Online Appendix – Modeling methods

ß-cell function ß-cell function was resolved from the OGTT using a mathematical model that describes the relationship between insulin secretion and glucose concentration, which has been illustrated in detail previously (1). The model expresses insulin secretion (in pmol per min per square meter of body surface area) as the sum of two components. The first component represents the dependence of insulin secretion on absolute glucose concentration at any time point during the OGTT, and is characterized by a dose-response function relating the two variables. The characteristic parameter of the dose-response, *i.e.*, the mean slope within the observed glucose range, is denoted as *ß-cell glucose sensitivity* by analogy with insulin sensitivity (slope of the dose-response of insulin-mediated glucose uptake *vs* insulin concentrations). Thus, glucose sensitivity as used here is not meant to measure the multiple cellular phenomena responsible for glucose sensing (or stimulus/secretion coupling) but only as a metrics to quantify the *in vivo* sensitivity of insulin secretion to glucose changes. In the mathematical model, the dose-response is modulated by a potentiation factor, which accounts for the fact that during an acute stimulation insulin secretion is higher on the descending phase of hyperglycemia than at the same glucose concentration on the ascending phase. As such, the potentiation factor encompasses several potentiating mechanisms (prolonged exposure to hyperglycemia, non-glucose substrates, gastrointestinal hormones, neural modulation). The potentiation factor is set to be a positive function of time, and is constrained to average unity during the experiment; thus, it expresses the relative potentiation of the secretory response to glucose. In normal subjects, the potentiation factor typically increases from the baseline value to the end of a 2-hour OGTT. The second insulin secretion component represents the dependence of insulin secretion on the rate of change of glucose concentration. This component is termed derivative component, and is expressed by a single parameter, denoted as *rate sensitivity*. Rate sensitivity is related to early insulin release (2). The model parameters were estimated from glucose and C-peptide concentration by regularized least-squares, as previously described (1; 3). Regularization involves the choice of smoothing factors which were selected to obtain glucose and C-peptide model residuals with standard deviations close to the expected measurement error (~1% for glucose and ~4% for C-peptide). Insulin secretion rates were calculated from the model every 5 min. The integral of insulin secretion during the 2-hour OGTT is denoted as *insulin output*. In this paper, only fasting insulin secretion rate, insulin output and ß-cell glucose sensitivity are presented.

Insulin sensitivity Insulin sensitivity was calculated using the Oral Glucose-derived Insulin Sensitivity index (OGIS) (4), which provides a validated estimate of the glucose clearance (in ml per min per square metre of body surface area) during the insulin-stimulated conditions of the euglycemic hyperinsulinemic clamp. Because insulin concentrations were not measured during the OGTT, a surrogate value was obtained from C-peptide concentrations by using a transformation derived from the comparison between insulin and C-peptide during the intravenous glucose tests of the DPT-1 study (n=5300). The predictive equation was insulin = $6.01 \cdot 10^{-5} \cdot (\text{C-peptide})^2 + 0.2094 \cdot (\text{C-peptide}) + 17.0$.

Time series In the subjects (n=208) with four or more OGTTs (median of 7 [4], for a total of 1903 tests), the time course of 2-hour glucose concentrations, ß-cell function parameters, and insulin sensitivity was analyzed using a function of time capable of representing a biphasic pattern with an initial phase in which the variable changes slowly with time and a late phase in which the change is accelerated. This five-parameter function, f(t), has the following expression:

$$f(t) = p_3 G + (p_4 - p_3)\frac{\frac{\log(\cosh(p_1(t-p_2)))-\log(\cosh(p_1 p_2))}{p_1} + \tanh(p_1 p_2)t}{1+\tanh(p_1 p_2)} + p_5 \quad (1)$$

The function is characterized by an initial slope, a final slope, a transition time, and a curvature around the transition time (as exemplified in Figure 3 of the main text). The transition time was defined as the time instant at which a significant change in slope was observed; this change was identified either at a maximum or a minimum of the function or at a time point in which the function increased (or decreased) beyond a pre-assigned threshold (2% of the data range) above (or below) the value extrapolated from the initial slope. The initial slope was obtained from the corresponding parameter of the biphasic function (p_3 in equation 1), while the final slope was calculated as the average slope over the last 0.2 years of the sequence. The parameters of the biphasic function were estimated with least squares from the sequences of the OGTT-derived indices in each individual subject.

Although the average time course of glucose concentrations was clearly biphasic (see Figure 4 of the main text), this was not the case in all subjects. To determine if a pattern was biphasic, we calculated the ratio of the standard deviation of the residuals obtained by fitting the data with the biphasic function to the corresponding standard deviation obtained using a single linear function. This index (SD ratio) is 1 when the pattern is linear (no difference in the residuals between the linear and biphasic function) and tends to zero (residual with the biphasic function much lower that that of the linear function) when the pattern is biphasic. A threshold of 0.75 for the SD ratio (corresponding to the 75th percentile of its distribution for both plasma glucose and glucose sensitivity values) was considered appropriate to distinguish between clearly biphasic sequences and linear or indeterminate patterns (see Table A1).

In progressors (n=30) with an unequivocally biphasic time course of 2-hour glucose concentrations, the transition times of ß-cell glucose sensitivity were positively associated with the transition times of 2-hour plasma glucose concentration (Figure A1). For both plasma glucose and glucose sensitivity, transition times were directly related to the length of the observation period (and, therefore, to the number of tests) before diabetes diagnosis or study end. Thus, shorter sequences had steeper final slopes more often than longer sequences, possibly reflecting the same underlying phenomenon, namely, rapid exhaustion of ß-cell functional reserve.

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Table A1 – Parameters of transition to diabetes.#

	Non-progressors (n=156)	**Progressors (n=52)**	***p*[§]**
2-hour plasma glucose (mmol/l)			
SD ratio	0.70 [0.26]	0.32 [0.50]	<0.0001
Initial slope (units/year)	0.17 [1.01]	0.15 [1.60]	ns
Final slope (units/year)	-0.15 [2.44]	12.69 [19.36]*	<0.0001
Transition time (years)	-1.20 [0.89]	-0.78 [1.10]	0.0166
Glucose sensitivity ($pmol \cdot min^{-1} \cdot m^{-2} \cdot mM^{-1}$)			
SD ratio	0.71 [0.33]	0.61 [0.35]	0.0195
Initial slope (units/year)	-0.2 [59.5]	2.8 [42.4]	ns
Final slope (units/year)	-8.2 [50.4]	-29.2 [27.2]*	<0.0001
Transition time (years)	-1.7 [1.8]	-1.5 [1.2]	0.0451
Insulin secretion ($pmol \cdot min^{-1} \cdot m^{-2}$)			
SD ratio	0.70 [0.29]	0.64 [0.37]	ns
Initial slope (units/year)	0.8 [48.8]	-0.6 [49.1]	ns
Final slope (units/year)	6.3 [37.3]	-10.5 [58.5]	0.0106
Transition time (years)	-1.7 [1.6]	-1.3 [1.2]	0.0083
Insulin sensitivity ($ml \cdot min^{-1} \cdot m^{-2}$)			
SD ratio	0.73 [0.26]	0.60 [0.42]	0.0046
Initial slope (units/year)	-9.6 [60.6]	-0.8 [53.6]	ns
Final slope (units/year)	-4.7 [61.0]	-60.4 [169.2]*	<0.0001
Transition time (years)	-1.7 [1.6]	-1.2 [1.2]	0.0002

SD ratio = ratio between the SD of the residuals of the biphasic function and the linear interpolation (1=no difference from linear function; 0.1=large difference)
[§] progressors *vs* non-progressors
* p<0.0001 *vs* initial slope

Figure A1 Positive association between the transition time of 2-hour plasma glucose concentrations and the transition time of ß-cell glucose sensitivity. The median values of the transition times are indicated by the arrows.