

Original Articles**Natural History of Glucose Homeostasis and β -Cell Function from Childhood to Early Adulthood in Transfusion-Dependent β -Thalassemia Patients with Early Iron Chelation: A Ten-Year Longitudinal Cohort Study**

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Abstract. Background: Iron overload from chronic transfusion therapy progressively impairs pancreatic β -cell function and insulin action in transfusion-dependent β -thalassemia (β -TDT), but longitudinal data from childhood through early adulthood are scarce.

Objectives: To describe, in a single retrospective cohort of patients with β -TDT followed for ten years, (i) the annual evolution of oral glucose tolerance test (OGTT)-derived glycemic values and (ii) insulin resistance and β -cell function indices from late childhood to early adulthood.

Methods: Twenty-three patients with TDT (12 males, 11 females) were followed from mean age of 10.2 ± 0.9 years (baseline) to 20.8 ± 0.85 years (ten-year follow-up). All patients received subcutaneous iron chelation with deferoxamine (DFO) monotherapy throughout. A standardized OGTT was done yearly. Plasma glucose and serum insulin were measured at 0, 30, 60, and 120 minutes after glucose load to evaluate glucose homeostasis and indices of insulin secretion and sensitivity. Results were compared with contemporaneous healthy controls (two separate, non-overlapping cross-sectional control groups at baseline and at ten years). Pearson and Spearman correlations examined bivariate relationships between glycemic and insulin parameters using one observation per patient ($n = 23$); the 230 annual OGTTs were analyzed with linear mixed-effects models, generalized estimating equations (GEE) and Kaplan–Meier estimates.

Results: In the whole cohort, fasting glucose rose from 91.6 ± 11.6 to 94.6 ± 10.7 mg/dL, 30-minute post-load glucose from 137.6 ± 26.8 to 147.6 ± 20.1 mg/dL, 60-minute and 120-minute post-load glucose (113.5 ± 23.5 to 147.6 ± 31.4 mg/dL and 101.6 ± 16.9 to 132.4 ± 15.3 mg/dL, respectively). HOMA1-% β fell from 119.8 ± 72.1 to 62.6 ± 24.9 , while HOMA1-IR declined from 1.81 ± 0.78 to 1.26 ± 0.59 , and ISSI-2 showed no linear trend, declining until about year 5 before partly recovering (1.82 ± 0.58 to 1.87 ± 0.57). At baseline, fasting glucose correlated positively with HOMA-IR ($r: 0.46$, $P = 0.027$) and inversely with HOMA1-% β ($r: -0.69$, $P < 0.001$) and ISSI-2 ($r: -0.72$, $P < 0.001$); 30-minute post-load glucose at baseline was associated with 1-hour post-load glucose at ten years ($r: 0.6545$, $P < 0.001$; $n = 23$). At 10 years of follow-up, dysglycemia was present in 11/23 patients (47.8%); 3 isolated impaired fasting glucose [IFG], 5 isolated impaired glucose tolerance [IGT], 3 combined IFG plus IGT, more frequently in males than in females (8/12 vs.

3/11; Fisher's exact test, $P = 0.10$). Notably, HOMA1-% β was significantly lower than in controls at ten years ($P < 0.0001$), whereas ISSI-2 was significantly lower than in controls at baseline and after 10 years of follow-up ($P < 0.0001$ and 0.0042 , respectively). No significant difference in the prevalence of dysglycemia between patients with good (mean serum ferritin: 607.4 ± 180.3 $\mu\text{g/L}$) and poor compliance (mean serum ferritin: 1998.6 ± 886.3 $\mu\text{g/L}$) with iron chelation therapy with DFO was documented (5/11 vs. 6/12; χ^2 : 0.0475, $P = 0.82$). In mixed-effects models of the annual OGTTs, 1-h and 2-h PG rose by 4.5 and 3.4 mg/dL per year (both $P < 0.001$), and 21 of 22 initially normoglycemic patients had a 1-h PG ≥ 155 mg/dL at least once (median 6 years). After adjusting for follow-up time, serum ferritin was not associated with glycemic status or the β -cell index.

Conclusions: Deterioration in glucose tolerance over the study period appeared to be associated mainly with a decline in surrogate indices of insulin secretory capacity rather than with a progressive increase in insulin resistance (IR), although HOMA- and OGTT-derived indices do not measure β -cell function directly. Reduced surrogate indices of β -cell function were already evident in childhood and were associated with an earlier, higher elevation of plasma glucose at 30 minutes after glucose load compared with controls. OGTT-derived indices of insulin secretion and sensitivity could help tailor screening intensity.

Keywords: Transfusion-dependent β -thalassemia; Glucose homeostasis; Insulin resistance; β -cell function; Deferoxamine; 1-h PG after glucose load; HOMA 1-IR; HOMA1-% β ; ISSI-2; Longitudinal cohort.

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Introduction. Transfusion-dependent β -thalassemia (β -TDT) requires lifelong regular blood transfusion, which corrects severe anemia but leads to progressive iron accumulation in multiple organs, including the endocrine pancreas.^{1,2} Iron deposition in pancreatic β -cells is thought to impair insulin secretion over time, while iron accumulation in the liver and other tissues may additionally affect insulin sensitivity.¹ Disturbances of glucose homeostasis in this setting can range from isolated impaired fasting glucose (IFG) to impaired glucose tolerance (IGT) and, in some patients, overt diabetes mellitus; a pooled meta-analysis of more than 16,000 patients with β -thalassemia major estimated the prevalence of diabetes mellitus at 6.5%, with IFG and IGT affecting a further 17.2% and 12.5%, respectively.³ A large ten-year retrospective cohort from England similarly identified diabetes as one of the three most prevalent non-hematologic complications of β -TDT, alongside cardiac disease and osteoporosis.⁴

Because these abnormalities are thought to accumulate gradually over many years of transfusion and chelation therapy, cross-sectional assessments at a single timepoint may not adequately capture how glucose homeostasis changes within the same individual as they move from childhood into early adulthood.^{5,6} The

International Network of Clinicians for Endocrinopathies in Thalassemia and Adolescent Medicine (ICET-A) has published position statements and recommendations emphasizing that glucose dysregulation in β -thalassemia typically evolves gradually, beginning with isolated IFG or IGT and, in a subset of patients, culminating in frank diabetes mellitus later in life.⁵ Longitudinal, within-patient data spanning the transition from childhood to adulthood — during which chelation adherence, pubertal development, and cumulative iron burden may all evolve simultaneously — are needed to clarify whether the dominant abnormality driving glycemic change is a decline in insulin secretory capacity, an increase in insulin resistance, or both, and whether chelation compliance modifies this trajectory.⁷⁻⁹ For a more accurate risk prediction of dysglycemia [impaired fasting glucose (IFG), impaired glucose tolerance (IGT), IFG plus IGT, and 1-h PG concentration ≥ 155 mg/dL as an additional simple marker for the development of dysglycemia], this retrospective study describes the timing of early changes in glucose metabolism, insulin resistance and β -cell function indices [homoeostasis model assessment (HOMA1-IR) of insulin sensitivity, HOMA1-% β of β -cell function, the insulinogenic index (IGI, the ratio of

the 30-minute increment in insulin to the 30-minute increment in glucose), the oral disposition index ($\text{oDI} = \text{IGI} \times \text{Matsuda index}$) and the insulin secretion-sensitivity index-2 (ISSI-2)] in patients with β -TDT who underwent a standardized annual oral glucose tolerance test (OGTT) from childhood to early adulthood.

Methods

Study design and patients. This single-center, retrospective longitudinal cohort study included 23 patients with transfusion-dependent β -thalassemia (12 males and 11 females). Patients were initially evaluated at a mean age of 10.2 ± 0.9 years, and final study evaluation was done after a mean follow-up of ten years, at a mean age of 20.8 ± 0.85 years.

All patients received regular red blood cell transfusions, with pre-transfusion hemoglobin levels maintained within the range observed during the study period (8.8 ± 0.24 g/dL at baseline and 9.1 ± 0.27 g/dL at ten years). Throughout the entire observation period, all patients received iron chelation with subcutaneous deferoxamine (DFO) monotherapy, with no other iron chelator used.

Regular subcutaneous DFO infusions were initiated around the age of 2 years. Initially, DFO was administered at a dose of 20 mg/kg body weight daily, usually overnight, using a micropump over 8–10 hours in patients receiving 2–4 units of packed red blood cells per month. The DFO dose was subsequently increased gradually to 40–50 mg/kg/day according to the ongoing transfusional iron burden.

Serum ferritin trends were reviewed at three-month intervals, and DFO dosing was adjusted accordingly. Patients' interviews to determine adherence to chelation therapy were conducted monthly at the time of blood transfusion, and patients were reminded of the importance of regular chelation. Based on agreement between the self-reported and prescribed chelation regimen, compliance was arbitrarily considered good when chelation therapy was given 5–6 days a week, moderate when given 4 days a week, and poor when given less than 3 days a week.

A minority of patients had undergone splenectomy (4/23, 17.4% at baseline; 6/23, 26.1% by ten years), and a small proportion had a positive family history of diabetes mellitus (type 1 or 2) at baseline (3/23, 13.0%), rising to 5/23 (21.7%) by the study endpoint. Mean age at first blood transfusion was 7.7 ± 5.0 months.

Study protocol: a. Inclusion and exclusion criteria. Patients were eligible for inclusion if they had a confirmed diagnosis of transfusion-dependent β -thalassemia, had received subcutaneous deferoxamine (DFO) monotherapy continuously from the age of 2 years without switching to or adding another iron chelator during the study period, and had complete

paired oral glucose tolerance test (OGTT) and anthropometric data available at both baseline and the ten-year follow-up assessment.

Patients were excluded if OGTT or anthropometric data were incomplete at either time point, or if a diagnosis of overt diabetes mellitus (thalassemia-related diabetes mellitus: Th-RDM) requiring insulin therapy had already been established before the baseline assessment, since this would preclude meaningful baseline OGTT classification.

Contemporaneous healthy children and young adults, matched approximately for age and sex, were assessed in parallel at each time point (16 controls at baseline, mean age 11 ± 1.0 years, 8 males and 8 females; 15 controls at ten years, mean age 22.1 ± 1.5 years, 8 males and 7 females) to provide a comparator for OGTT-derived indices. All were brothers, sisters, or cousins of β -TDT patients or healthy volunteer adult subjects. None were overweight or β -thalassemia carriers. Unlike the patients, who were followed longitudinally as the same 23 individuals at both time points, the baseline and ten-year control groups consisted of different, non-overlapping individuals; therefore, the controls provide a cross-sectional reference range rather than a longitudinally followed comparison group.

Eight of the 23 patients (34.8%) had also been included in our previous long-term ICET-A analyses of glucose–insulin homeostasis;^{8,9} the remaining 15 patients (65.2%) have not been reported previously. The relationship with previous publications is detailed in the discussion.

b. Clinical and laboratory assessments. Body mass index (BMI) was recorded at baseline and 10-year follow-up and categorized as underweight (below the 5th percentile or <19 kg/m²), overweight (75th–95th percentile or 25–30 kg/m²), or obese (above the 95th percentile or >30 kg/m²). Serum ferritin (with 95% confidence intervals) and alanine aminotransferase (ALT, with 95% confidence intervals) were measured at both time points as markers of iron burden and hepatic status. Pubertal status (spontaneous pubertal development, arrested puberty, hypogonadism) and, in females, the occurrence of secondary amenorrhea were recorded.

All patients underwent a standardized annual (12 ± 3 months) oral glucose tolerance test (OGTT) with plasma glucose measured at 0 (fasting), 30, 60, and 120 minutes after glucose load, together with insulin sampled at the same time points, including a fasting insulin measurement, to allow calculation of the insulin and glucose areas under the curve required for ISSI-2. Prediabetes included 2 categories, namely, impaired fasting glucose (IFG) and impaired glucose tolerance (IGT), according to the American Diabetes Association (ADA) for the development of dysglycemia.¹⁰ Moreover, 1-hour plasma glucose (PG) concentration ≥ 155 mg/dL

served as a simple marker for the development of dysglycemia.¹¹ PG was measured by the glucose oxidase method using a glucose reaction analyzer (Glucose Analyzer, Ames). Plasma insulin levels were measured using a commercial solid-phase radioimmunoassay (Coat-A-Count Insulin Kit; Diagnostic Products Corporation, Los Angeles, CA, USA). The intra- and inter-assay coefficients of variation were 4.1% and 6.9%, respectively, with an analytical sensitivity of 2 μ U/mL.

Iron overload (IOL) was assessed every 3–4 months, in the early years by serum ferritin and in the last years by electrochemiluminescence immunoassays. The 90th percentile of reported normal values in females and males are 201 and 243 μ g/L, respectively. IOL was arbitrarily classified as: mild, SF: < 1,000 μ g/L, moderate SF: 1,000–2,000 μ g/L, and severe > 2,000 μ g/L.

c. Assessment of IR and β -cell function. The homeostasis model assessment for insulin resistance (HOMA-IR), the whole-body insulin sensitivity, estimated with the Matsuda-DeFronzo index, and Insulin Secretion-Sensitivity Index-2 (ISSI-2) were used as validated methods for quantifying IR and β -cell function. HOMA1-IR was calculated as [fasting insulin (μ U/mL) $\times$ fasting plasma glucose (mg/dL)/405]. Homeostasis model assessment of β -cell function (HOMA1-% β) was calculated as $360 \times \text{fasting insulin } (\mu\text{U/mL}) / [\text{fasting plasma glucose (mg/dL)} - 63]$ (the mg/dL-adjusted form of the standard $20 \times \text{insulin} / [\text{glucose (mmol/L)} - 3.5]$ equation), according to Wallace et al.¹², the insulinogenic index (IGI, the ratio of the 30-minute increment in insulin to the 30-minute increment in glucose), the oral disposition index (oDI = IGI $\times$ Matsuda index). β -cell compensation was assessed by insulin secretion-sensitivity index-2 (ISSI-2), which is an OGTT-based measure that is analogous to the disposition index obtained from the intravenous glucose tolerance test (IVGTT) against which it has been directly validated by Retnakaran et al.^{13,14}

The ISSI-2 index is defined as the ratio of the area under the insulin curve to the area under the PG curve (as a measure of β -cell response), calculated using the trapezoidal rule, multiplied by the Matsuda-DeFronzo index.^{15,16} The oral disposition index is a sensitive measure of β -cell function, and a natural decline in such function likely begins in early adulthood and progresses with age.

d. Definition of dysglycemia. Impaired fasting glucose (IFG) and impaired glucose tolerance (IGT) were defined using standard fasting and 2-hour post-load plasma glucose cut-points consistent with the American Diabetes Association's Standards of Care in Diabetes.¹⁰

Statistical analysis. Data analysis was carried out using R version 4.2.1 (R Core Team, 2022) and Statistics

Kingdom calculators; Melbourne, Australia, 2017 (<http://www.statskingdom.com>). Data are reported as mean $\pm$ standard deviation or standard error (SD or SE), count, percentage (%) and 95% confidence interval (CI) for serum ferritin and ALT. The Kolmogorov–Smirnov test and Q–Q plot were used to assess the normality of the data distribution. One-way analysis of variance (ANOVA) with Tukey's post-hoc test was performed to compare means of continuous variables with normal distribution; the Mann–Whitney U test was used for continuous variables with skewed distributions. The association between categorical variables was tested using the χ^2 test. Pearson linear correlation coefficient (r) was determined for normally distributed variables, whereas Spearman's rank correlation coefficient [ρ (rho)] was used for variables that remained skewed after log transformation. A correlation coefficient < 0.10 was considered negligible, 0.10 – 0.39 weak, 0.40 – 0.69 moderate, 0.70–0.89 strong and 0.90 – 1.00 very strong.

Patients were compared with the contemporaneous control group separately at each time point (23 patients vs. 16 controls at baseline; 23 patients vs. 15 controls at ten years) using the unpaired Student's t-test (equivalent to one-way ANOVA for two groups) or the Mann–Whitney U test, as appropriate; because the two control groups consisted of different individuals, no longitudinal analysis was performed in controls. Within-patient differences between baseline and ten-year values in the same 23 patients (pre-transfusion hemoglobin, serum ferritin, ALT and family history of diabetes) were tested with the t-test for continuous variables and the χ^2 test for proportions, or Fisher's exact test when expected cell counts were below 5. The annual OGTTs yielded ten repeated, non-independent measurements per patient; these were analyzed with repeated-measures methods (see below), whereas the bivariate correlation analyses were performed with one observation per patient ($n = 23$), either within the baseline assessment or between baseline and ten-year values. Trends in prevalence were tested with GEE logistic models (exchangeable correlation), baseline vs the ten-year assessment with the exact McNemar test, and time to first abnormal test with Kaplan–Meier estimates.

Longitudinal change across the ten annual OGTTs (230 tests) was analyzed with linear mixed-effects models (outcome $\sim$ year, with a random intercept and slope for each patient, restricted maximum likelihood), and is reported as the slope per year with its 95% CI; the Friedman test was used as a non-parametric check. A change in slope was tested with piecewise mixed models with a knot at year 4, and curvature of ISSI-2 with a quadratic term. Within-patient associations between serum ferritin and glycemic or β -cell indices were assessed with repeated-measures correlation (rmcorr; $df = 206$) and, to separate the effect of ferritin from that of time, with mixed models including year and serum

ferritin (per 1,000 µg/L). Between-patient associations were assessed with Spearman's ρ on each patient's ten-year mean values, and glucose trajectories were compared across tertiles of ten-year mean ferritin by likelihood-ratio tests of the group and group $\times$ year terms. The ability of baseline 30-minute PG to identify patients with 1-h PG ≥ 155 mg/dL at ten years was explored with logistic regression and receiver operating characteristic (ROC) analysis (bootstrap 95% CI, 5,000 resamples). As sensitivity analyses, HOMA1-% β models were repeated after excluding visits with fasting PG <70 mg/dL, at which the HOMA formula is unreliable, and P values were adjusted with the Holm method. Repeated-measures analyses were performed in Python 3 (statsmodels 0.15, pingouin 0.7, SciPy 1.17).

Statistical tests were two-sided, and a P value < 0.05 was considered statistically significant.

Ethics. All participants gave informed consent in accordance with the Declaration of Helsinki and its 2020 amendments (www.wma.net) after a detailed explanation of the OGTT procedures and the test's nature and purpose. In our retrospective observational study, patients underwent only routine diagnostic procedures in accordance with current recommendations and guidelines.^{5,7} No additional interventions were provided. Researchers were only allowed to access the database for analysis purposes, and the database did not contain any personal identifying information. In accordance with the Italian Data Protection Authority General Authorization

no. 9/2014 (Available at: <https://www.garanteprivacy.it/web/guest/home/docweb/-/docweb-display/docweb/3786078>; accessed on 1 April 2026), the requirement for Ethics Committee approval was waived given the retrospective nature of the analysis and the use of fully anonymized data.

Results. All 23 patients with TDT (12 males, 11 females) completed both the baseline and ten-year follow-up assessments; none were lost to follow-up. Table 1 presents baseline demographic, hematologic, iron/hepatic, and OGTT-derived glycemic and insulin parameters, along with ten-year follow-up values and comparisons with contemporaneous controls. Impaired fasting plasma glucose (IFG) was already present at baseline in 7/23 patients (30.4%).

Overall, iron overload (IOL), assessed by serum ferritin (SF) at the end of follow-up, was consistently lower than baseline (1328.0 ± 951.4 vs. 2001.3 ± 1166.5 µg/L; P = 0.037), although a subset of 12 patients had poorer compliance with DFO monotherapy (SF: 1998.6 ± 886.3 µg/L).

Spontaneous puberty occurred in 14 of 23 patients (60.9%), arrested puberty/hypogonadism was present in 7/23 patients (30.4%), and secondary amenorrhea affected 3 of 11 females (27.3%) by the ten-year assessment (**Table 1**).

At baseline, fasting glucose correlated positively with HOMA-IR (r: 0.46, P = 0.027) and inversely with HOMA1-% β (r: -0.69, P < 0.001) and ISSI-2 (r: -0.72,

Table 1. Demographic, hematologic, iron/hepatic, and OGTT-derived glycemic parameters at baseline and ten-year follow-up.

Variable (mean $\pm$ SD)	Baseline (n=23)	Control group (n = 16)	10 year follow-up (n=23)	Control group (n = 15)
Age (years)	10.2 $\pm$ 0.9	11 $\pm$ 1.0	20.8 $\pm$ 0.85	22.1 $\pm$ 1.5
Gender (M/F)	12/11	8M/8F	12/11	8M/7F
Positive family history of diabetes	3/23 (13.0%)	—	5/23 (21.7%) vs. baseline: P = 0.26	—
Past splenectomy	4/23 (17.4%)	—	6/23 (26.1%)	—
Age at first packed red blood cell transfusion (months)	7.7 $\pm$ 5.0	—	—	—
BMI, kg/m ²			22.4 $\pm$ 2.5	
Underweight	1/23	—	0/23	—
Overweight	1/23	—	1/23	—
Obese	0/23	—	0/23	—
Pre-transfusional hemoglobin (g/dL)	8.8 $\pm$ 0.24	—	9.1 $\pm$ 0.27 vs.: baseline: P = 0.0003	—
Serum ferritin, µg/L [95% CI]	2001.3 $\pm$ 1166.5 [1524.5–2478.0]	—	1328.0 $\pm$ 951.4 [939.1–1716.8] vs.: baseline: P = 0.037	—
Alanine aminotransferase, IU/L [95% CI]	31.5 $\pm$ 14.2 [29.1–37.0]	—	28.4 $\pm$ 11.1 [23.7–33.0] vs.: baseline: P = 0.41	—
Iron chelation (subcutaneous DFO)	23/23 (100%)	—	23/23 (100%)	—

Fasting plasma glucose (mg/dL)	91.6 ± 11.6	79.1 ± 10.6 P = 0.0016	94.6 ± 10.7	81.8 ± 9.4 P < 0.001
PG 30 min post-load (mg/dL)	137.6 ± 26.8	117.1 ± 23.3 P = 0.018	147.6 ± 20.1	116.2 ± 21.3 P < 0.001
PG 60 min post-load (mg/dL)	113.5 ± 23.5	101.5 ± 20.2 P = 0.15	147.6 ± 31.4	109.5 ± 27.4 P < 0.001
PG 120 min post-load (mg/dL)	101.6 ± 16.9	87.0 ± 16.5 P = 0.011	132.4 ± 15.3	88.4 ± 20.4 P < 0.0001
HOMA1-IR	1.81 ± 0.78	1.0 ± 0.57 P = 0.0022	1.26 ± 0.59	1.22 ± 0.55 P = 0.82
HOMA1-%β	119.8 ± 72.1	100 ± 47.1 P = 0.347	62.6 ± 24.9	118.1 ± 31.8 P < 0.0001
ISSI-2	1.82 ± 0.58	3.5 ± 1.6 P < 0.0001	1.87 ± 0.57	2.94 ± 1.5 P = 0.0042
Final short stature (< 3rd centile)	—	—	6/11 (females) 3/12 (males)	—
Spontaneous pubertal development	—	—	14/23 (8 males)	—
Arrested puberty	—	—	2/23 (females)	—
Hypogonadism	—	—	7/23 (5 males)	—
Secondary amenorrhea	—	—	3/11 females	—

Abbreviations = BMI: body mass index; DFO: deferoxamine; PG: plasma glucose; HOMA1-IR: homeostasis model assessment of insulin resistance; HOMA1-%β: homeostasis model assessment of β-cell function; ISSI-2: insulin secretion-sensitivity index-2. **Note:** P values in the control-group columns compare patients with the contemporaneous control group at the same time point (baseline: 23 patients vs. 16 controls; ten years: 23 patients vs. 15 controls). P values labeled 'vs. baseline' in the ten-year column refer to the within-patient comparison of baseline and ten-year values in the same 23 patients. The baseline and ten-year control groups consisted of different individuals assessed cross-sectionally.

P < 0.001); 30-minute post-load glucose at baseline was associated with 1-hour post-load glucose at ten year (r: 0.6545, P < 0.001; n = 23 for all correlations).

Baseline 30-minute PG also discriminated between patients with and without 1-h PG ≥155 mg/dL at ten years (area under the ROC curve 0.79, 95% CI 0.57–0.96). A cut-off of about 140 mg/dL identified 10 of 12 patients who later had 1-h PG ≥155 mg/dL (sensitivity 83%, specificity 82%), and each 10 mg/dL increase in baseline 30-minute PG raised the odds 1.79-fold (95% CI 1.06–3.03; P = 0.030). Because this threshold was derived and tested in the same cohort, and the comparison did not survive Holm correction (P = 0.15), the finding is exploratory.

Over the ten-year retrospective follow-up, fasting and post-load plasma glucose rose progressively at every OGTT timepoint, with the largest absolute increase seen at 120 minutes (101.6 ± 16.9 to 132.4 ± 15.3). Interestingly, 30-minute PG was already higher than in controls at baseline (137.6 ± 26.8 vs. 117.1 ± 23.3 mg/dL; P = 0.018), whereas 1-h PG was not (113.5 ± 23.5 vs. 101.5 ± 20.2 mg/dL; P = 0.15) (Table 1). During follow-up, 30-minute PG changed little until about year 4 (+0.9 mg/dL per year; P = 0.42) and rose thereafter (+3.4 mg/dL per year; P < 0.001), whereas 1-h PG rose steadily from the start (+4.0 and +4.9 mg/dL per year before and after year 4; Table 2). The early abnormality was therefore an elevated 30-minute glucose at baseline rather than an earlier rise in its trajectory. Both trajectories are illustrated in **Figure 1**.

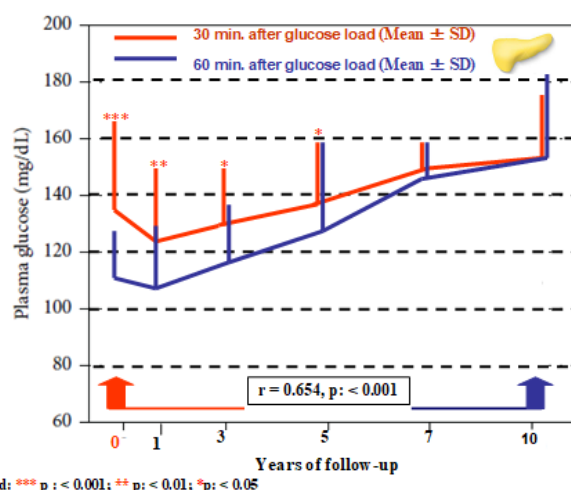


Figure 1. Trajectories of plasma glucose (PG) at 30 and 60 minutes after glucose load from baseline to the end of follow-up. Data are presented as mean ± SD.

HOMA1-IR decreased numerically from baseline to the end of follow-up (1.81 ± 0.78 to 1.26 ± 0.59), consistent with reduced fasting insulin secretion rather than increased insulin resistance. However, the comparison with contemporaneous controls was no longer statistically significant at ten years (P=0.82), whereas it was significant at baseline (P=0.0021). In contrast, HOMA1-%β, the surrogate marker of β-cell secretory capacity, fell by nearly half over the same period (119.8 ± 72.1 to 62.6 ± 24.9) and was significantly lower than in controls at ten years (**Table 1**). ISSI-2 changed little in absolute terms (1.82 ± 0.58 to 1.87 ±

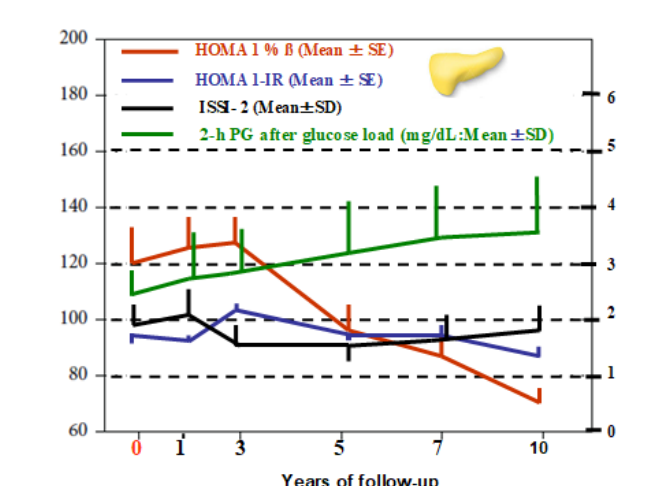


Figure 2. Trajectories of Homeostasis Model Assessment (HOMA1-IR) insulin sensitivity, HOMA1-%β-cell function, insulin secretion-sensitivity index-2 (ISSI-2) and 2-h PG after glucose load from baseline to the end of follow-up. Data are presented as mean ± SD and SE.

0.57) but remained significantly different from controls at both assessments (**Figure 2** and **Table 1**).

At last follow-up, dysglycemia was present in 11/23 patients (47.8%: 3 isolated IFG, 5 isolated IGT, 3 combined IFG + IGT). Dysglycemia was more frequent in males than in females (8/12, 66.7% vs. 3/11, 27.3%), although the difference was not statistically significant (Fisher's exact test, $P = 0.10$). An isolated 1-h hyperglycemia (≥ 155 mg/dL) after glucose load was documented in 3 patients with good adherence to ICT with DFO and 3 patients with poor adherence to ICT with DFO (total: 26.1%; mean ± SD: 169.6 ± 10.6 mg/dL). None developed Th-related DM (**Figure 3**). No renal or liver dysfunction was seen in 10 young adult patients, with a mean serum ferritin of 572.4 ± 145.2 µg/L.

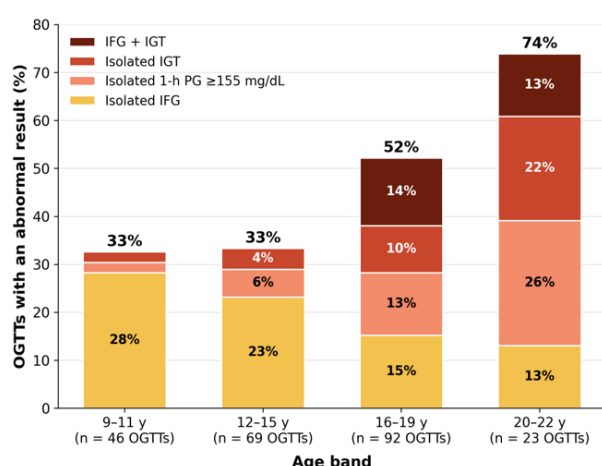


Figure 3. Prevalence of dysglycemia in the different age groups of β-TDT patients. *Note:* Figure redrawn from the annual OGTT data. Each stacked bar shows the percentage of OGTTs within the age band that fell into each mutually exclusive category (isolated IFG, isolated IGT, isolated 1-h PG ≥ 155 mg/dL, IFG plus IGT); the total is shown above each bar. The 20–22-year band corresponds to the ten-year assessment ($n = 23$).

In the whole cohort, fasting glucose rose from 91.6 ± 11.6 to 94.6 ± 10.7 mg/dL, 30-minute post-load glucose from 137.6 ± 26.8 to 147.6 ± 20.1 mg/dL, 60-minute and 120-minute post-load glucose (113.5 ± 23.5 to 147.6 ± 31.4 mg/dL and 101.6 ± 16.9 to 132.4 ± 15.3 mg/dL, respectively).

Baseline and ten-year values were each compared with their respective age-matched control groups (baseline vs. controls: fasting glucose, $P = 0.0016$; 30-minute glucose, $P = 0.018$; 60-minute glucose, $P = 0.15$ [not significant]; 120-minute glucose, $P = 0.011$; HOMA1-IR, $P = 0.0021$; HOMA1-%β, $P < 0.001$; ISSI-2, $P < 0.0001$; ten-year vs. controls: fasting glucose, $P < 0.001$; 30-minute glucose, $P < 0.001$; 60-minute glucose, $P < 0.0001$; 120-minute glucose, $P < 0.0001$; HOMA1-IR, $P = 0.82$ [not significant]; HOMA1-%β, $P < 0.0001$; ISSI-2, $P = 0.0042$) (**Table 1**).

Notably, HOMA1-%β was significantly lower than in controls at ten years ($P < 0.0001$), whereas ISSI-2 was significantly lower than in controls at baseline and after 10 years of follow-up (**Table 1**).

No significant difference in dysglycemia prevalence was observed between patients with good (mean serum ferritin 607.4 ± 180.3 µg/L) and poor (1998.6 ± 886.3 µg/L) compliance with DFO iron chelation therapy (5/11 vs. 6/12; χ^2 : 0.0475, $P = 0.82$).

In brief, ten years after baseline, patients in this cohort showed worsening glucose tolerance, with a significant decline in the fasting surrogate index of β-cell function (HOMA1-%β), while insulin resistance (HOMA1-IR) was no longer significantly different from controls.

Over ten years of annual OGTTs (230 tests in 23 patients), fasting PG remained stable (91.6 ± 11.6 vs 94.6 ± 10.7 mg/dL; $P = 0.29$), whereas 1-h and 2-h PG each rose by 30% (113.5 ± 23.5 to 147.6 ± 31.4 and 101.6 ± 16.9 to 132.4 ± 15.3 mg/dL; both $P < 0.001$; mixed-model slopes +4.5 and +3.4 mg/dL per year (**Table 2** and **Figure 4**). The proportion with 1-h PG ≥ 155 mg/dL increased from 4% to 52% and with IGT from 0% to 35% ($P = 0.001$ and 0.008); no patient developed diabetes. Nearly every patient crossed the 1-h threshold at least once: 21 of the 22 patients normal at baseline reached 1-h PG ≥ 155 mg/dL by the ten-year assessment (Kaplan–Meier 95%; median 6 years). IGT appeared at least once in 14 of 23 (61%; median 7 years) (**Figure 5**).

HOMA1-%β declined by 48% (119.8 ± 72.1 to 62.6 ± 24.9 ; $P < 0.001$), mainly after year 4, while HOMA1-IR decreased (1.81 ± 0.78 to 1.26 ± 0.59 ; $P = 0.017$) and ISSI-2 declined until about year 5 before partly recovering (quadratic term $P < 0.001$). Serum ferritin fell by about one third (**Table 1**). After adjustment for follow-up time, ferritin was not associated with any glycemic or β-cell index, and glucose trajectories did not differ across ferritin tertiles.

Piecewise models showed that HOMA1-%β was stable from baseline to year 4 (-1.7% per year, $P = 0.57$)

Table 2. Longitudinal trends in OGTT glucose, insulin indices and serum ferritin across the ten annual OGTTs (23 patients, 230 tests): linear and piecewise mixed-effects models.

Variable	Slope per year (95% CI)	P	Slope, baseline–year 4	Slope, year 4–ten years	P, change in slope
Fasting PG (mg/dL)	+0.8 (0.4 to 1.2)	<0.001	+1.0	+0.7	0.67
30-min PG (mg/dL)	+2.3 (1.5 to 3.2)	<0.001	+0.9	+3.4	0.15
1-h PG (mg/dL)	+4.5 (3.5 to 5.6)	<0.001	+4.0	+4.9	0.70
2-h PG (mg/dL)	+3.4 (2.6 to 4.1)	<0.001	+4.8	+2.3	0.088
HOMA1-IR	−0.038 (−0.071 to −0.004)	0.026	+0.079	−0.124	0.002
HOMA1-%β	−7.2 (−10.2 to −4.2)	<0.001	−1.7	−11.3	0.038
ISSI-2	−0.017 (−0.039 to 0.005)	0.139	−0.105	+0.048	<0.001
Serum ferritin (μg/L)	−100 (−126 to −73)	<0.001	−111	−92	0.72

Note: Linear mixed-effects models with random intercept and slope (REML); piecewise models with a knot at year 4 (random intercept, maximum likelihood). After Holm correction for nine outcomes, all trends remained significant except HOMA1-IR ($P = 0.052$). Excluding visits with fasting PG <70 mg/dL did not change the HOMA1-%β slope (−7.1% per year, $P < 0.001$).

Table 3. Association of serum ferritin with OGTT glucose and insulin indices across the ten annual OGTTs.

Outcome	Within-patient rmcrr, r (P)	Year-adjusted mixed model, β per 1,000 μg/L (P)	Between-patient ρ, ten-year means (P)
Fasting PG	−0.22 (0.001)	−0.3 (0.73)	0.17 (0.45)
1-h PG	−0.33 (<0.001)	−3.5 (0.12)	−0.07 (0.74)
2-h PG	−0.34 (<0.001)	−1.5 (0.31)	0.16 (0.46)
HOMA1-IR	0.07 (0.29)	0.12 (0.089)	0.21 (0.35)
HOMA1-%β	0.32 (<0.001)	9.2 (0.055)	0.09 (0.69)
ISSI-2	0.18 (0.009)	0.05 (0.26)	−0.13 (0.55)

Note: rmcrr, repeated-measures correlation (230 observations, $df = 206$); mixed models include year and serum ferritin as fixed effects with a random intercept per patient; ρ, Spearman's rank correlation of each patient's ten-year mean values ($n = 23$).

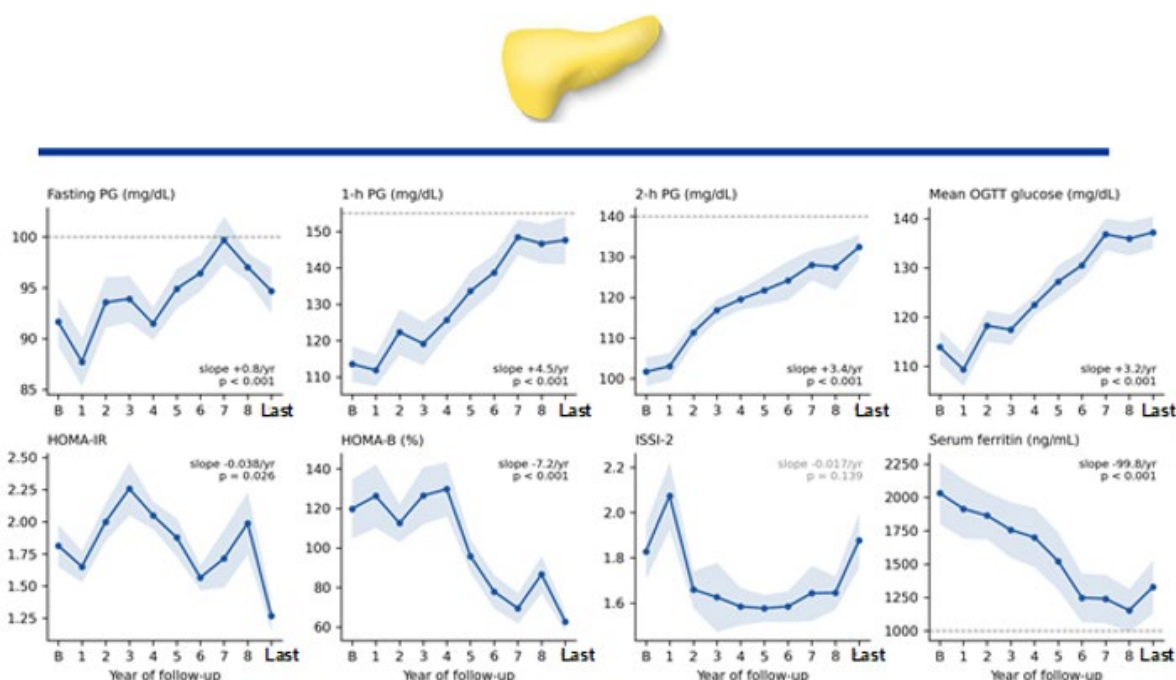


Figure 4. Annual trajectories (mean $\pm$ SE, $n = 23$) of fasting, 1-h and 2-h PG, mean OGTT glucose, HOMA1-IR, HOMA1-%β, ISSI-2 and serum ferritin, with mixed-model slopes and P values. Panels show mean $\pm$ SE; dashed lines mark FPG: 100 mg/dL, 1-h PG: 155 mg/dL, and 2-h PG: 140 mg/dL, and serum ferritin: 1,000 μg/L

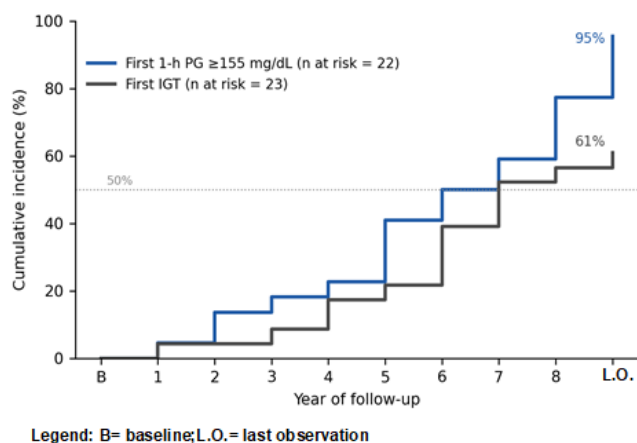


Figure 5. Cumulative incidence (%) of 1-h PG ≥ 155 mg/dL and 2-h PG ≥ 140 mg/dL (IGT), after glucose load (Kaplan–Meier estimates of the first abnormal test in patients without the abnormality at baseline; n = 22 and 23, respectively).

Within patients, serum ferritin correlated inversely with post-load glucose (repeated-measures $r = -0.33$ for 1-h PG and -0.34 for 2-h PG, both $P < 0.001$) and positively with HOMA1-% β ($r = 0.32$, $P < 0.001$). These associations reflected the opposite time trends of the two variables: after adjustment for follow-up year, ferritin was not associated with any glycemic or β -cell index (**Table 3**). Between-patient correlations of ten-year mean values, cross-sectional correlations at baseline and at ten years, and comparisons across tertiles of ten-year mean ferritin ($\leq 1,105$, $1,208$ – $1,482$ and $\geq 1,534$ $\mu\text{g/L}$; all group $\times$ year $P \geq 0.15$) were likewise non-significant and declined thereafter (-11.3% per year, $P < 0.001$; change in slope $P = 0.038$), in parallel with a late fall in HOMA1-IR (change in slope $P = 0.002$). In contrast, 1-h PG rose at a similar rate before and after year 4 ($+4.0$ and $+4.9$ mg/dL per year; change in slope $P = 0.70$) (**Table 2**).

Discussion. In this ten-year longitudinal study, we followed children with transfusion-dependent β -thalassemia (β -TDT), early treated with iron chelation therapy (~ 2 years of age), from late childhood into early adulthood and examined changes in glucose homeostasis using repeated oral glucose tolerance tests (OGTTs) and indices of insulin sensitivity and β -cell function. Three findings were particularly noteworthy. First, glucose tolerance progressively deteriorated during follow-up, with dysglycemia identified in 11 of 23 patients (47.8%) by early adulthood. Second, the longitudinal pattern was characterized predominantly by lower surrogate β -cell function indices rather than a sustained increase in insulin resistance. Third, abnormalities in early post-load glucose, particularly the 30-minute glucose concentration, were evident before conventional dysglycemia and were associated with higher 1-hour glucose concentrations later. Together, these findings suggest that glucose regulation abnormalities may

results support a more nuanced approach to assessing classification risks associated with PG levels traditionally considered normal.

Large multicenter series, including Jobanputra et al.⁴, have not consistently shown a clear sex disparity in thalassemia-related dysglycemia. Consistent with this, the higher frequency of dysglycemia in our male patients (66.7% vs. 27.3%) did not reach statistical significance, although the small sample size limits the power of this comparison.

The most prominent metabolic change in our cohort was the progressive decline in surrogate indices of β -cell function. HOMA1-% β decreased by about 48% during follow-up, whereas HOMA1-IR changed only modestly and was not significantly different from healthy controls at the final assessment. ISSI-2, an OGTT-derived measure that integrates insulin secretion with insulin sensitivity, remained impaired compared with controls, which is consistent with reduced β -cell functional reserve. These findings support the view that impaired insulin secretion contributes to glucose dysregulation in transfusion-dependent thalassemia. However, the relative contributions of β -cell dysfunction and insulin resistance may vary by age, iron burden, liver disease, and treatment. Previous studies have reported abnormalities in both insulin secretion and insulin sensitivity in β -TDT, while the ICET-A recommendations recognize glucose dysregulation as a multifactorial complication of chronic transfusional iron overload.⁷

When interpreting these findings, note that HOMA-derived indices and OGTT-derived indices assess different aspects of glucose metabolism. HOMA1-% β is based on fasting glucose and insulin concentrations and therefore primarily reflects basal glucose-insulin homeostasis. In contrast, ISSI-2 captures insulin and glucose responses during an OGTT, along with insulin sensitivity, and therefore provides information about β -cell performance under stimulated conditions.¹⁵ Accordingly, HOMA1-% β and ISSI-2 should be viewed as complementary rather than competing measures. The marked decline in HOMA1-% β in our cohort, together with persistently impaired ISSI-2, provides a more complete picture of declining β -cell reserve than either index alone. Nevertheless, ISSI-2 differed little between baseline and ten years (1.82 ± 0.58 vs. 1.87 ± 0.57). However, the annual data showed a transient decline until about year 5 followed by partial recovery. Its difference from controls was already present at baseline, whereas the fall in HOMA1-% β , a fasting index, largely reflects lower fasting insulin concentrations. Because neither index measures insulin secretion directly, and C-peptide, intravenous glucose tolerance test, or clamp data were not available, our data support, but do not prove, a progressive decline in β -cell function.

The annual OGTTs add a temporal dimension to these observations. Post-load glucose rose steadily from the first years of follow-up, whereas HOMA1-% β remained stable for about four years and declined steeply only thereafter. In other words, deterioration in stimulated glucose disposal preceded the fall in the fasting index of β -cell function, suggesting that a reduced β -cell response to a glucose load occurs earlier than reduced basal insulin secretion. Because the turning point fitted equally well at years 3–5, it should be regarded as approximate.

Our findings also extend previous observations by providing longitudinal information within the same individuals over almost a decade. Earlier studies of glucose metabolism in β -thalassemia have frequently been cross-sectional or have compared different age groups. For example, abnormalities in glucose tolerance and β -cell function have been reported in children and young adults with β -TDT, but the temporal sequence of these abnormalities has been less clearly defined. Therefore, this study adds an important dimension by showing that metabolic deterioration can be tracked from childhood through adolescence into early adulthood in the same patients. The findings support the view that β -cell dysfunction is not simply a late consequence of established diabetes but may emerge earlier during the course of transfusion-dependent disease.

The relationship between the present cohort and our group's previously published ICET-A series merits explicit clarification. Eight of the 23 patients (34.8%) in the present study had also been included in our previous long-term ICET-A analyses of glucose–insulin homeostasis;^{8,9} the remaining 15 patients (65.2%) have not been reported previously. The present analysis differs from these and from our earlier reports on isolated 30-minute and 1-hour post-load hyperglycemia^{17,18} in three respects: (i) patient selection, as only patients who started DFO monotherapy at about 2 years of age and never switched to, or added, another iron chelator were included; (ii) design, as the same 23 patients were assessed with paired baseline and ten-year OGTT-derived indices of insulin sensitivity and secretion (HOMA1-IR, HOMA1-% β and ISSI-2), each compared with contemporaneous controls; and (iii) focus, as the earlier reports examined single-timepoint OGTT abnormalities rather than within-patient trajectories of these indices.

One of the most interesting observations in our study was the relationship between early post-load glucose concentrations and subsequent glucose metabolism. Baseline 30-minute glucose was associated with the 1-hour glucose concentration ten years later, suggesting that the early glucose excursion during an OGTT may contain information about future metabolic deterioration. Although 30-minute glucose is not currently part of the conventional diagnostic definition of dysglycemia, studies in other populations have shown that

intermediate post-load glucose measurements can improve identification of individuals at increased metabolic risk. In particular, elevated 30-minute glucose has been associated with subsequent development of diabetes. In contrast, 1-hour glucose has been shown to identify individuals with metabolic abnormalities who may not yet meet conventional 2-hour OGTT criteria.^{5–7,16} Importantly, a longitudinal study in β -TDT also showed that elevated 1-hour glucose may identify patients at increased risk of future glucose dysregulation. Our findings, therefore, support further evaluation of intermediate OGTT measurements as potential early markers of metabolic deterioration in β -TDT.^{17,18} In an exploratory ROC analysis, a baseline 30-minute PG of about 140 mg/dL identified 10 of 12 patients who had a 1-h PG ≥ 155 mg/dL ten years later; this threshold was derived in a small cohort and requires validation in independent series before it can be used clinically.

The finding that higher fasting glucose was associated with lower HOMA1-% β and ISSI-2 at baseline further suggests that abnormalities in β -cell function are already detectable during late childhood, before overt diabetes develops. This observation has practical implications because glucose dysregulation in β -TDT is generally gradual, and conventional fasting glucose measurements may remain relatively normal during the early stages. The biological mechanisms underlying these findings are likely to be multifactorial, with transfusional iron overload playing an important role.^{19–22} Pancreatic iron deposition has been demonstrated in patients with transfusion-dependent thalassemia and has been associated with abnormalities in glucose metabolism. Iron-related pancreatic injury may impair insulin secretion through oxidative and cellular injury, reducing β -cells' ability to respond appropriately to glucose.^{23–26} The relationship between pancreatic iron and β -cell dysfunction is also supported by studies using MRI-based assessment of pancreatic iron, in which greater pancreatic iron burden was associated with impaired glucose metabolism and lower measures of β -cell function. These observations provide a plausible biological context for the lower surrogate indices of β -cell function observed in our cohort.

At the same time, the relatively stable HOMA1-IR in our patients suggests that increasing insulin resistance was not the dominant longitudinal feature during the study period. This does not exclude insulin resistance as a contributor, particularly with hepatic iron overload or liver dysfunction, and previous studies show that insulin resistance can contribute substantially to glucose intolerance in thalassemia. Rather, our findings suggest that, within this particular pediatric cohort followed under a relatively uniform treatment strategy, deterioration in β -cell secretory capacity was more apparent than progressive worsening of insulin resistance.

We also observed no significant reduction in serum ferritin during follow-up, despite the emergence of dysglycemia in a substantial proportion of patients. This finding deserves careful interpretation. Improvement in serum ferritin is clinically important, but ferritin is an indirect marker of total body iron and does not reliably reflect iron deposition in individual organs. Studies using pancreatic MRI have demonstrated that pancreatic iron burden may not be accurately predicted by serum ferritin or hepatic iron measurements alone. In one study, pancreatic T2* was significantly associated with glucose dysregulation, whereas serum ferritin did not adequately distinguish patients with and without metabolic abnormalities.¹⁹ Thus, the persistence of dysglycemia despite improved ferritin in our cohort should not necessarily be interpreted as failure of chelation therapy. Rather, it highlights the possibility that pancreatic injury may persist despite improved systemic iron indices and reinforces the potential value of organ-specific iron assessment when clinically available. Our repeated-measures analysis reveals another pitfall. Within patients, lower ferritin was associated with higher glucose, a relationship opposite to the expected effect of iron; it disappeared after adjusting for follow-up time, suggesting it reflected two concurrent but independent trends (falling ferritin with chelation and rising glucose with age) rather than a protective effect of iron. Serum ferritin should therefore not be used alone to estimate dysglycemia risk in individual patients.

This study has several strengths. Most importantly, the same patients were followed for ten year, allowing us to examine individual metabolic trajectories rather than relying solely on cross-sectional comparisons between different age groups. The standardized annual OGTT protocol provided repeated measurements of glucose and insulin responses, while the inclusion of both fasting and OGTT-derived indices allowed assessment of complementary aspects of β -cell function and insulin sensitivity. In addition, the availability of contemporaneous healthy controls provided a useful reference for interpreting the metabolic changes observed during the transition from childhood to early adulthood. The relatively uniform use of deferoxamine monotherapy also reduced variability related to different chelation strategies within the cohort.

Several limitations should be considered when interpreting these findings. First, the study was conducted at a single center and included a relatively small number of patients, which limits statistical power and the generalizability of the findings. Second, the observational design does not allow us to establish a causal relationship between iron overload and progressive β -cell dysfunction. Third, pancreatic MRI was not routinely available throughout the study period. Consequently, serum ferritin was used as the principal longitudinal marker of iron burden, despite its limitations

as a surrogate for pancreatic iron. Fourth, because all patients received deferoxamine monotherapy, the findings may not be directly applicable to patients receiving oral or combination chelation regimens. Finally, we did not have additional measures of metabolic function, including C-peptide, continuous glucose monitoring, and more comprehensive dynamic assessments of insulin secretion. In addition, we could not model age, pubertal stage, and BMI as time-varying covariates in the repeated-measures analyses, so part of the rise in post-load glucose may reflect normal maturation and pubertal insulin resistance. Furthermore, the control groups were cross-sectional and included different individuals at the two time points, and 8 of the 23 patients had been included in previous ICET-A analyses. These limitations should be considered when evaluating the potential value of 30-minute glucose as an exploratory early marker and the mechanisms underlying β -cell dysfunction.

Conclusions and Implications for screening practice.

In conclusion, this ten-year longitudinal study suggests that deterioration of glucose homeostasis in transfusion-dependent β -thalassemia is mainly associated with progressive impairment of surrogate indices of β -cell function, with relatively stable insulin sensitivity during the observation period. Importantly, abnormalities in early post-load glucose, particularly the 30-minute glucose concentration, were associated with later metabolic deterioration and may provide an early signal of increased risk. These findings support continued OGTT-based surveillance beginning in childhood and suggest that intermediate glucose measurements may provide additional information beyond conventional fasting and 2-hour values (**Figure 6**). However, the validity of our results should be further confirmed by larger-scale studies conducted across diverse populations. Future studies incorporating pancreatic MRI are needed to evaluate threshold indices of insulin secretion and sensitivity that could help identify patients most likely to progress to clinically significant dysglycemia and guide more individualized monitoring and intervention.

Author contributions. Conceptualization: VDS. Methodology: VDS, SD, PT, ATS and MF. Investigation and data collection: VDS. Statistical analysis: ATS and VDS; Writing — original draft: ATS. Revision of manuscript and editing: VDS, SD, MF, and PZ. Supervision: VDS, SD, MF, and PT. Preparation of final version: ATS, Figures preparation: ATS, MF and VDS. All authors have read and approved the final version of the manuscript, ensuring that questions related to the accuracy and integrity of any part of the work were appropriately analyzed and discussed. The authors declare that no Generative AI was used in the creation of this manuscript.

Progressive β -cell dysfunction precedes dysglycemia from childhood to early adulthood in transfusion-dependent β -thalassemia

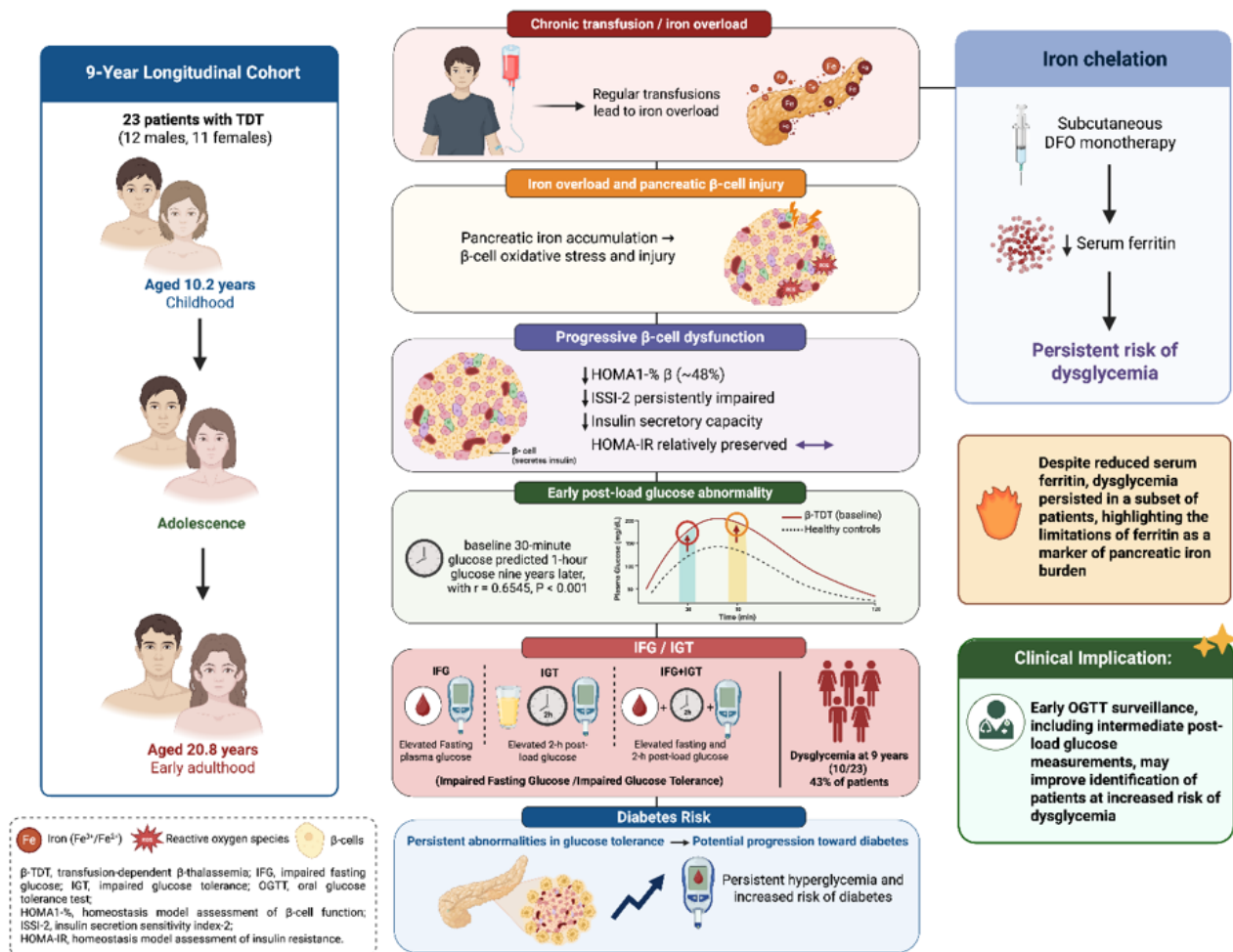


Figure 6. Graphical abstract of the most relevant changes in glucose dysregulation and β -cell function from childhood to early adulthood in transfusion-dependent β -thalassemia patients treated with subcutaneous deferoxamine (DFO) monotherapy. This schematic/conceptual summary presents the study's clinical and biochemical findings; it does not depict histological or pancreatic MRI iron assessment, neither of which was performed in this cohort.

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