Appendix

The analysis of the glucose and C-peptide curves during the hyperglycemic clamps follows the general strategy described in a previous publication (1) with some modifications. The kinetics of C-peptide is described with a two-compartment model, in which the two pools (1 and 2) exchange with each other and the irreversibile loss of the hormone is from pool 1, the same where C-peptide concentration is measured. C-peptide kinetic parameters are computed according to the equations by Van Cauter et al. (2).
Herein are the equations describing the model of glucose induced insulin secretion during a hyperglycemic clamp:

$$dcp_1(t)/dt = ISR(t) + cp_2 \cdot k_{12} - (k_{01} + k_{21}) \cdot cp_1 \qquad (1)$$

where ISR = insulin secretion rate, cp_1 = C-peptide mass in the sampling (accessibile) compartment, cp_2 = C-peptide mass in the remote compartment, k_{12} and k_{21} = rate constants of the exchange between the two C-peptide compartments, and k_{01} = rate constant of the irreversibile loss of C-peptide from the accessibile compartment.

$$ISR(t) = BSR + SR1^{st}(t) + SR2^{nd}(t) \qquad (2)$$

where BSR = basal insulin secretion rate, $SR1^{st}$ = first-phase insulin secretion rate, and $SR2^{nd}$ = second-phase insulin secretion rate.

$$BSR = CP_b \cdot V_1 \cdot k_{01} \qquad (3)$$

where CP_b is basal C-peptide concentration and V_1 is the volume of the accessibile compartment of C-peptide.

$$SR1^{st}(t) = X1^{st}(t) \cdot \tau^{-1} \qquad (4)$$

$$dX1^{st}(t)/dt = \sigma 1^{st} \cdot \{[dG(t)/dt]/[\log(1.1+ t)]\} - X1^{st}(t) \cdot \tau^{-1} \quad \text{if } dG(t)/dt > 0 \qquad (5)$$

$$dX1^{st}(t)/dt = - X1^{st}(t) \cdot \tau^{-1} \quad \text{if } dG(t)/dt \le 0 \qquad (6)$$

where $\sigma 1^{st}$ = glucose sensitivity of first-phase insulin secretion, G = plasma glucose concentration, $X1^{st}$ = C-peptide (insulin) mass made available for first-phase insulin secretion, τ = time constant of first-phase insulin secretion, and the term $\log(1.1 + t)$ accomodates the time-associated decline of $\sigma 1^{st}$ documented in humans during a hyperglycemic stimulus. The response to the rate of increase of glucose is detected at the sampling site after a pure time delay (distinct from τ), which is another unknown parameter estimated by the model.

$$SR2^{nd}(t) = X2^{nd}(t) \cdot \delta^{-1} \qquad (7)$$

$$dX2^{nd}(t)/dt = \sigma 2^{nd} \cdot \{1 + \iota \cdot [(\int G(t) \cdot dt) - \alpha]\} \cdot [G(t) - \theta] - X2^{nd}(t) \cdot \delta^{-1}$$
$$\text{if } \{1 + \iota \cdot [(\int G(t) \cdot dt) - \alpha]\} \ge 1 \qquad (8)$$

$$dX2^{nd}(t)/dt = \sigma 2^{nd} \cdot [G(t) - \theta] - X2^{nd}(t) \cdot \delta^{-1} \quad \text{if } \{1 + \iota \cdot [(\int G(t) \cdot dt) - \alpha]\} < 1 \qquad (9)$$

where $\sigma 2^{nd}$ = glucose sensitivity of second-phase insulin secretion, $X2^{nd}$ = C-peptide (insulin) mass made available for second-phase insulin secretion, δ = time constant of second-phase insulin secretion, θ = glucose threshold above which β-cell responds with second-phase insulin secretion to plasma glucose concentration, ι is the glucose sensitivity of an additional component (a gain or "booster") of second phase secretion which comes into play when the integral of the hyperglycemic stimulus crosses a

threshold value equal to α. This model was implemented in the SAAM 1.2 software (3). The unknown parameters estimated by the model were: $\sigma 1^{st}$ = glucose sensitivity of first phase secretion, τ = time constant of first phase secretion, $\sigma 2^{nd}$ = glucose sensitivity of second phase secretion, δ= time constant of second phase secretion, ι = the gain in glucose sensitivity of second phase secretion due the integrative component, and α = the threshold for the appearance of the gain in glucose sensitivity of second phase secretion due the integrative component. For model identification, CP_b and θ were assumed to be equal to pre-test C-peptide and glucose concentrations, respectively.

The additional component (ι) of insulin secretion used in the present model is somewhat analogous to the potentiation factor of the insulin secretion model introduced by Mari and Ferrannini (4; 5) and to the integrative component of the PID (Proportional-Integrative-Derivative) model introduced by Steil and coll (6). The model we previously employed for the analysis of hyperglycemic clamps (1) used only equation 9 to describe the entire time course of second phase secretion, in close agreement with the model developed by Cobelli and Toffolo (7; 8), but the description of insulin secretion dynamics in children was somewhat wanting in the final part of the study (last 20-40 min).

The model used in the present paper was adopted after comparing its performance to the previous model in a data set of 97 hyperglycemic clamps, including also those used for the present publication. Two criteria were used: 1. the weighted residuals of the present model fit to the data were compared to the weighted residuals of the previous, more parsimonious model (table 1); 2. the Akaike information criterion (AIC) (9) was computed and used to compare the two models (table 2). AIC allows to compare models with a different number of variables, because it takes into account both the goodness of fit and the reduction in the degrees of freedom associated with the use of a greater number of variables to describe the same set of data (9). In both cases, the model used in the present paper showed a superior performance which reached the statistical significance.

Table 1. Weighted residuals of the earlier model and of the present model applied to the same set of hyperglycemic clamps. The closer to 0 are the weighted residuals, the better is the fit of the model to the data.

Weighted residuals of model fit to C-peptide data of hyperglycemic clamps (n=97)											
		Time (min)									
		2’	4’	6’	8’	10’	20’	40’	60’	80’	100’	120’
Earlier Model	Mean	-0.55	+1.01	+0.39	-0.91	-0.50	+1.43	-0.15	-0.72	-0.00	+0.34	+0.67
	SD	0.86	1.27	1.19	1.07	1.17	1.40	1.39	1.38	1.54	1.26	1.46
Present Model	Mean	-0.65	+1.13	+0.59	-0.85	-0.65	+0.67	+0.07	-0.05	+0.29	+0.02	-0.11
	SD	0.91	1.35	1.11	1.06	1.17	0.96	0.91	0.88	1.2	1.09	1.20
P Value by ANOVA	Overall: 0.003	0.45	0.53	0.23	0.69	0.38	0.001	0.20	0.001	0.13	0.06	0.001

Table 2. The Akaike Information Criterion median values of the earlier model (1) and of the present model applied to the same set of hyperglycemic clamps. On purely statistical grounds, the model with the lower Akaike Information Criterion is the one to be preferred (9).

Akaike Information Criterion			
	Earlier Model	Present Model	P Value (by Wilcoxon Test)
Median (I.Q. Range)	+ 0.39 (- 0.12 - + 0.75)	+ 0.16 (- 0.16 - + 0.56)	0.0001

Appendix References

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