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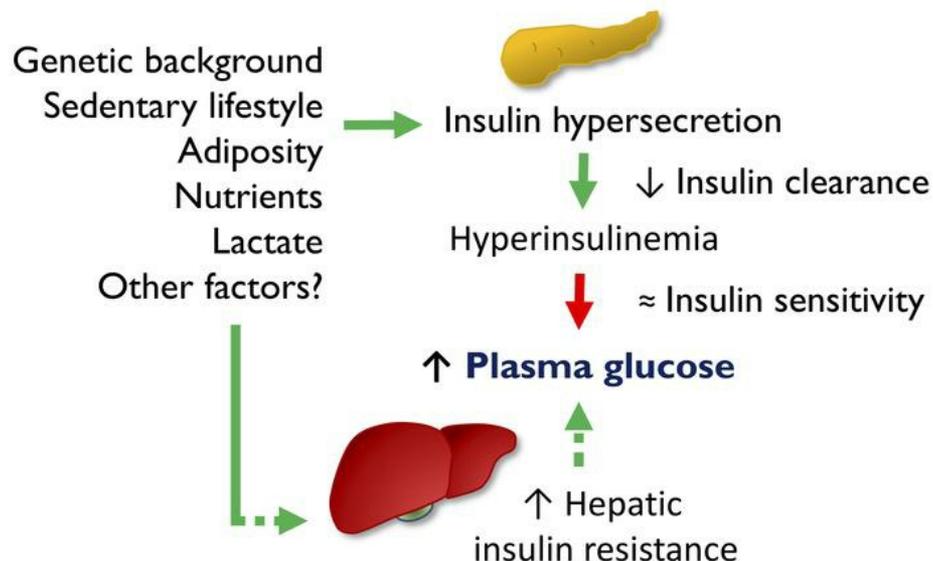
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Identification, pathophysiology, and clinical implications of primary insulin hypersecretion in nondiabetic adults and adolescents

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BACKGROUND. Excessive insulin secretion may lead to glucose dysregulation. Our aim was to identify primary (independent of insulin resistance) insulin hypersecretion in subjects with normal glucose tolerance and its role in the progression of dysglycemia.

METHODS. In 1,168 adults, insulin secretion rate (ISR) and β cell function were estimated by C-peptide modeling during an oral glucose tolerance test (OGTT) and an i.v. glucose tolerance test. Whole-body insulin sensitivity was measured by a hyperinsulinemic-euglycemic clamp. After regressing ISR on insulin sensitivity, subjects in the upper tertile of the distribution of residuals were defined as primary hypersecretors. This approach was applied to a biethnic cohort of 182 obese adolescents, who received an OGTT, a hyperglycemic, and a euglycemic clamp.

RESULTS. Adult hypersecretors showed older age, more familial diabetes, sedentary lifestyle, increased fat mass, and worse lipid profile compared with the rest of the cohort, despite virtually identical BMI and insulin sensitivity. Insulin secretion was increased by 53% due to enhanced (+23%) β cell glucose sensitivity. Despite the resulting hyperinsulinemia, glucose tolerance was worse in hypersecretors among both adults and adolescents, coupled with higher indices of liver insulin resistance and increased availability of gluconeogenic substrates. At the 3-year follow-up, adult hypersecretors had increased incidence of impaired glucose tolerance/type 2 diabetes.

CONCLUSION. Primary insulin hypersecretion, independent of insulin resistance, is associated with a worse clinical and metabolic phenotype in adults and adolescents and predicts deterioration of glucose control over time.

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Introduction

The prevailing view of the natural history of type 2 diabetes (T2D) is that an early decline of insulin sensitivity precedes and causes a progressive increase in insulin secretion, which initially compensates for insulin resistance (IR) to maintain glucose tolerance. At a later time, β cell function declines and hyperglycemia ensues (1). However, animal studies (2–8) and a few human studies (9–13) have suggested an alternative model, which postulates that, in some individuals, an inappropriate increase of insulin secretion, independent of IR, might be the first event triggering T2D progression (14–17). This paradigm, named primary insulin hypersecretion, hinges upon the demonstration that the hypersecretion is not just the compensatory response to IR. This requires that IR is directly and independently measured and that an unbiased criterion is then used to define the hypersecretory state, measured by an equally direct method.

Aims of the present study were (a) to identify primary insulin hypersecretion, (b) to describe the clinical and metabolic phenotype of subjects with primary insulin hypersecretion, (c) to assess its consequences for glucose homeostasis, and (d) to search for potential mechanisms. To this end, insulin secretion rate (ISR) and β cell function were estimated — by C-peptide/glucose modeling — in a large cohort of clinically healthy European Caucasians with normal glucose tolerance (NGT) during both an oral glucose tolerance test (OGTT) and an i.v. glucose tolerance test (IVGTT), while insulin sensitivity was measured by the hyperinsulinemic-euglycemic clamp technique. To validate the findings of this approach and to put them in the perspective of the natural history of T2D, we conducted a similar analysis on the euglycemic clamp data of an independent cohort of White American and African American adolescents in whom β cell function was estimated during both an OGTT and a hyperglycemic clamp.

Results

Adult cohort. In participants of the relationship between insulin sensitivity and cardiovascular disease (RISC) study ($n = 1,168$) (Supplemental Figure 1; supplemental material available online with this article; <https://doi.org/10.1172/jci.insight.124912DS1>), insulin sensitivity, expressed as the M value from the hyperinsulinemic-euglycemic clamp normalized by the steady-state plasma insulin concentrations (M/I), was 133 (interquartile range [IQR], 86) $\mu\text{mol}\cdot\text{min}^{-1}\cdot\text{kg}_{\text{FFM}}^{-1}\cdot\text{nM}^{-1}$ (median [IQR]). In the same subjects, total insulin output over the 2 hours of the OGTT (ISR_{OGTT}) was 39 [IQR, 17] nmol/m^2 . As shown in Figure 1A, the relationship between M/I and ISR_{OGTT} was reciprocal and nonlinear; the best fit of the data was a log-linear relationship ($\text{ISR}_{\text{OGTT}} = 99 - 12 \ln[\text{M}/\text{I}]$, $r = 0.43$, $P < 0.0001$), which was statistically superior to a linear or log-log fit. From this regression, primary insulin hypersecretion was defined as the ISR_{OGTT} values in the upper tertile of the distribution of the regression residuals (HyperS, $n = 389$). In the comparison between HyperS subjects and the rest of the cohort (NormS, $n = 779$), insulin sensitivity (M/I) was virtually identical, as expected (Table 1). Using the acute insulin response to i.v. glucose ($\text{AIR}_{\text{IVGTT}}$) as a measure of insulin secretion, HyperS can be similarly identified as the upper tertile of the distribution of the regression residuals against M/I (Figure 1B). Insulin hypersecretion identified by this approach was defined as primary to underscore its independence from insulin sensitivity, although it could be explained by other factors discussed below.

The clinical and metabolic phenotype of HyperS (on the OGTT) showed prevalence of women, marginally higher age (by 1.7 years, on average), higher percent fat mass for a similar BMI, more familial diabetes, and a worse serum lipid profile. In addition, γ -glutamyltransferase levels (γ -GT) levels were higher, and estimated physical activity was lower. In multiple logistic analysis, positive predictors of HyperS were female sex (odds ratio [OR], 2.74 [95% CI, 2.02–3.63]), post-OGTT glucose (OR, 1.89 [95% CI, 1.64–2.19]), and fasting triglycerides (OR, 1.34 [95% CI, 1.16–1.57]), while HDL cholesterol (OR, 0.84 [95% CI, 0.75–1.00]) was a negative predictor.

By design, all measures of insulin secretion — fasting as well as post-OGTT — were 40%–50% higher in HyperS than NormS and remained significantly higher after adjusting for anthropometric and metabolic differences (Table 2). Furthermore, the acute insulin response (AIR) to an i.v. glucose bolus ($\text{AIR}_{\text{IVGTT}}$) also was 26% enhanced in HyperS vs. NormS. With regard to β cell function, insulin hypersecretion was associated with a 23% increase in β cell glucose sensitivity (i.e., the relation of insulin secretion to glucose levels; β -GS) (Figure 2A), which persisted after controlling for baseline differences (Table 2). Despite similar insulin sensitivity and higher β -GS and insulin concentrations, glucose tolerance was worse in HyperS than NormS (Figure 3).

Adolescent cohort. As a validation data set, we analyzed a cohort of obese nondiabetic adolescents ($n = 182$), in whom an OGTT, a hyperinsulinemic-euglycemic clamp, and a hyperglycemic clamp were performed in each subject. In this group, the definition of HyperS was the same as that of the adult cohort, i.e., the top tertile of the residuals from the regression of ISR_{OGTT} on M/I ($\text{ISR}_{\text{OGTT}} = 144 - 23 \ln[\text{M}/\text{I}]$, $r = 0.52$, $P < 0.0001$). In this group, there was a higher prevalence of hypersecretors among White Americans than African Americans (Table 3). As in adults, both fasting and post-OGTT insulin secretion were ~70% higher in HyperS than NormS (Table 4). β -GS was enhanced by ~50% (Figure 2B), and rate sensitivity was also higher, to a similar extent; nonetheless, glucose tolerance was significantly worse (Figure 3). In line with this pattern, during the hyperglycemic clamp both the AIR (AIR_{HGC}) and the second phase insulin secretory response (ISR_{HGC}) were higher in HyperS than NormS (Figure 4). Furthermore, β -GS calculated from the hyperglycemic clamp data was increased by 43%, similar to the value derived from the OGTT.

Mechanisms of altered glucose tolerance in HyperS. When the rate of appearance of ingested glucose is the same, the only determinant of glycemia other than peripheral tissue glucose utilization (which the clamp-de-

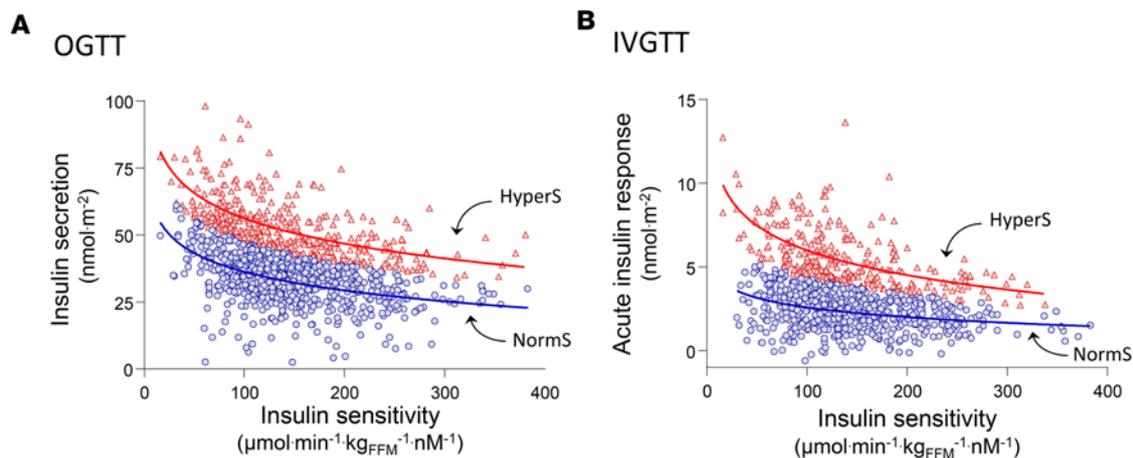


Figure 1. Identification of primary insulin hypersecretion. (A) Relationship between total insulin secretion during a 75-g OGTT (ISR_{OGTT}) and insulin sensitivity (M/I) and in the RISC study cohort ($n = 1,168$). (B) Relationship between M/I and acute insulin response during an i.v. glucose tolerance test (AIR_{IVGTT}) in the same cohort. Primary insulin hypersecretion was defined as either the ISR_{OGTT} or AIR_{IVGTT} values in the upper tertile of the distribution of the residuals from the regression of log-linear data ($ISR_{OGTT} = 99 - 12 \ln[M/I]$, $r = 0.43$, $P < 0.0001$, and $AIR_{IVGTT} = 10 - 14 \ln[M/I]$, $r = 0.35$, $P < 0.0001$, respectively). Using this cutoff, subjects were classified as hypersecretors (HyperS, red triangles; $n = 389$) or normosecretors (NormS, blue circles; $n = 779$).

rived M value measures) is endogenous glucose production (EGP). Though no direct measurements of EGP during the OGTT were available, the liver IR index (which has been shown to correlate with tracer-derived EGP measurements during fasting and clamp; ref. 18) was significantly higher in HyperS than NormS, a difference that persisted after multiple adjustments in both adults and adolescents (Table 5). In line with this was also a reduction in the metabolic clearance rate of insulin, which largely reflects hepatic insulin degradation (19). Finally, a proxy of hepatic steatosis, the fatty liver index (20) (available only in the adult cohort), was higher in HyperS than NormS, in agreement with their increased γ -GT levels (Table 1).

The higher liver IR index in HyperS aligns with the finding of raised circulating concentrations of gluconeogenic substrates, including lactate, arginine, and glutamate. The increased plasma levels of a major free fatty acids (FFA) (i.e., oleate) suggest an increased rate of lipolysis in HyperS vs. NormS (Table 5).

Of a total of 1,168 subjects in the adult cohort, 185 (16%) had impaired fasting glycemia (IFG) diagnosed according to the American Diabetes Association (ADA) criteria (fasting plasma glucose between 100–125 mg/dl). The prevalence of IFG was well balanced between HyperS and NormS (17% vs. 15%, respectively, $P = 0.31$). In addition, when we analyzed NGT and IFG separately, in both groups the liver IR index was still higher in HyperS than NormS (NGT, 2.14 [IQR, 0.45] vs. 1.85 [IQR, 0.02], respectively, $P < 0.0001$; IFG, 2.28 [IQR, 0.44] vs. 2.00 [IQR, 0.41], respectively, $P < 0.0001$).

Follow-up. In the adult cohort at follow-up ($n = 953$), significantly more HyperS than NormS progressed to impaired glucose tolerance (IGT) or T2D (17.4% vs. 10.3%, $P < 0.01$) (Figure 5). HyperS were ~80% more likely to develop dysglycemia than NormS (OR, 1.82 [95% CI, 1.24–2.69], $P = 0.003$). This finding persisted after adjustment for sex, age, percent fat mass, BMI, familial diabetes, fasting glucose, and M/I (OR, 1.55 [95% CI, 1.03–2.33], $P = 0.036$). Baseline differences in percent fat mass, HDL cholesterol, and triglycerides between HyperS and NormS were maintained at follow-up (Table 6). With regard to β cell function, post-OGTT insulin release was still higher in HyperS vs. NormS, but it was reduced as compared with baseline only in HyperS ($P = 0.0001$ for the time \times group interaction). This difference was also reflected in the post-OGTT plasma insulin concentrations ($P < 0.0001$ for the time \times group interaction). Likewise, β -GS was still higher in HyperS, but it was reduced as compared with baseline (though the group \times time interaction fell short of statistical significance).

Discussion

In this study, we identified primary insulin hypersecretion in normotolerant adults by separately measuring insulin sensitivity and secretion. The attribute of primary, therefore, only indicates hypersecretion that is not compensatory to IR. Moreover, while using the regression residuals to adjust secretion for sensitivity is a rigorous statistical method, the quantile criterion (i.e., upper tertile represents hypersecretors) is arbitrary,

Table 1. Clinical and metabolic characteristics of adult subjects with primary insulin hypersecretion (HyperS) or normal insulin secretion (NormS) in the RISC cohort^A

	HyperS (<i>n</i> = 389)	NormS (<i>n</i> = 779)	P value
M/I ($\mu\text{mol}\cdot\text{min}^{-1}\cdot\text{kg}_{\text{FFM}}^{-1}\cdot\text{nM}^{-1}$)	127 (91)	135 (83)	ns
M ($\mu\text{mol}\cdot\text{min}^{-1}\cdot\text{kg}_{\text{FFM}}^{-1}$)	53 (28)	55 (26)	ns
Whole-body glucose disposal ($\mu\text{mol}\cdot\text{min}^{-1}\cdot\text{kg}_{\text{FFM}}^{-1}$)	71 (39)	68 (33)	0.04
Sex (F/M, %)	61/39	52/48	0.005
Age (years)	44.7 ± 8.5	43.0 ± 8.2	0.001
BMI (kg/m^2)	25.6 ± 4.3	25.1 ± 3.7	ns
Waist/hip ratio (cm/cm)	0.85 ± 0.09	0.86 ± 0.11	ns
Fat mass (%)	28.8 ± 9.6	26.4 ± 8.7	<0.0001
Systolic BP (mmHg)	117 ± 12	117 ± 12	ns
Hypertension (yes/no, %)	4/96	3/97	ns
Physical activity (counts/ 1×10^3)	283 (148)	314 (186)	0.005
Smoker (yes/no, %)	58/42	53/47	ns
Alcohol consumption (yes/no, %)	85/15	84/16	ns
Familial diabetes (yes/no, %)	30/70	24/76	0.03
Fasting glucose (mmol/l)	5.1 ± 0.6	5.0 ± 0.5	0.02
Post-OGTT glucose (mmol/l)	7.1 ± 1.3	6.4 ± 1.2	<0.0001
Fasting insulin (pmol/l)	35 (26)	27 (19)	<0.0001
Post-OGTT insulin (pmol/l)	292 (190)	164 (95)	<0.0001
Fasting glucagon (pmol/l)	9.0 ± 4.0	8.9 ± 4.1	ns
Total cholesterol (mmol/l)	5.0 ± 0.9	4.8 ± 0.9	0.001
HDL cholesterol (mmol/l)	1.4 ± 0.4	1.5 ± 0.4	0.006
LDL cholesterol (mmol/l)	3.0 ± 0.8	2.8 ± 0.8	0.002
Triglycerides (mmol/l)	1.0 (0.7)	0.9 (0.5)	<0.0001
Fasting FFA (mmol/l)	0.5 (0.3)	0.5 (0.3)	ns
Steady-state FFA ($\mu\text{mol}/\text{l}$)	30 (35)	25 (25)	<0.0001
Alanine transaminase (U/l)	19 (14)	19 (13)	ns
Aspartate transaminase (U/l)	20 (8)	20 (8)	ns
γ -Glutamyltranspeptidase (U/l)	18 (16)	17 (13)	0.005

^AEntries are mean ± SD or median (interquartile range); BP, blood pressure; FFA, free fatty acids; HDL, high-density; LDL, low-density lipoprotein; M, average glucose infusion rate during the euglycemic clamp, normalized to the fat-free mass (FFM); M/I, M value normalized for the mean plasma insulin; OGTT, oral glucose tolerance test.

as would be choosing any other threshold. Within these boundaries, we could clearly identify 2 groups of subjects with virtually identical whole-body insulin sensitivity in whom the size and impact of primary insulin hypersecretion could be examined. Incidentally, using fasting rather than post-glucose insulin secretion in the analysis yielded essentially similar results (data not shown). To further test its consistency, the approach was applied to a cohort of youths from a biethnic background.

In adults, individuals identified as primary hypersecretors shared a clinical phenotype of increased adiposity, a more sedentary lifestyle, and mild dyslipidemia. As expected from the analysis strategy, measures of both fasting and glucose-stimulated insulin secretion were higher in both adult and adolescent hypersecretors as compared with the respective normosecretors: a ~30%–40% higher estimated daily basal insulin output (40 [IQR, 25] U/24 hours vs. 29 [IQR, 18] U/24 hours, $P < 0.0001$) and a ~45%–55% higher insulin release in response to oral glucose (16 [IQR, 7] U/2 hours vs. 11 [IQR, 5] U/2 hours, $P < 0.0001$). Importantly, these differences were significant after adjustment for individual anthropometric and metabolic characteristics. Moreover, augmented insulin secretion was also detected in response to more specific β cell stimuli such as the IVGTT and the hyperglycemic clamp, confirming the intrinsic nature of the β cell hypersecretion. The potentially novel, and unexpected, finding was that β -GS was enhanced in hypersecretors of both study cohorts (by ~20% in adults and ~50% in adolescents) in the face of reduced glucose tolerance (Figure 3). β -GS quantitates the dependence of insulin release on glucose concentrations during stimulation (Figure 2), regardless of IR (21). While IGT is generally associated with reduced β cell glucose sensing (22, 23), the association was paradoxically inverted in hypersecretors. Glucose rate sensitivity, an

Table 2. β Cell function in the adult cohort^A

	HyperS	NormS	P value ^B
Fasting ISR ($\text{pmol}\cdot\text{m}^{-2}\cdot\text{min}^{-1}$)	86 (42)	61 (29)	<0.0001
ISR _{OGTT} ($\text{nmol}\cdot\text{m}^{-2}$)	52 (12)	34 (11)	<0.0001
AIR _{IVGTT} ($\text{nmol}\cdot\text{m}^{-2}$)	3.63 (2.44)	2.89 (2.31)	<0.0001
β -GS ($\text{pmol}\cdot\text{min}^{-1}\cdot\text{m}^{-2}\cdot\text{mM}^{-1}$)	134 (89)	109 (74)	<0.0001
Potential factor (ratio)	1.75 (1.18)	1.71 (1.35)	ns
Rate sensitivity ($\text{pmol}\cdot\text{mM}^{-1}\cdot\text{m}^{-2}$)	950 (1,278)	699 (1,310)	0.0002

^A β -GS, β cell glucose sensitivity; ISR_{OGTT}, total insulin secretion during the 2-hour OGTT; AIR_{IVGTT}, acute insulin response to i.v. glucose; OGTT, oral glucose tolerance test. ^BAdjusted for center, age, sex, BMI, percent fat mass, and fasting plasma glucose. Entries are mean \pm SD or median (interquartile range).

index of early insulin release, also was increased in both adult and adolescent hypersecretors, thereby confirming the generalized hyperreactivity of the β cell in these individuals.

Previous clinical studies have argued for the occurrence of true insulin hypersecretion in prediabetes (10, 14–16) and for its primacy in the development of glucose intolerance (9–12, 14–17, 24, 25). However, in none of them has the effect of IR been adequately discounted. In fact, not only is IR strongly related to insulin secretion in a reciprocal manner (10), but the compensatory hyperinsulinemia feeds back negatively on insulin action. Prolonged hyperinsulinemia can lead to generalized IR by causing receptor and postreceptor defects (2, 3, 24, 26, 27). It also adversely affects body composition by increasing intracellular fat storage (24, 28). Furthermore, experimental euglycemic hyperinsulinemia applied for 72 hours does induce IR in human volunteers (29). More convincing evidence for primary hypersecretion has emerged from interventional clinical studies showing, for example, that diazoxide — an agonist of the K^+/ATP channel (30) — lowers plasma glucose levels at the same time as it selectively curtails insulin secretion (30–33). Likewise, early after bariatric surgery, both plasma glucose and insulin concentrations fall markedly before there is any detectable change in insulin sensitivity (16, 34, 35). Conversely, short-term (5 days) high-fat feeding in healthy volunteers raises fasting glucose levels with no change in peripheral insulin action (M from euglycemic clamps), muscle mitochondrial function, and general and specific oxidative phosphorylation gene expression (9). However, detailed analysis of β cell function (glucose sensitivity, rate sensitivity, and potentiation) has not been previously attempted, to our knowledge.

β Cell glucose hypersensitivity may be explained by multiple mechanisms: excess circulating substrates

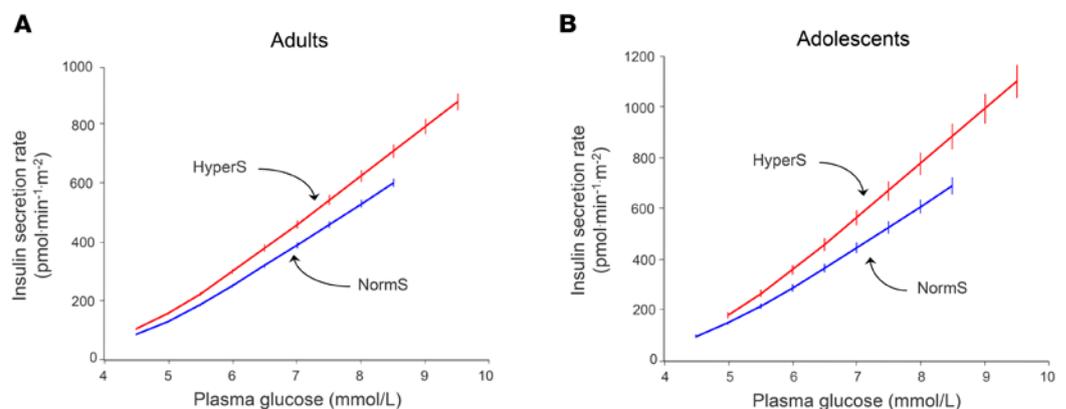


Figure 2. Dose-response of insulin secretion in adults and adolescents. (A) Dose-response of insulin secretion rate during a 75-g OGTT in hypersecretors (*HyperS*, red line, $n = 389$) and normosecretors (*NormS*, blue line, $n = 779$) in the RISC study cohort. (B) Dose-response of insulin secretion rate during a 75-g OGTT in *HyperS* ($n = 61$) and *NormS* ($n = 121$) in the adolescent group. Note the steeper slopes of adolescents vs. adults.

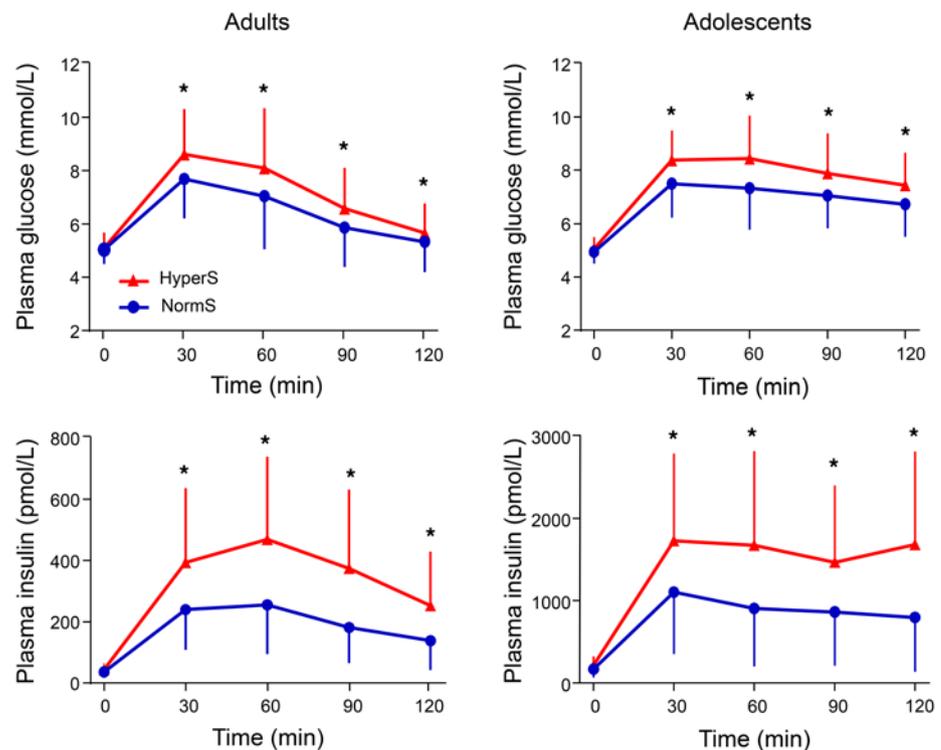


Figure 3. Plasma glucose and insulin concentrations during a 75-g OGTT in adults and adolescents. Insulin hypersecretors (*HyperS*) are shown as red triangles ($n = 389$ in adults and $n = 61$ in adolescents), while normosecretors (*NormS*) are shown as blue circles ($n = 779$ in adults and $n = 121$ in adolescents). Repeated-measure ANOVA was performed including group (*HyperS* vs. *NormS*), time, and group \times time interaction as factors, followed by post hoc pairwise comparisons using Tukey HSD tests. Plots are mean \pm SD. Asterisks denote values that are significantly ($*P \leq 0.05$) different between *HyperS* and *NormS*.

(36), such as lactate (16, 37), lipids (38–42), and amino acids (16, 39, 43, 44); genetic and ethnic factors (45–51); environmental agents (14, 52, 53); gut hormones (54, 55); and neural signals (56, 57). In particular, autonomic nervous system activity affects insulin secretion by the direct action on the β cell of several neurotransmitters (56–59), which also exert control on food intake (60). Of note, in genetic models of congenital hyperinsulinism (e.g., glucokinase activating mutations; refs. 61, 62), there is associated hypoglycemia, both fasting and postcibal, along with suppressed plasma FFA and ketone levels.

The combination of hyperinsulinemia and normal insulin sensitivity increases glucose disposal (Table 1) and should, consequently, reduce glucose levels. Therefore, the higher plasma glucose levels of hypersecretors can only be explained by an increased rate of endogenous glucose release (Table 1 and Table 3) or reduced hepatic glucose uptake (or both) in the face of hyperinsulinemia, descriptively hepatic IR. In fact, we show that, in both adults and adolescents, indices of hepatic IR were significantly increased in hypersecretors as compared with normosecretors even after controlling for confounders. Moreover, circulating levels of gluconeogenic substrates — such as lactate, alanine, and glutamate — were all raised, along with lipid substrates, which provide the energy for gluconeogenesis (63). Under conditions of increased glucose utilization, peripheral tissues release more lactate (64), thereby completing an overactive Cori cycle (and glucose-alanine cycle; ref. 65) as well as an effective energy shuttle (66). Except for the preserved peripheral tissue insulin sensitivity, the resulting metabolic picture resembles that of early T2D (67), including mild increments in serum triglycerides and liver enzymes (Table 1) (68).

Overall, the current findings are compatible with our proposed sequence schematized in Figure 6. Multiple factors determine β cell hypersensitivity and insulin hypersecretion. The more corpulent phenotype of the hypersecretors does suggest that increased energy intake may be a prevalent etiology among a host of inherited and acquired influences (69). Hepatic IR may ensue before peripheral IR (9), leading to inappropriate EGP and mild hyperglycemia, coupled with increased cycling of substrates between peripheral

Table 3. Clinical and metabolic characteristics of obese adolescents with primary insulin hypersecretion (HyperS) or normal insulin secretion (NormS)

	HyperS (n = 61)	NormS (n = 121)	P value
M/I ($\mu\text{mol}\cdot\text{min}^{-1}\cdot\text{kg}_{\text{FFM}}^{-1}\cdot\text{nM}^{-1}$)	36 (26)	40 (31)	ns
Sex (F/M, %)	64/36	60/40	ns
Age (years)	15 \pm 2	15 \pm 2	ns
BMI (kg/m^2)	34.9 \pm 4.9	35.1 \pm 6.3	ns
Ethnicity (W/AA, %)	67/33	45/55	0.006
Fat mass (%)	45 \pm 5	43 \pm 7	ns
NGT/IGT (%)	62/38	83/17	0.003
Fasting glucose (mmol/l)	5.1 \pm 0.4	4.9 \pm 0.4	0.018
Post-OGTT glucose (mmol/l)	7.7 \pm 1.0	6.9 \pm 1.0	<0.0001
Fasting insulin (pmol/l)	191 (180)	140 (113)	<0.001
Post-OGTT insulin (pmol/l)	1,249 (945)	644 (586)	<0.0001
Total cholesterol (mmol/l)	4.14 \pm 0.88	3.84 \pm 0.82	0.028
HDL cholesterol (mmol/l)	1.03 \pm 0.23	1.05 \pm 0.22	ns
LDL cholesterol (mmol/l)	2.51 \pm 0.73	2.26 \pm 0.73	ns
Triglycerides (mmol/l)	1.33 \pm 0.75	1.16 \pm 0.76	0.020

W, white; AA, African American; NGT, normal glucose tolerance; and IGT, impaired glucose tolerance. Entries are mean \pm SD or median (interquartile range).

tissues and the liver. The antecedence of hepatic over peripheral IR is physiologically compatible with the notion that the liver is about 3 times more sensitive to insulin than are peripheral tissues (70); the temporal dissociation is supported by studies showing more rapid development of hepatic than peripheral IR in response to short-term high-fat feeding in healthy volunteers (9).

The follow-up data in the adult cohort demonstrate that primary insulin hypersecretion is a risk factor for progression of dysglycemia. In fact, over just 3 years, insulin output and β -GS had declined (Table 6), and more subjects had progressed to glucose intolerance (Figure 5) in hypersecretors as compared with normosecretors.

One limitation of the statistical approach is that the actual prevalence of primary insulin hypersecretion cannot be established precisely. By way of example, if glucose-stimulated insulin secretion is adjusted for all its potential confounders and measured determinants (center, age, sex, BMI, M/I, and mean post-OGTT plasma glucose levels), the distribution of the residuals shows that only about 1 in 10 adults has

Table 4. β Cell function in obese adolescents^A

	HyperS	NormS	P value ^B
Oral glucose tolerance test (OGTT)			
Fasting ISR ($\text{pmol}\cdot\text{m}^{-2}\cdot\text{min}^{-1}$)	173 (67)	96 (58)	<0.0001
ISR _{OGTT} ($\text{nmol}\cdot\text{m}^{-2}$)	85 (30)	49 (19)	<0.0001
β -GS ($\text{pmol}\cdot\text{min}^{-1}\cdot\text{m}^{-2}\cdot\text{mM}^{-1}$)	183 (90)	124 (96)	<0.0001
Potentiator factor (ratio)	1.1 (0.3)	1.0 (0.5)	ns
Rate sensitivity ($\text{pmol}\cdot\text{mM}^{-1}\cdot\text{m}^{-2}$)	2,009 (1,780)	1,382 (1,541)	0.002
Hyperglycemic clamp^C			
AIR _{HCC} ($\text{nmol}\cdot\text{m}^{-2}$)	9.9 (5.6)	6.8 (4.4)	0.0001
ISR _{HCC} ($\text{nmol}\cdot\text{m}^{-2}$)	106 (35)	75 (28)	0.0001
2 nd -phase plasma insulin (pmol/l)	2,370 (1,650)	1,518 (1,380)	0.0001
β -GS _{HCC} ($\text{pmol}\cdot\text{min}^{-1}\cdot\text{m}^{-2}\cdot\text{mM}^{-1}$)	113 (52)	79 (33)	<0.0001

^AAIR_{HCC}, acute insulin response during the hyperglycemic clamp; β -GS_{HCC}, β cell glucose sensitivity during the hyperglycemic clamp; ISR_{HCC}, insulin secretion rate during the hyperglycemic clamp; M, average glucose infusion rate during the euglycemic clamp, normalized to the fat-free mass (FFM). ^BAdjusted for age, sex, race, BMI, percent fat mass, and plasma glucose concentration. ^Cn = 122. Entries are median (interquartile range).

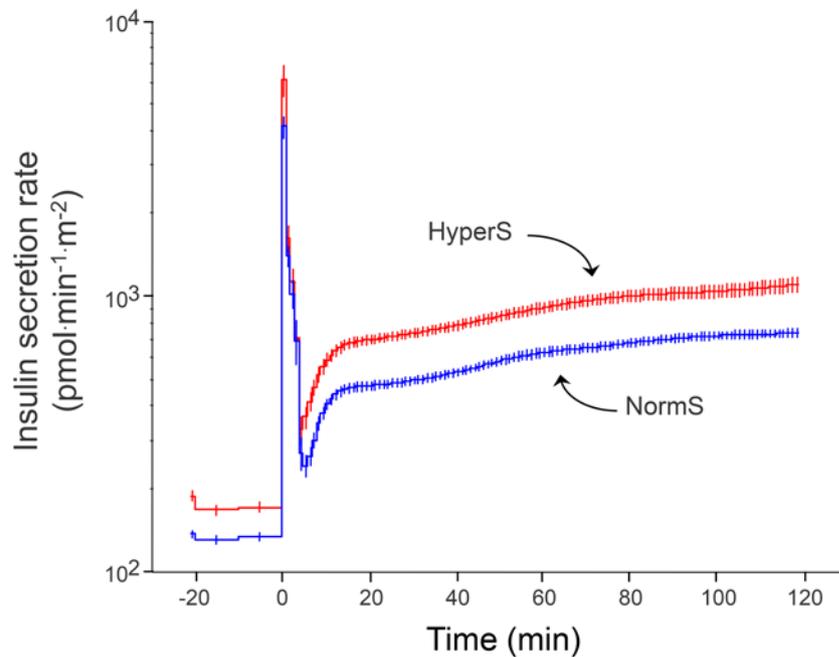


Figure 4. Insulin secretion rates during a hyperglycemic clamp in obese adolescents. Insulin hypersecretors (*HyperS*) are shown in red ($n = 61$), and normosecretors (*NormS*) are shown in blue ($n = 121$). Plots are mean \pm SEM.

a value above 1 SD of the entire cohort (i.e., an excess insulin secretion ranging from 14–50 $\text{nmol}\cdot\text{m}^{-2}$ during the 2-hour OGTT). Whether different clinical phenotypes would become apparent by using different threshold criteria is unlikely but cannot be ruled out.

Another limitation is that EGP was not directly measured and liver IR could only be estimated. Of note in this regard is that hypersecretors had very similar fasting glucose but definitely higher stimulated glucose levels in comparison with normosecretors (Figure 3 and Figure 5). This pattern could result from a difference in oral glucose absorption. However, it is unlikely that gastric emptying would be uniformly faster in hypersecretors without distorting the OGTT glucose profile (as is the case, for example, after bariatric surgery; ref. 34). Thus, the higher glucose excursions in the hypersecretors must result from impaired suppression of endogenous glucose release during the OGTT. While this is a probable mechanism, as demonstrated in the small study by Brøns et al. (9), its direct measurement (using multiple glucose tracers) in large numbers of subjects would be technically formidable. The observed differences in suppressed FFA (Table 1) and circulating gluconeogenic precursors, if numerically small, go toward supporting this mechanism in our subjects.

Another limitation is that the *HyperS* youths in this cohort did not show the differences in clinical phenotype emerging in adults (Table 3). A potential explanation is that, by selection, these adolescents were all obese (by ~ 10 BMI units more than the adults); however, we cannot rule out an effect of age per se. Finally, in our adolescents, insulin hypersecretion showed a slightly higher prevalence in White Americans than African Americans. The latter observation is apparently in contradiction with previous reports, showing that African Americans have higher fasting and glucose-stimulated insulin levels than White Americans (45, 47–49). However, hyperinsulinemia in African Americans is driven mainly by an approximately 15%–30% lower hepatic insulin clearance (47, 49) and by a greater IR (48, 49), which would reduce the relative contribution of primary insulin hypersecretion to the overall insulin concentration.

In conclusion, we identified primary insulin hypersecretion in normotolerant adults and adolescents and described its adverse metabolic effects independent of insulin sensitivity, suggesting potential underlying mechanisms. Our data support a causal pathogenic role of the hypersecretory state in the early derangements of glucose homeostasis that characterize the natural history of T2D, and they warrant further studies to identify mechanisms leading to primary insulin hypersecretion and potential therapeutic approaches.

Methods

Study design and participants. The RISC study is a multicenter, prospective, observational study with examinations at baseline ($n = 1,566$) and after a 3-year follow-up ($n = 1,059$). Details of study design and protocol have been reported (71). Participants were clinically healthy Europeans between 30 and 60 years of age. Exclusion criteria

Table 5. Liver indices and circulating substrates

	HyperS	NormS	P value ^A
Liver indices (adults)			
Liver insulin resistance index (units)	2.2 (0.5)	1.9 (0.4)	<0.0001
Fatty liver index (units)	22 (42)	16 (32)	0.009
Insulin clearance (l·min ⁻¹ ·m ⁻²)	1.5 (0.6)	1.7 (0.7)	<0.0001
Liver indices (adolescents)			
Liver insulin resistance index (units)	4.8 (0.4)	4.5 (0.4)	<0.0001
Insulin clearance (l·min ⁻¹ ·m ⁻²)	0.5 (0.3)	0.6 (0.3)	ns
Gluconeogenic substrates (adults)			
Lactate (R.I.) ^B	48.3 (27.9)	44.8 (20.0)	0.002
Alanine (R.I.) ^B	8.7 (3.8)	8.2 (3.3)	0.03
Arginine (μg/ml)	13.6 (4.3)	12.9 (4.1)	0.001
Glutamate (μg/ml)	15.5 (9.5)	13.7 (7.5)	<0.0001
Oleate (μg/ml)	82.7 (41.4)	78.6 (39.4)	0.02
Palmitate (μg/ml)	31.2 (14)	30.3 (14.1)	ns

^AInsulin sensitivity parameters are adjusted for center, age, sex, BMI, percent fat mass, and fasting plasma glucose.

^BR.I., relative intensity units (available in 399 subjects; ref. 72). Entries are median (interquartile range).

were chronic diseases (e.g., diabetes, dyslipidemia, hypertension, chronic lung, hepatic or kidney diseases, and neoplastic and inflammatory diseases), class III obesity, presence of carotid stenosis >40%, and treatment for hypertension, diabetes, dyslipidemia, obesity, and steroid therapy. A standardized examination protocol included anthropometry, blood pressure measurements, a fasting blood test, a hyperinsulinemic-euglycemic clamp, an OGTT, and an IVGTT. Brachial blood pressure was measured by a digital electronic tensiometer (model 705cp; Omron), with regular or large cuffs according to the arm circumference, in subjects seated for at least 10 minutes. Physical activity was measured objectively by a small single-axis accelerometer (Actigraph model AM7164-2.2; Computer Science and Applications). The acceleration signal was digitized with 10 samples per second, registered as counts over 1-minute intervals. Information regarding medical history, drug use, alcohol and cigarette consumption, and family history of diabetes (i.e., any first-degree family member with T2D) was collected using standardized self-reported questionnaires. For the purpose of this study, we excluded individuals with diabetes ($n = 28$) or IGT ($n = 119$) detected during the screening and those with missing information on the clamp-derived insulin sensitivity ($n = 208$) or OGTT-derived insulin secretion ($n = 43$). This resulted in a study population of 1,168 participants for the cross-sectional study. Among those, 215 individuals did not attend follow-up, resulting in a population of 953 participants for the prospective analysis (Supplemental Figure 1).

As a validation cohort, we analyzed data from 182 obese nondiabetic adolescents recruited at the Pediatric Clinical and Translational Research Center of the Children's Hospital of Pittsburgh. Participants were White American or African American adolescents between 10 and 20 years of age. All participants were in good health, as assessed by medical history, physical examination, and routine hematological and biochemical tests. Individuals with serious medical conditions or receiving treatments known to affect glucose or lipid metabolism were excluded.

GTTs and clamp procedures. In all participants, an OGTT with 75 g glucose (1.75 g/kg in adolescents, up to 75 g) was performed after an overnight fast. Blood samples were collected at baseline and at 30, 60, 90, and 120 minutes after glucose ingestion for measurement of glucose, insulin, and C-peptide concentrations. Insulin sensitivity was evaluated during a hyperinsulinemic-euglycemic clamp. Exogenous insulin was administered as a primed-continuous infusion at a rate of 240 pmol·min⁻¹·m⁻² in adults and 480 pmol·min⁻¹·m⁻² in adolescents. Plasma glucose was clamped at 4.5–5.5 mmol/l with a variable 20% dextrose infusion adjusted every 5 minutes. During the clamp, blood was sampled at least every 20 minutes for determination of insulin concentrations. To evaluate the AIR to i.v. glucose, an IVGTT was performed in 828 adults at the end of the hyperinsulinemic-euglycemic clamp. A glucose bolus (0.3 mg/kg body weight) was administered over 1 minute, and plasma glucose and C-peptide concentrations were measured at 2, 4, 6, and 8 minutes after the bolus. In adolescents, first- and second-phase ISRs were assessed during a 2-hour hyperglycemic clamp in which plasma glucose was increased rapidly to 12.5 mmol/l by a bolus infusion of 25% dextrose and maintained at that level by a variable rate infusion of 20% dextrose.

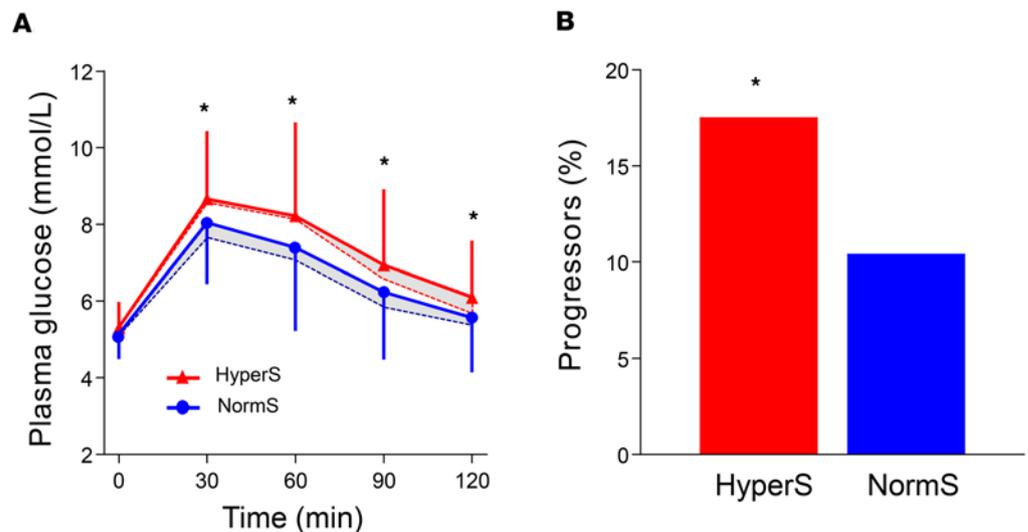


Figure 5. Glucose tolerance at follow-up in the adult cohort. (A) Plasma glucose concentrations during a 75-g OGTT at baseline (dotted lines) and follow-up (full lines) in insulin hypersecretors (*HyperS*, $n = 313$) and normosecretors (*NormS*, $n = 640$) in the RISC study cohort. Follow-up plasma glucose levels were analyzed by repeated-measure ANOVA including group (*HyperS* vs. *NormS*), time, and group \times time interaction as factors, followed by post hoc pairwise comparisons using Tukey HSD tests. Plots are mean \pm SD. (B) Incidence of dysglycemia at follow-up (progressors) in *HyperS* and *NormS* in the RISC study cohort. Asterisks denote values that are significantly ($*P \leq 0.05$) different between *HyperS* and *NormS*.

Insulin sensitivity, insulin secretion, and insulin clearance parameters. Whole-body insulin-mediated glucose disposal was calculated as the average glucose infusion rate (M value) during the final 40 minutes of the hyperinsulinemic-euglycemic clamp, normalized to the fat-free mass (FFM); insulin sensitivity was calculated as the ratio of M to the mean plasma insulin concentration measured during the same interval (M/I, in units of $\mu\text{mol}\cdot\text{kg}_{\text{FFM}}^{-1}\cdot\text{min}^{-1}\cdot\text{nM}^{-1}$). Whole-body glucose disposal during the OGTT was estimated as the product of insulin-mediated glucose clearance (M/steady-state plasma glucose during the final 40 minutes of the euglycemic clamp) and the mean plasma glucose concentration during the OGTT. ISR (expressed in $\text{pmol}\cdot\text{min}^{-1}\cdot\text{m}^{-2}$) was estimated from C-peptide deconvolution (72), and total insulin output was calculated by integrating ISR over the duration of the test. β Cell function parameters were calculated by mathematical modeling of ISR and glucose concentrations, as previously reported (73). This model describes the relationship between ISR and glucose concentration as the sum of 2 components. The first component represents the dependence of ISR on absolute glucose concentration through a dose-response function relating the 2 variables. The slope of this dose-response function, named β -GS, is calculated. The dose-response is modulated by a potentiation factor, which accounts for the physiological processes that can acutely modify insulin secretion (e.g., protracted hyperglycemia, nonglucose substrates, gastrointestinal hormones, neural modulation). The potentiation factor, which is set to average 1 during the entire test, measures the relative potentiation or inhibition of ISR; its excursion is quantified by the ratio between the 2-hour and the baseline value (potentiation ratio). The second component of β cell function represents the dependence of ISR on the rate of change of glucose concentration and is determined by a single parameter (rate sensitivity), which is related to early insulin release. With the IVGTT, the AIR to i.v. glucose (8 minutes) was expressed as the incremental insulin secretion/glucose area ratio over the same time interval (in $\text{nmol}\cdot\text{m}^{-2}$). During the hyperglycemic clamp, first-phase ISR (AIR) was calculated as the AUC between 2.5 and 12.5 min, while second-phase ISR was calculated from 15–120 minutes.

Insulin clearance, expressed in $\text{l}\cdot\text{min}^{-1}\cdot\text{m}^{-2}$, was calculated as the ratio of the average ISR during the OGTT and the average plasma insulin levels during the OGTT.

Definitions. To identify primary insulin hypersecretion, the M/I value from the hyperinsulinemic-euglycemic clamp was regressed against the total insulin output over the 2-hour OGTT (ISR_{OGTT}) (Figure 1). Primary insulin hypersecretion was defined as the ISR_{OGTT} values in the upper tertile of the distribution of

Table 6. Clinical and metabolic characteristics of adults with primary insulin hypersecretion (HyperS) or normal insulin secretion (NormS) at follow-up^A

	HyperS (n = 313)		NormS (n = 640)		P value ^A		
	Follow-up	Δ	Follow-up	Δ	Group	Time	G × T
BMI (kg/m ²)	25.8 ± 4.3	+0.4	25.1 ± 4.8	+0.4	ns	<0.0001	ns
Fat mass (%)	30.0 ± 8.9	+8.3	27.5 ± 9.2	+10.4	0.0002	<0.0001	ns
Systolic BP (mmHg)	121 ± 16	+3	120 ± 15	+3	ns	<0.0001	ns
Fasting insulin (pmol/l)	36 (27)	+1	29 (20)	+2.5	<0.0001	0.04	ns
PostOGTT insulin (pmol/l)	284 (203)	-14	178 (107)	+29	<0.0001	0.046	<0.0001
Total cholesterol (mmol/l)	5.0 ± 0.9	0	5.0 ± 0.9	+0.1	ns	0.007	0.006
HDL cholesterol (mmol/l)	1.4 ± 0.4	0	1.5 ± 0.4	0	0.01	0.01	ns
LDL cholesterol (mmol/l)	3.0 ± 0.8	0	3.0 ± 0.8	+0.1	ns	ns	0.003
Triglycerides (mmol/l)	1.0 (0.9)	0	0.9 (0.6)	+0.1	<0.0001	0.03	0.046
Fasting FFA (mmol/l)	0.6 (0.3)	+0.1	0.6 (0.3)	+0.1	ns	<0.0001	ns
Fasting ISR (pmol·m ⁻² ·min ⁻¹)	84 (48)	0	63 (29)	+3	<0.0001	ns	ns
ISR _{OGTT} (nmol/m ²)	50 (21)	0	37 (15)	+4	<0.0001	ns	0.0001
β-GS (pmol·min ⁻¹ ·m ⁻² ·mM ⁻¹)	125 (91)	-8	108 (76)	+3	<0.0001	ns	ns
Potential factor (ratio)	1.7 (1.3)	-0.1	1.7 (1.3)	-0.1	ns	0.03	ns
Rate sensitivity (pmol·mM ⁻¹ ·m ⁻²)	980 (1,400)	+168	788 (1,208)	+107	<0.0001	0.04	ns

Δ, mean change from baseline. ^ARepeated-measures ANOVA for the effect of Group (HyperS vs. NormS), Time, and Group × Time interaction (G × T). Entries are mean ± SD or median (interquartile range).

the residuals from the regression of log-linear data ($ISR_{OGTT} = 99 - 12 \ln[M/I]$, $r = 0.43$, $P < 0.0001$ for adults; $ISR_{OGTT} = 144 - 23 \ln[M/I]$, $r = 0.52$, $P < 0.0001$ for adolescents), which was statistically superior to a linear or log-log fit.

NGT (fasting glucose < 7.0 mmol/l and 2-hour glucose < 7.8 mmol/l), IFG (fasting glucose between 5.6–7.0 mmol/l and 2-hour glucose < 7.8 mmol/l), IGT (fasting glucose < 7.0 mmol/l and 2-hour glucose between 7.8 and 11.1 mmol/l), and T2D (fasting glucose ≥ 7.0 mmol/l and 2-hour glucose ≥ 11.1 mmol/l) were defined according to the 2018 ADA criteria (74). Based on the observed changes of glucose tolerance at follow-up, participants were classified as progressors if they stepped up from NGT to IGT or T2D.

Surrogate markers of hepatic IR and liver fat content. The liver IR index was calculated in adults and adolescents with an algorithm based on OGTT insulin levels, percent fat mass, BMI, and HDL cholesterol (18). The liver IR index has been shown to correlate with the tracer-derived EGP relative to fasting plasma insulin (EGP × FPI) at baseline ($r = 0.65$, $P < 0.001$) and during clamp ($r = 0.59$, $P < 0.001$) in nondiabetic subjects (18).

The fatty liver index, available only in the adult cohort, was calculated from BMI, waist circumference, triglycerides, and γ-GT (20). The fatty liver index has been validated against liver ultrasound and has been proven an accurate predictor of hepatic steatosis in the general population (accuracy = 0.84 [95% CI, 0.81–0.87]) (20).

Body composition. Body composition and FFM were assessed using a Body Composition Analyzer (model TB-300; Tanita) in adults and by dual-energy X-ray absorptiometry in adolescents. Waist circumference was measured as the narrowest circumference between the lower rib margin and anterior superior iliac crest. Hip circumference was measured around the widest portion of the buttocks, and the waist/hip circumference ratio was calculated.

Biochemical measurements. Plasma glucose was measured by the glucose oxidase technique. Plasma samples were divided into aliquots and stored at –80°C until analysis. Plasma insulin and C-peptide were measured by fluoroimmunoassay in adults (AutoDELFIA Insulin kit; Wallac Oy) and by radioimmunoassay in adolescents (Linco, catalog 1011). Plasma FFA were measured in the fasting state and at timed intervals during the hyperinsulinemic-euglycemic clamp by a fluorimetric method (Wako); the values during the last 40 minutes of the clamp were averaged to express insulin inhibition of circulating FFA (steady-state FFA). Plasma concentrations of arginine, glutamate, oleate, and palmitate were measured by targeted metabolomics. Plasma lactate and alanine were measured in 399 adult participants using a mass spectrometry, nontargeted biochemical profiling approach, as previously reported (75).

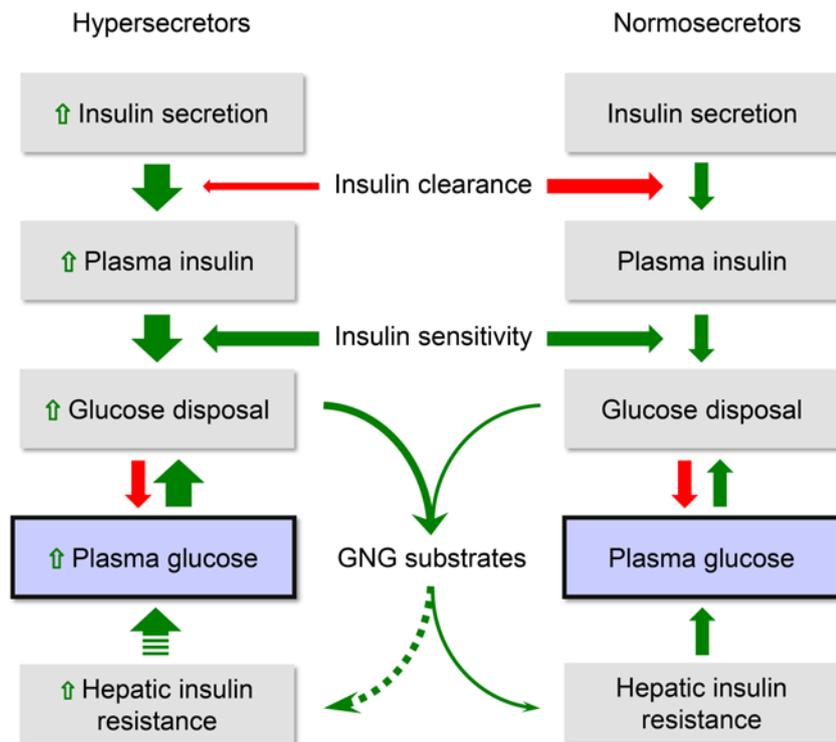


Figure 6. Schematic summary of the insulin/glucose system in primary insulin hypersecretors and normosecretors. Green arrows indicate stimulation, and red arrows denote inhibition. Dotted arrows indicate speculative mechanisms.

Statistics. Continuous variables are presented as mean \pm SD, and nominal variables are expressed as percentages. Variables with a skewed distribution are presented as median (interquartile range; IQR) and were log-transformed in multivariate analyses. Differences between groups were tested by Mann-Whitney U test for continuous variables or χ^2 for nominal variables. General linear models were used to test for differences while controlling for potential confounders. Multivariate logistic regression was used to identify factors associated with the hypersecretory state; OR and 95% CI were reported. Repeated measures were analyzed by 2-way repeated-measure ANOVA including group (HyperS vs. NormS), time (either “time during the OGTT” or “baseline vs. follow-up”), and group \times time interaction as factors. Post hoc pairwise comparisons were performed by Tukey’s honest significant difference (HSD) tests. Statistical analyses were performed using JMP Pro 13.2.1 software (SAS Institute). A 2-sided $P < 0.05$ was considered statistically significant.

Study approval. The studies were conducted according to the principles expressed in the Declaration of Helsinki and approved by the local ethics committee of each center. Written informed consent was obtained prior to recruitment from all adult participants, as well as from parents of adolescent participants, who also gave their written informed assent.

Author contributions

DT contributed study design, data analysis and interpretation, and manuscript write-up; AN contributed study design, data collection, analysis and interpretation, and manuscript editing; SA contributed data collection and manuscript editing; AM contributed mathematical modeling of insulin secretion and β cell function parameters, data analysis and interpretation, and manuscript editing; EF contributed study design and supervision, funding, data collection, analysis and interpretation, manuscript write-up, and critical revision. All authors read and approved the final submitted version of the manuscript. DT and EF are the guarantors of this work and, as such, have full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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