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Insulin Resistance and Hyperinsulinemia in Homozygous β-Thalassemia

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Insulin resistance and hyperinsulinemia have been reported in homozygous β-thalassemia before the development of diabetes. However, insulin sensitivity (S_I) has never been studied in normoinsulinemic patients. Furthermore, whether hyperinsulinemia is due to increased β-cell secretion or to decreased hepatic insulin extraction is poorly understood. We estimated S_I, β-cell secretion, and hepatic insulin extraction using the minimal model analysis of a frequently sampled intravenous glucose tolerance test (2.8 g/m^2) in two groups of nondiabetic pubertal patients with homozygous β-thalassemia (seven hyperinsulinemic and seven normoinsulinemic patients) and seven control subjects matched for age, body mass index, and pubertal stage. In both thalassemic groups, S_I was reduced by approximately 40% (3.52 ± 0.57 and 3.74 ± 0.66 *v* 6.89 ± 1.02 $10^{-4} \cdot min^{-1}$ [μU/mL], $P = .011$) and was inversely correlated with iron overload ($r = -.707$, $P = .006$). All parameters of β-cell secretion were not significantly different in patients and controls. On the other hand, basal posthepatic insulin delivery (BDR) was more than doubled in hyperinsulinemic patients with respect to normoinsulinemic patients or controls (15.1 ± 2.4 *v* 6.1 ± 1.1 and 7.5 ± 2.3 pmol/L · min^{-1}, $P = .012$), and the same was true for total posthepatic insulin delivery ([TID] 6.3 ± 1.0 *v* 2.9 ± 0.5 and 2.9 ± 0.7 pmol/L · 240 min^{-1}, $P = .015$). Hepatic insulin extraction was significantly lower in hyperinsulinemic patients than in normoinsulinemic patients or controls (49.3% ± 9.4% *v* 73.0% ± 3.7% and 77.4% ± 3.9%, $P = .011$), and in patients it was inversely correlated with iron overload ($r = -.829$, $P = .0001$). In conclusion, insulin resistance is present even in normoinsulinemic patients, β-cell responsiveness to glucose is normal, and hyperinsulinemia is mainly due to decreased hepatic insulin extraction. In nondiabetic thalassemic patients, these defects are possibly related to iron overload.

TRANSFUSION-DEPENDENT patients with homozygous β-thalassemia may develop impaired glucose tolerance or overt diabetes despite regular iron chelation with desferrioxamine.[1-7] Insulin resistance and hyperinsulinemia have been reported in these patients before the development of diabetes.[6,7] However, it is not clear whether hyperinsulinemia is the consequence or the cause of insulin resistance. In fact, insulin resistance could be the primary event and hyperinsulinemia could develop as a compensatory mechanism to maintain blood glucose near normal.[7] Alternatively, hyperinsulinemia could occur first, since it per se is able to induce insulin resistance.[8]

Hyperinsulinemia has been related either to increased insulin secretion[7] or to decreased liver degradation of the hormone,[6] but the relative contribution of these two mechanisms is poorly understood in thalassemia. This issue is relevant because the liver is the main site of insulin metabolism and one of the primary target organs of the oxidative damage from iron overload.[9]

The aim of the present study was to evaluate simultaneously insulin sensitivity (S_I), β-cell secretion, and hepatic insulin extraction in two groups of nondiabetic transfusion-dependent thalassemic patients with a normal or hyperinsulinemic response to oral glucose, to evidence early impairment of glucose disappearance, C-peptide secretion, and insulin delivery and kinetics. We used the minimal model method,[10] which proved to be a suitable noninvasive tool to estimate S_I,[11] glucose effectiveness,[12] insulin secretion, and hepatic insulin extraction[13] from the measured plasma concentration of glucose, insulin, and C-peptide after an intravenous bolus of glucose.

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SUBJECTS AND METHODS

Subjects

From 135 outpatients attending the Pediatric Thalassemia Center of Turin, 14 pubertal patients (Tanner stages II through IV) with homozygous β-thalassemia were selected using the following criteria: no personal or family history of diabetes or hypertension; normal blood pressure; no overt cardiac disease; no medication other than desferrioxamine; good compliance with chelation therapy (five or more desferrioxamine infusions per week); normal creatinine clearance; negative anti–islet-cell antibodies; no recent illness; and oral glucose tolerance test (OGTT) negative for diabetes (National Diabetes Data Group criteria[14]). All were treated with an identical computerized transfusion scheme: a mean hemoglobin level of 120 g/L was maintained by administering filtered red blood cell concentrates (mean packed cell volume, 0.81) to keep the hemoglobin level between 95 and 145 g/L. Desferrioxamine (30 to 50 mg/kg/d) was administered by nightly continuous subcutaneous infusion over 8 to 10 hours, with the addition of ascorbic acid (2 mg/kg by mouth at the start of desferrioxamine infusion). All patients were in good general health and normally active. Several had mild elevations in serum aspartate aminotransferase but normal serum levels of albumin and direct bilirubin. Thalassemic patients were divided into two groups. The hyperinsulinemic group consisted of seven patients whose plasma insulin levels (both fasting and after glucose loading) were more than 2 SD higher than the mean of a reference group of 50 healthy subjects (Tanner stages II to IV, no family history of diabetes or hypertension, and normal glucose tolerance). A comparable group of seven patients was formed by matching a normoinsulinemic subject (defined as having plasma insulin levels, both fasting and after glucose loading, within 1 SD of the mean of the 50 index subjects) of similar age, sex, and body mass index for each subject with hyperinsulinemia. Seven normal subjects chosen from the 50 index subjects served as controls. They were similar to the patients in age, sex, body mass index, and pubertal stage. Clinical data of the study subjects are summarized in Table 1. Iron overload from transfusions (calculated as the difference between the total amount of iron introduced and the total amount of iron excreted with chelation[15]) was significantly higher in hyperinsulinemic than in normoinsulinemic patients ($P = .033$), suggesting a lower compliance with desferrioxamine therapy in the former. All the procedures used were in accordance with the Helsinki Declaration of 1975. Informed consent was obtained from all subjects and their parents.

Table 1. Characteristics of Thalassemic Patients and Control Subjects

	Thalassemic Patients		Control Subjects
	Normoinsulinemic	Hyperinsulinemic	
n	7	7	7
Age (yr)	14 ± 1	14 ± 1	15 ± 1
Sex (M/F)	2/5	2/5	1/6
BMI (kg/m²)	19 ± 1	19 ± 1	19 ± 1
AST (U/L)	53 ± 8	40 ± 6	—
Serum cholinesterase (U/L)	7,801 ± 1,564	8,940 ± 2,349	—
Iron overload (g)	25 ± 3	39 ± 5*	—
Fasting plasma glucose (mmol/L)	4.2 ± 0.1	4.7 ± 0.2	4.2 ± 0.1
Fasting plasma insulin (pmol/L)	42 ± 6	126 ± 10†	48 ± 5
Fasting plasma C-peptide (pmol/L)	1.34 ± 0.18	1.49 ± 0.22	1.37 ± 0.13

NOTE. Results are the mean ± SEM.

Abbreviations: BMI, body mass index; AST, serum aspartate aminotransferase.

Student's *t* test: *$P = .033$.

Bonferroni's *t* test after one-way ANOVA: †$P = .0001$ *v* controls and *v* normoinsulinemic patients.

OGTT

An OGTT was performed according to the recommendations of the National Diabetes Data Group.[14] Glucose was ingested in a dose of 1.75 g/kg up to a maximum of 75 g, and blood samples were obtained at 0, 30, 60, 90, and 120 minutes for measurement of plasma glucose and insulin.

Frequently Sampled Intravenous Glucose Tolerance Test

The test was performed about 7 to 10 days after the OGTT. For each subject, the experimental protocol started between 8:00 and 8:30 AM after an overnight fast. A butterfly needle was inserted into an antecubital vein, and patency was maintained with a slow saline drip. After a 20-minute rest period, basal blood samples were drawn at −8 and −3 minutes, after which glucose (12.8 $g \cdot m^{-2}$ surface area) was injected over 1 minute starting at time 0. Additional samples were obtained from a contralateral antecubital vein at times 2, 3, 4, 5, 6, 8, 10, 13, 16, 20, 25, 30, 40, 60, 80, 100, 120, 150, 180, and 240 minutes. Samples were rapidly collected via a three-way stopcock connected to the butterfly needle.

Assays

The plasma glucose level was measured in duplicate by the glucose oxidase method (Glucose Analyzer II, Beckman Instruments, Fullerton, CA). The coefficient of variation was ±1.6%. The plasma insulin level was measured in duplicate by solid-phase antibody radioimmunoassay (Cornig Kit, Medfield, MA), and the plasma C-peptide level was measured in duplicate by radioimmunoassay (Byk-Mallinkrodt Kit, Dietzenbach, Germany). The between- and within-assay variabilities for insulin and C-peptide were 9% and 6% and 11% and 8%, respectively.

Data Analysis

Data from the intravenous glucose tolerance test were submitted to computer programs that calculate the characteristic metabolic parameters by fitting glucose, insulin, and C-peptide values to the minimal models that describe the time courses of glucose, insulin, and C-peptide concentrations.[10,13] The models assume a first-order linear kinetics for both insulin and C-peptide and a glucose-controlled biphasic release from the β cell. They have previously been described in detail.[10-13,16,17] The glucose disappearance model, by accounting for the effect of insulin and glucose on glucose disappearance, provides two parameters: S_I (10^{-4} per minute per microunit per milliliter),[11] and S_G, the glucose effectiveness (per minute).[12] The minimal models of insulin secretion and kinetics account for the effect of glucose on C-peptide and insulin concentrations during a frequently sampled intravenous glucose tolerance test by describing both the ability of the β cells to secrete C-peptide in response to the glycemic stimulus, and the kinetics of C-peptide and insulin after their entry into the peripheral circulation.[13] These models provide the time courses of C-peptide secretion [CPS(t)] and posthepatic insulin delivery [IDR(t)] in addition to the following parameters: Φ_1 (picomoles per liter per minute/milligram per deciliter), which describes the dynamic (suprabasal) first-phase β-cell (prehepatic) sensitivity to glucose; Φ_2 (picomoles per liter per minute/milligram per deciliter), which describes the second phase; and n and k_{01}, which are the insulin and C-peptide peripheral fractional clearance rates (per minute), respectively. They are the disappearance rates of the peptides in unit time per

unit volume. Because insulin and C-peptide are secreted in equimolar fashion, CPS(t) also represents the time course of β-cell (prehepatic) insulin secretion. Therefore, the time course of the percent hepatic insulin extraction may be computed as the difference between CPS(t) and IDR(t), normalized to CPS(t).[13,16]

Calculations

The integral over 240 minutes of CPS(t) gives TIS (picomoles per liter in 240 minutes), ie, the total amount of insulin released per unit volume by the β cell; the integral of IDR(t) gives TID (picomoles per liter in 240 minutes), which is the total amount of insulin per unit volume entering the peripheral circulation during the test.[16] Basal prehepatic C-peptide and insulin production ([BSR] picomoles per liter per minute) and posthepatic insulin delivery per unit volume ([BDR] picomoles per liter per minute) were calculated as $k_{01}C_b$ and nI_b, respectively, with C_b and I_b being the fasting concentrations of C-peptide and insulin.[16] The average weighted hepatic insulin extraction (WeHm) is computed as the integral of the insulin extraction time course divided by the length of the test (240 minutes). The total area under the concentration curve of C-peptide was calculated by integrating (trapezoidal rule) the concentration time courses during the observation period (240 minutes).

The estimation of model parameters was performed according to the MINMOD program,[17] which was adapted to include also the C-peptide model.[16]

Statistical Analysis

All values are expressed as the mean ± SEM. Statistical analysis was performed using Bonferroni's t test when significant differences were found by one-way ANOVA. Nonparametric statistics (Kruskal-Wallis test and Spearman's correlation) were also used where indicated.[18]

RESULTS

OGTT

Plasma glucose and insulin values during the OGTT are reported in Fig 1. Plasma glucose values were not significantly different in the three groups both during fasting and the OGTT. Plasma insulin values of hyperinsulinemic patients were approximately twice those of normoinsulinemic patients and controls both during fasting ($P = .004$) and the OGTT ($P = .0001$).

Frequently Sampled Intravenous Glucose Tolerance Test

The time courses of plasma concentrations of glucose, insulin, and C-peptide in the three groups are shown in Fig 2. In the hyperinsulinemic group, the insulin peak was twofold greater than in the normoinsulinemic group despite a similar C-peptide first phase. During the duration of the test, insulin levels remained higher in hyperinsulinemic than in normoinsulinemic patients or control subjects (area under the curve, 46.7 ± 5.3 v 21.5 ± 2.6 and 19.0 ± 1.4 nmol/L in 240 minutes, $P = .0001$).

The area under the C-peptide curve was 199 ± 13 nmol/L in 240 minutes in controls, 211 ± 25 in normoinsulinemic patients, and 185 ± 17 in hyperinsulinemic patients.

In both thalassemic groups, S_I was reduced by approximately 40% with respect to the controls ($P = .011$; Table 2). In thalassemic patients, S_I was inversely correlated with iron overload ($r = -.707$, $P = .006$). No correlations were found between S_I and glucose tolerance (120-minute plasma glucose after the OGTT) or liver function tests.

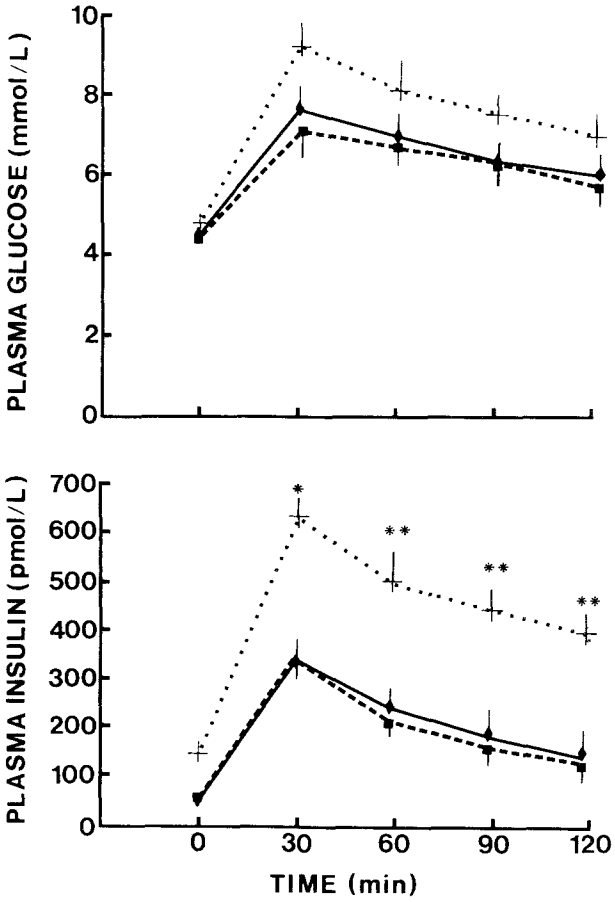

Fig 1. Response of plasma glucose and insulin to 75 g oral glucose in seven normoinsulinemic (■) and seven hyperinsulinemic (+) patients with thalassemia and seven control subjects (◆). Mean ± SEM. Bonferroni's t test after one-way ANOVA: at time 0, *$P = .004$; and at time 30, **$P = .0001$ v normoinsulinemic and control subjects.

S_G did not exhibit significant changes among the three groups of subjects (Table 2) and did not correlate with iron overload.

β-Cell Secretion

BSR, TIS, and Φ_1 and Φ_2 were not significantly different in patients and controls (Table 2).

BDR and TID were similar in normoinsulinemic patients and controls, but were more than doubled in hyperinsulinemic patients ($P = .012$ and $P = .015$, respectively; Table 2).

The hepatic insulin extraction (WeHm) was similar in normoinsulinemic patients and controls, but it was significantly reduced (~35%) in hyperinsulinemic patients ($P = .011$; Table 2). In the patients, WeHm exhibited a strong negative correlation with iron overload ($r = -.829$, $P = .0001$; Fig 3).

DISCUSSION

In this study, the minimal model method[11,13] was used to obtain a quantitative description of S_I, β-cell secretion, and

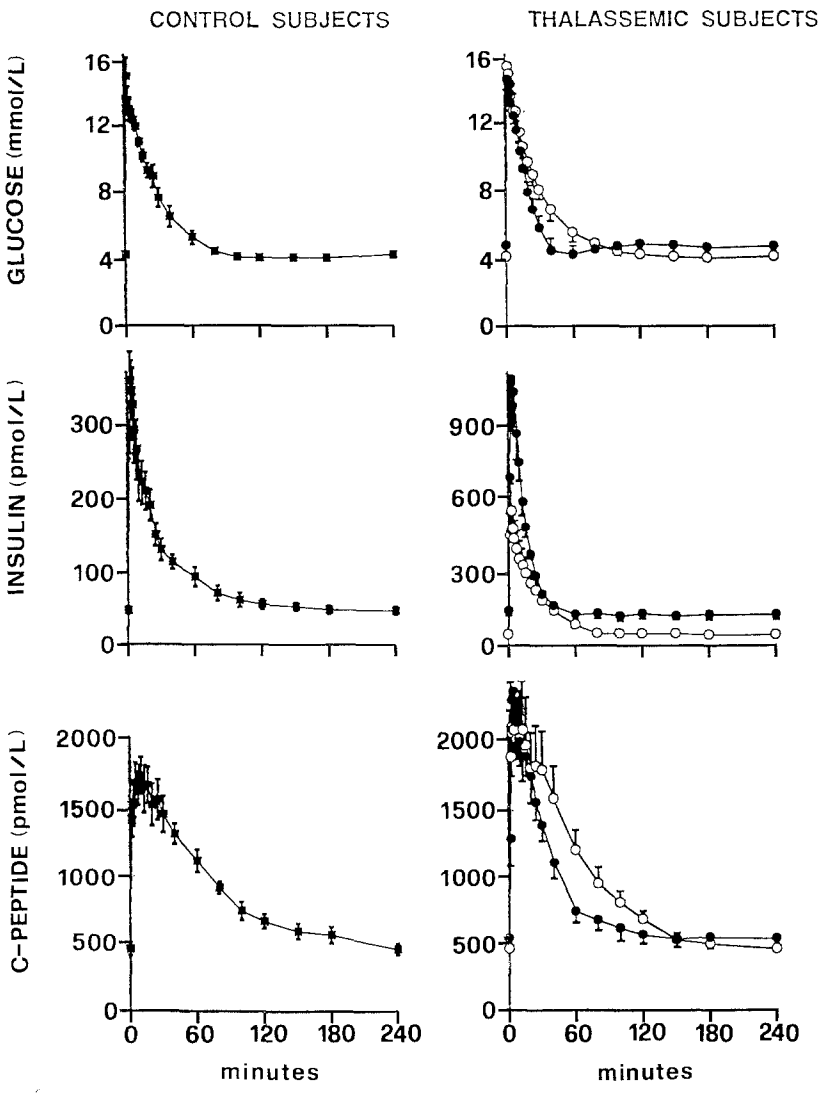

Fig 2. Time courses of plasma glucose, insulin, and C-peptide values during the frequently sampled intravenous glucose tolerance test in seven normoinsulinemic (○) and seven hyperinsulinemic (●) patients with thalassemia and seven control subjects (■). Glucose (2.8 g/m²) was injected at time 0. Note the different scale of the insulin plots.

Table 2. Model-Derived Parameters of S_I, S_G, β-Cell Secretion, Posthepatic Insulin Delivery, and Hepatic Insulin Extraction in Thalassemic Patients and Control Subjects

	Thalassemic Patients		Control Subjects
	Normoinsulinemic	Hyperinsulinemic	
S_I	3.52 ± 0.57*	3.74 ± 0.66*	6.89 ± 1.02
S_G	0.028 ± 0.005	0.036 ± 0.006	0.022 ± 0.003
Φ_1	183.0 ± 37.1	182.9 ± 43.7	123.7 ± 18.6
Φ_2	0.039 ± 0.011	0.053 ± 0.010	0.054 ± 0.031
BSR	24.3 ± 3.9	33.2 ± 4.6	29.8 ± 5.7
TIS	11,622 ± 1,148	13,453 ± 1,335	13,183 ± 2,178
k_{01}	0.058 ± 0.011	0.069 ± 0.006	0.064 ± 0.009
BDR	6.1 ± 1.1	15.1 ± 2.4†	7.5 ± 2.3
TID	2,991 ± 534	6,260 ± 1,069†	2,957 ± 758
n	0.15 ± 0.003	0.12 ± 0.0033	0.15 ± 0.003
WeHm	73.0 ± 3.7	49.3 ± 9.4†	77.4 ± 3.9

NOTE. Results are the mean ± SEM.

Bonferroni's *t* test after one-way ANOVA: **P* = .011 *v* control subjects; †*P* = .012 *v* normoinsulinemic patients and *v* control subjects.

hepatic insulin extraction in thalassemic patients. This method was used mainly due to its low invasiveness and because it is able to provide a simultaneous picture of the time courses of the β-cell and liver processes involving insulin under dynamic conditions.[13] The other noninvasive method to assess insulin degradation is the C-peptide to insulin molar ratio, but because of the different kinetics of the two peptides, this ratio reflects insulin extraction only under steady-state conditions and not during dynamic tests, as in response to a glucose load.[19] The minimal model approach has been used in a variety of pathophysiological conditions characterized by either reduced S_I, impaired β-cell secretion, and/or reduced hepatic insulin extraction.[20,21]

In the present study, S_I was markedly reduced (~40%) in both thalassemic groups. This result is in agreement with that obtained in patients comparable to ours.[6,7] Although the precise mechanism of insulin resistance in thalassemia cannot be elucidated, we found that S_I was inversely correlated with iron overload, suggesting that the defect in

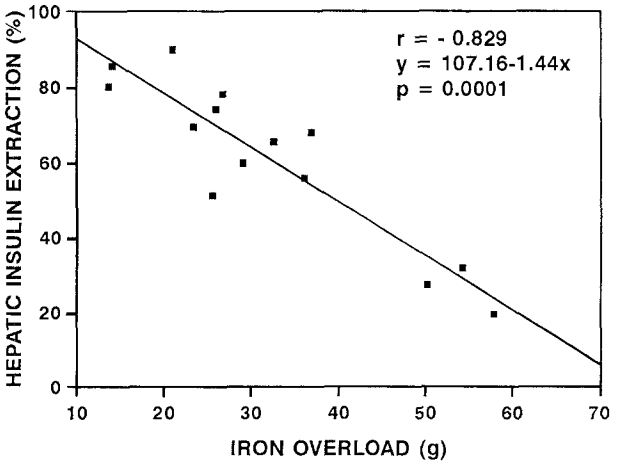

Fig 3. Spearman's correlation between hepatic insulin extraction and iron overload in 14 thalassemic patients.

insulin action may depend on a chronic toxic effect of iron. Other states of excessive tissue iron, eg, hemochromatosis, are also associated with hyperinsulinemia and insulin resistance.[22] In these conditions, two organs might theoretically be involved: the liver and the peripheral muscle tissue, where an increased iron content has been reported.[9] However, in chronic liver disease such as cirrhosis, basal hepatic glucose production is normal or decreased and almost completely suppressed by insulin.[23,24] Furthermore, model-derived sensitivity (S_I) is dominated by extrahepatic insulin effects,[16] and muscle is the principal site of overall glucose disposal.[25] A peripheral defect in glucose utilization therefore seems to have a primary role in the insulin resistance of thalassemic patients.

A new finding from the present study is that S_I is also reduced in normoinsulinemic patients. A reduced insulin sensitivity in the presence of normoinsulinemia has to our knowledge never been reported in other conditions of insulin resistance. In fact, this picture does not fulfill the classic definition of insulin resistance proposed by Yalow and Berson,[26] which implies normal or impaired glucose tolerance and hyperinsulinemia. With a similar degree of S_I but less peripheral insulin, one would expect to find a slower glucose disappearance from plasma in the normoinsulinemic group with respect to the hyperinsulinemic group. This is what we found during the intravenous glucose tolerance test (Fig 2), at variance with the OGTT (Fig 1), where plasma glucose levels were consistently lower in the normoinsulinemic group. The apparent discrepancy between the two tests may depend on several factors, such as a different arterial-portal glucose gradient or the activation of the enteroinsular axis.[27] The pathophysiologic mechanism of insulin resistance in normoinsulinemic patients is unknown and cannot be elucidated from the present study. We could speculate that iron overload in liver and muscle may have resulted in both a reduced glucose production and a decreased glucose uptake, respectively. However, S_I is a measure of the ability of insulin both to augment glucose utilization and to inhibit hepatic glucose output, and the model does not allow any segregation between these two processes, which can only be quantitatively estimated by tracer techniques. Whatever the mechanism, the appearance of a decreased S_I when plasma glucose and insulin are still in the normal range seems peculiar in the early stages of impaired glucose metabolism in thalassemia.

S_G was not significantly different in the three groups, showing that the impairment in glucose disposition processes was only due to a reduced insulin effect. S_G measures the ability of glucose per se to increase glucose utilization[28] and to decrease endogenous glucose production[29] in the absence of changes in plasma insulin, ie, at the basal hormone level. Given that in thalassemic patients S_G tends to increase, we may hypothesize that it happens in the attempt to compensate for the overall reduced insulin effect.

As for hyperinsulinemia, a simultaneous evaluation of β-cell secretion and hepatic insulin extraction in thalassemic patients is relevant because the liver is both the main site of insulin metabolism and one of the primary target organs of tissue damage from iron overload.[9] A normal β-cell secretion can be demonstrated via a model-independent but imprecise measure, ie, the area under the C-peptide concentration curve, which was found to be virtually the same in the three groups. Since C-peptide clearance also was not different, the similar C-peptide patterns are only due to similar β-cell secretion. The model-derived analysis of insulin and C-peptide kinetics showed that basal and glucose-stimulated β-cell secretion (BSR, Φ_1, Φ_2, TIS) were normal in both groups of patients (Table 2). On the other hand, the BDR and TID were more than doubled in hyperinsulinemic patients with respect to normoinsulinemic patients and controls (Table 2). Furthermore, a 36% reduction of hepatic insulin extraction (WeHm) was found in the hyperinsulinemic group. Finally, hepatic insulin extraction was inversely correlated with the degree of iron overload (Fig 3), suggesting that the liver is more susceptible than the β cell to damage from iron accumulation. In our patients, a reduced hepatic insulin extraction rather than an increased β-cell secretion therefore seems to be the main mechanism of hyperinsulinemia. This conclusion is in accordance with recently published results obtained using a modified frequently sampled intravenous glucose tolerance test analyzed with minimal models.[6]

Until more information is available, it is possible to speculate on a plausible pathogenetic sequence of glucose metabolism impairment in thalassemia. In the early stages of the disease, β-cell secretion and hepatic insulin extraction are normal, but insulin sensitivity decreases with progressive iron overload. At this stage, a reduced hepatic glucose production (liver damage) is associated with a decreased glucose uptake (defect of peripheral insulin action from muscle damage). As liver damage worsens, hyperinsulinemia develops mainly as a result of reduced hepatic insulin extraction with possible concomitantly increased β-cell secretion, which function as compensatory mechanisms to overcome insulin resistance. Eventually, insulin secretion decreases (β-cell damage from iron over-

load and/or β-cell exhaustion) and overt diabetes develops.[2,7]

In summary, this study indicates that in thalassemia, insulin resistance is present even in normoinsulinemic patients, β-cell responsiveness to glucose is normal, and hyperinsulinemia is mainly due to reduced hepatic insulin extraction. These defects are possibly related to iron overload.

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