in clinical practice.

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Many but not all studies have found a nearly linear association between fasting plasma glucose levels >100 mg/dl and mortality (1). Plasma glucose 2 h after an oral glucose load is also a strong predictor of mortality regardless of fasting plasma glucose level (2,3) and may actually be a better predictor of mortality than the fasting level (4).

It has been proposed that in early stages of glucose metabolism dysregulation, fasting and 2-h plasma glucose measures during an oral glucose tolerance test (OGTT) may be normal or slightly elevated, but the amount of insulin necessary to maintain this equilibrium is supraphysiological (5). This state of "insulin resistance" may have detrimental consequences on health. However, there is little evidence that hyperinsulinemia—either fasting or with a glucose challenge—is a risk factor for mortality (6). Thus, the clinical usefulness of measuring insulin remains uncertain.

Using data from the Baltimore Longitudinal Study of Aging (BLSA), we examined longitudinal change in glucose and insulin in response to OGTT and the association of different glucose and insulin measurements on mortality.

## RESEARCH DESIGN AND METHODS

— BLSA participants are community-dwelling volunteers with above-average education, income, and access to medical care (7). Participants underwent extensive evaluations biannually. Participants who were nondiabetic at the initial OGTT evaluation and with at least one OGTT with both glucose and insulin measurements were included in this study. The research protocol was approved by the institutional review board at the Johns Hopkins Bayview Medical Center, and all participants provided written informed consent.

Analysis was restricted to OGTTs performed before 1995 (when laboratory procedures were modified). Overall, 1,510 BLSA participants (aged 17–95 years) were eligible, of whom 109 had diabetes at the time of their initial OGTT (defined as fasting glucose ≥7.0 mmol/l [126 mg/dl], 2-h OGTT glucose measurement ≥11.1 mmol/l [200 mg/dl], history of diabetes, or current use of oral hypoglycemic agents or insulin), leaving 1,401 participants with 3,727 observations (424 subjects with one observation, 343 with two observations, 262 with three observations, and 372 with four or more observations).

### OGTT

OGTT assessments began in 1959 using a 1.75 g glucose/kg body wt glucose challenge, which was changed to 40 g/m$^2$ body surface area in 1977 following recommended guidelines. Consistent with previous research (2,3), to convert OGTT results across methods we regressed glucose and insulin levels at each OGTT time point on a fourth-order polynomial of the glucose load, which was centered at 75 g. Adjusted measurements no longer depended on glucose load.

Participants were observed overnight in the research ward; they began fasting at 8:00 P.M. and received the OGTT between 7:00 and 8:00 A.M. Blood samples were

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**Abbreviations:** BLSA, Baltimore Longitudinal Study of Aging; BMA, Bayesian model averaging; HOMA, homeostatis model assessment; OGTT, oral glucose tolerance test.

**Table 1—*Characteristics of participants at first evaluation***

| | Survivors | Decedents | *P** |
|---|---|---|---|
| Subjects (*n*) | 931 | 470 | |
| Women (%) | 42.6 | 24.9 | |
| Age (years) | 45.2 ± 15.5 | 69.3 ± 11.7 | <0.0001 |
| Age (censor, death) (years) | 68.3 ± 15.9 | 83.0 ± 10.8 | <0.0001 |
| Time follow-up (years) | 23.2 ± 5.2 | 13.7 ± 6.7 | <0.0001 |
| OGTT | | | |
| Glucose (mmol/l) | | | |
| Fasting | 5.3 ± 0.4 | 5.5 ± 0.4 | 0.48 |
| 20 min | 7.4 ± 1.2 | 7.6 ± 1.1 | 0.06 |
| 40 min | 9.0 ± 1.7 | 9.2 ± 1.6 | 0.03 |
| 60 min | 8.5 ± 2.1 | 9.4 ± 2.0 | 0.71 |
| 80 min | 7.7 ± 2.1 | 8.9 ± 2.0 | 0.11 |
| 100 min | 7.1 ± 1.8 | 8.3 ± 1.9 | 0.002 |
| 120 min | 6.5 ± 1.5 | 7.6 ± 1.7 | 0.0001 |
| Insulin (pmol/l) | | | |
| Fasting | 53 ± 34 | 53 ± 35 | 0.42 |
| 20 min | 224 ± 153 | 209 ± 153 | 0.27 |
| 40 min | 373 ± 250 | 348 ± 239 | 0.50 |
| 60 min | 376 ± 249 | 400 ± 282 | 0.87 |
| 80 min | 349 ± 242 | 403 ± 278 | 0.67 |
| 100 min | 317 ± 239 | 385 ± 286 | 0.28 |
| 120 min | 272 ± 207 | 341 ± 256 | 0.12 |
| HOMA-IR units | 2.13 ± 1.43 | 2.37 ± 1.84 | 0.38 |
| Glucose area (mmol · $min^{-1}$ · $l^{-1}$)† | 917 ± 172 | 1005 ± 165 | 0.46 |
| Insulin area (pmol · $min^{-1}$ · $l^{-1}$)† | 36,468 ± 21,462 | 39,270 ± 23,772 | 0.92 |
| Covariates | | | |
| Weight (kg) | 72.8 ± 12.3 | 72.8 ± 14.2 | 0.68 |
| Height (cm) | 172.1 ± 9.8 | 170.8 ± 9.0 | 0.02 |
| BMI (kg/$m^2$) | 24.4 ± 3.5 | 24.8 ± 3.4 | 0.08 |
| Waist (cm) | 82.6 ± 11.5 | 87.8 ± 10.0 | <0.0001 |
| Systolic blood pressure (mm/Hg) | 119.5 ± 15.9 | 136.2 ± 20.5 | <0.0001 |
| Diastolic blood pressure (mm/Hg) | 77.2 ± 10.2 | 81.2 ± 10.8 | <0.0001 |
| Current smokers (%) | 17.7 | 14.1 | 0.0003 |

Data are means ± SD unless otherwise indicated. *Adjusted for age and sex. †Glucose and insulin area are the integrated areas under the curve.

drawn at 0, 20, 40, 60, 80, 100, and 120 min. Glucose levels were measured using the ferricyanide reduction method (Technico autoanalyzer) before 1977 and the glucose oxidase method from 1977 to present with a Beckman glucose analyzer (1977–1983), the Abbott Laboratories ABA 200 ATC Series II Biochromatic Analyzer (1983–1992), and the Abbott Spectrum CCX (1992 to present). Plasma insulin was measured using radioimmunoassay (8). The lower limit of detection for this assay is 15 pmol/ml and the inter- and intra-assay coefficients of variation are 11.5% and 6%, respectively (9).

### Covariates

Height, weight, and seated blood pressure were measured by standard methods. BMI was calculated as weight in kilograms divided by the square of height in meters. Waist circumference (centimeters) was measured as the smallest circumference between the umbilicus and lower ribs. Smoking history was assessed using a standard questionnaire.

### Mortality

Deaths were ascertained by telephone follow-up of inactive participants, correspondence from relatives, and search of the National Death Index. Cause of death was determined by the consensus of three physicians reviewing available information.

### Statistical analyses

Data are summarized as means ± SD unless otherwise stated. Differences in baseline characteristics (Table 1) between survivors and decedents were tested by age- and sex-adjusted ANCOVA or logistic regressions, as appropriate.

From the 3,727 observations, 2.0% of the OGTT glucose and insulin measurements and 0.6% of covariate data were missing. Missing data were imputed using the multivariate imputation by chained equations (MICE) program (10) with five replicated datasets. Survival models were based on the first imputation while all other analyses used the average of the five imputations. The remaining four imputed datasets produced the same conclusions.

Basal insulin resistance was estimated by the homeostatis model assessment (HOMA), calculated using fasting glucose and insulin levels as an assessment of basal insulin resistance (11). Integration of the glucose and insulin OGTT curves was calculated by the standard trapezoid method.

Proportional hazards models using all longitudinally collected OGTT data were used to examine the relationship between glucose and insulin levels at each OGTT time point and time to death with three hierarchical models that adjusted for date and sex, added age, and added other covariates. The survival model used all longitudinally collected OGTT data with a time-dependent approach based on the Anderson-Gill formulation of a counting process using the survival functions developed by Therneau and Grambsch(12).

To examine which OGTT measurements independently predicted mortality, we used a standard backward elimination to obtain a parsimonious model with all variables showing $P < 0.10$. Additionally, we fitted a Bayesian model averaging (BMA) (13) to identify the best set of predictors for mortality across all feasible models. Because BMA used the initial evaluation, the effects of diabetes on mortality would be underestimated due to the exclusion of those who were diabetic at initial evaluation. Therefore, we repeated the BMA analysis including diabetic participants.

A two-tailed $P$ value $< 0.05$ was used to indicate statistical significance. All analyses and graphs were completed using R version 2.4.1 (R Project for Statistical Computing, http://www.r-project.org).

**RESULTS** — There were 470 deaths and 931 surviving participants as of January 2006. Baseline characteristics are presented in Table 1. Those who died were older initially, had shorter follow-up time, tended to have higher BMI and sys-

**Table 2—*Hazard ratios from time-dependent survival models for glucose (per mmol/l) and insulin (per 100 pmol/ml) measurements from the longitudinally collected OGTT***

| | All-cause mortality | | | Cardiovascular mortality |
|---|---|---|---|---|
| | Model 1: univariate date, sex | Model 2: univariate age, date, sex | Model 3: univariate age, date, sex, covariates* | Model 4: univariate age, date, sex, covariates* |
| Glucose | | | | |
| Fasting | 1.34 (1.14–1.59) | 1.12 (0.96–1.32) | 1.18 (0.99–1.39) | 1.11 (0.80–1.55) |
| 20 min | 1.06 (0.99–1.14) | 1.02 (0.94–1.10) | 1.01 (0.94–1.10) | 1.06 (0.91–1.23) |
| 40 min | 1.00 (0.95–1.05) | 0.98 (0.94–1.04) | 0.99 (0.94–1.04) | 0.99 (0.89–1.10) |
| 60 min | 1.10 (1.06–1.14) | 1.03 (0.98–1.07) | 1.03 (0.99–1.08) | 1.02 (0.94–1.11) |
| 80 min | 1.16 (1.12–1.20) | 1.04 (0.99–1.08) | 1.04 (0.99–1.08) | 1.04 (0.96–1.12) |
| 100 min | 1.20 (1.16–1.24) | 1.05 (1.00–1.09) | 1.05 (1.00–1.09) | 1.06 (0.98–1.14) |
| 120 min | 1.22 (1.17–1.27) | 1.06 (1.02–1.10) | 1.06 (1.01–1.11) | 1.08 (1.00–1.18) |
| Insulin | | | | |
| Fasting | 1.18 (0.91–1.52) | 1.34 (1.03–1.74) | 1.42 (1.07–1.89) | 1.03 (0.60–1.76) |
| 20 min | 0.97 (0.92–1.03) | 1.00 (0.95–1.06) | 1.01 (0.96–1.06) | 0.99 (0.90–1.08) |
| 40 min | 0.96 (0.92–1.00) | 1.00 (0.96–1.04) | 1.00 (0.96–1.03) | 1.02 (0.96–1.09) |
| 60 min | 1.00 (0.97–1.04) | 1.01 (0.98–1.05) | 1.01 (0.98–1.04) | 1.00 (0.94–1.06) |
| 80 min | 1.04 (1.01–1.07) | 1.03 (0.99–1.06) | 1.02 (0.99–1.06) | 0.98 (0.92–1.04) |
| 100 min | 1.07 (1.04–1.10) | 1.04 (1.01–1.07) | 1.04 (1.01–1.07) | 1.01 (0.95–1.07) |
| 120 min | 1.06 (1.03–1.09) | 1.01 (0.98–1.04) | 1.01 (0.98–1.05) | 1.02 (0.96–1.08) |
| HOMA-IR | 1.05 (0.99–1.10) | 1.07 (1.01–1.13) | 1.09 (1.02–1.16) | 1.02 (0.90–1.16) |
| Glucose integrated/100 | 1.16 (1.11–1.21) | 1.03 (0.99–1.08) | 1.03 (0.98–1.08) | 1.00 (1.00–1.01) |
| Insulin integrated/1,000 | 1.00 (1.00–1.01) | 1.00 (1.00–1.01) | 1.00 (1.00–1.001) | 1.01 (0.97–1.06) |

Data are hazard ratios (95% CI). Each row is a proportional hazard model with only the specific measure from the OGTT entered as the risk variable in the analysis. The model uses the longitudinally collected measure and covariates as time-dependent variables. Results are from the first imputation. Note results were essentially the same for imputations 2–5. *Covariates included systolic and diastolic blood pressure, BMI, waist circumference, smoking status, and cholesterol. HOMA-IR, HOMA of insulin resistance.

tolic and diastolic blood pressure, and were less likely to smoke than those who survived.

At the initial evaluation, average glucose values during the OGTT were higher in those who died than in those who survived (Table 1). After adjusting for age and sex, glucose levels at 40, 100, and 120 min were higher in those who died than in survivors, while insulin levels did not differ at any time point. In adjusted analyses, HOMA, integrated glucose, and integrated insulin were not statistically different according to survivorship (Table 1).

In analyses that considered longitudinal trends, all OGTT glucose levels increased with follow-up time ($P < 0.0001$), but the rate of change was lowest for the fasting state (0.07 mmol · $l^{-1}$ · 10 $years^{-1}$) and increased progressively for subsequent OGTT time points (e.g., rate at 60 min was 0.37 and at 120 min was 0.42 mmol · $l^{-1}$ · 10 $years^{-1}$). Corresponding rates for insulin were 1.32 for fasting, 12.90 for 60 min, and 128.92 pmol · $l^{-1}$ · 10 $years^{-1}$ for 120 min.

Proportional hazards models using all longitudinally collected data evaluated the individual relative risk of mortality for each OGTT measure (Table 2). Adjusting for age, date, and sex, 100- and 120-min glucose, fasting insulin, 100-min insulin, and HOMA were associated with all-cause mortality. These associations persisted after further adjustments for BMI, waist circumference, diastolic and systolic blood pressure, lipids, and smoking. Of the 326 deaths, 134 were considered cardiovascular deaths. In adjusted analyses, only 120-min glucose and integrated glucose were significant predictors of mortality (Table 2, model 4).

For each OGTT measure, Fig. 1*A* presents risk ratios with 95% CI comparing risk of mortality associated with being at the 75th percentile versus the 25th percentile adjusted for age, date, and sex. The greatest discernable risk was found with the 100- and 120-min glucose measurements. Figure 1*B* shows a similar result for cardiovascular mortality.

From the longitudinal survival model including all glucose and insulin measures, we removed variables not independently associated with mortality. In the final parsimonious model, 100-min insulin (risk ratio 1.11, 95% CI 1.05–1.17 per 100 pmol/ml increase), 120-min insulin (0.90, 0.85–0.92 per 100 pmol/ml increase), 40-min glucose (0.92, 0.87–0.97 per mmol/l increase), 120-min glucose (1.10, 1.05–1.16 per mmol/l increase), and HOMA (1.07, 1.00–1.13) were independently associated with greater mortality. Because the selection of variables with this method may be strongly influenced by collinearity, we performed a confirmatory analysis using BMA, which assesses uncertainty by exploring multiple models and providing weighted probabilities that coefficients are not equal to zero. Using data from the first evaluation, 120-min glucose was the only variable in the BMA model with a probability >10% (16.4%) that the regression coefficient was not zero (i.e., no association with mortality). When the 109 participants classified as diabetic at baseline were included in the BMA, the probability that the 120-min glucose regression coefficient was not zero was 93.4%, while that for fasting insulin was only 15.5%. Fasting glucose

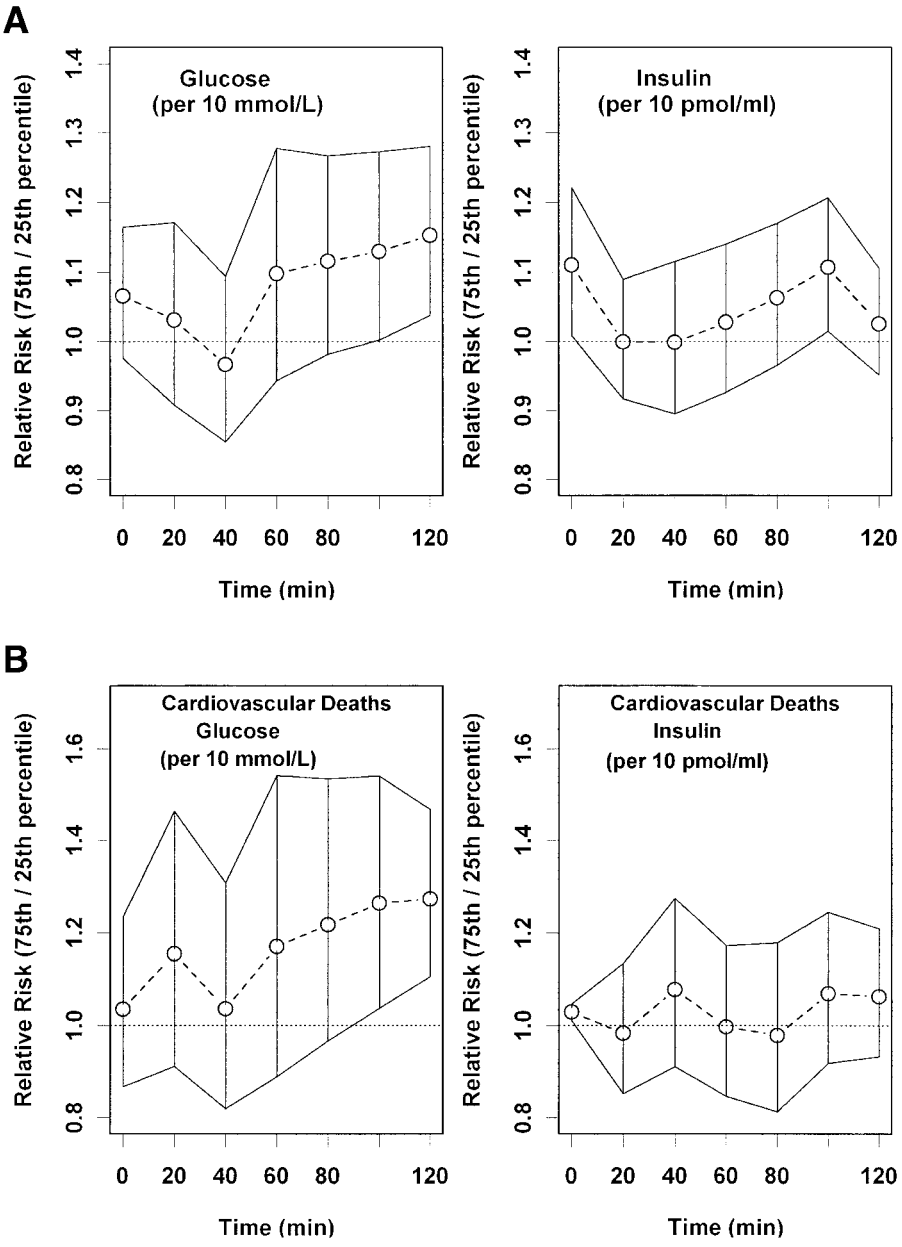


**Figure 1—** *Age, date, and sex-adjusted risk ratio for mortality comparing the 75th percentile versus the 25th percentile for the time-specific measurement, adjusted for insulin and glucose OGTT. The graph shows the median risk ratio and 95% CI at each time point for all-cause mortality* (A) *and cardiovascular mortality* (B).

was not an independent predictor of mortality in these models.

**CONCLUSIONS** — In a large population of men and women aged 17–95 years at initial OGTT, higher 120-min glucose level was the only OGTT measure consistently and independently associated with mortality.

Independent of age and sex, fasting glucose, 120-min glucose, fasting insulin, and 100-min insulin were associated with higher mortality. When considering all glucose and insulin measurements together, only the 120-min glucose remained prognostic for mortality, independent of age, date, and sex. Although 100- and 120-min insulin, 40-min glucose, and HOMA were statistically independent predictors of mortality in the parsimonious model, the prognostic information contributed by the other four variables was almost negligible. This observation is consistent with the study by Sorkin et al. (3), who found that the 120-min glucose measurement improved mortality prediction using fasting glucose alone. However, these authors did not consider the predictive value of the 120-min measure alone.

Why might 120-min glucose be the most robust predictor of mortality? It is possible that as long as β-cells secrete enough insulin to maintain euglycemia, consequences of elevated blood glucose are avoided, regardless of the amount of insulin necessary to maintain glucose homeostasis (14). Although this interpretation contrasts, at least in part, with the study by Sorkin et al. (3), which focused on categorical characterizations (i.e., normal, impaired, and diabetic), we suggest that a more refined risk assessment can be obtained by examining the continuous-range 120-min glucose values.

Alternatively, 120-min glucose could be a marker of poor health. Deteriorating health may cause a progressive inability to respond to a glucose challenge, possibly due to a loss of resiliency in the hypothalamic-pituitary-adrenal axis resulting in prolonged secretion of glucocorticoids following challenge (15).

Our findings do not imply that insulin resistance is not critical in the development of glucose impairment and diabetes. HOMA of insulin resistance was associated with mortality independent of the covariates, although its predictive value substantially diminished after adjusting for 120-min glucose. Since calculating HOMA of insulin resistance does not require an OGTT, its clinical usefulness remains high. Interestingly, in preliminary analyses, fasting insulin was a significant, independent predictor of mortality. The ratio between fasting insulin and glucose during the OGTT is a marker of prompt homeostatic response, whereas fasting insulin depends on long-term response, which has 8–10 h to reach a steady state and is also influenced by change in hormonal level characteristic of the morning awakening (e.g., cortisol, catecholamines). The insulin level required to reach steady state over a long time period may be a better indicator of overall homeostatic capability. In addition, the insulin assay used in this study detects the proinsulin des (64–65) and proinsulin split (65–66), which may partially mask the effect of insulin on mortality.

The BLSA population is well-educated and tends to be health-seeking, which may limit generalizability of these findings to similar populations. However, previous reports from the BLSA involving

glucose (2,3,16) and other systems (6,17,18) have been consistent with observations from more general populations. In conclusion, consistent with the study by Sorkin et al. (3), we find that the OGTT is an important tool for assessing glucose homeostasis. Information provided by fasting glucose can be usefully complemented by evaluating 120-min glucose. Although investigators have proposed that the shape of the OGTT response curve (19) and OGTT indexes of insulin release and sensitivity may be informative (20,21,22), we found no evidence in support of these hypotheses.

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