No Effect of Deferoxamine Therapy on Glucose Homeostasis and Insulin Secretion in Individuals With NIDDM and Elevated Serum Ferritin

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Deferoxamine has been proposed as a potentially important therapy for individuals with NIDDM and mild elevations in serum ferritin. Previously, iron chelation therapy with intravenous deferoxamine over a 5–13-wk period has been reported to normalize serum ferritin and markedly improve glycemic control. To confirm these results and to study potential beneficial effects of deferoxamine on insulin secretion, 9 individuals with NIDDM and elevated serum ferritin levels were treated twice weekly with deferoxamine infusion, following a previously described protocol. Although 8 of 9 subjects achieved normal or near-normal serum ferritin values after deferoxamine therapy, we found little evidence that it produced beneficial effects on glycemic control. Fasting glucose levels pre- and post-deferoxamine therapy were unchanged (11.6 ± 1.2 and 11.3 ± 1.5 mM, respectively, $P = 0.80$). GHb levels declined slightly after deferoxamine therapy (9.3 ± 0.7 vs. 8.8 ± 0.7%, $P < 0.05$); however, this effect was small and was not associated with elimination of or even substantial reduction in insulin or oral hypoglycemic therapy. Deferoxamine therapy did not significantly alter fasting insulin or C-peptide levels, nor stimulated insulin or C-peptide responses to intravenous arginine or glucose. During follow-up studies 1.5–8 mo after deferoxamine therapy, serum ferritin levels again were elevated in 5 of 8 subjects who showed an initial response. Thus, although deferoxamine therapy reduced serum ferritin levels in our subjects, we were unable to confirm a previous report that this effect was associated with any meaningful improvement in glycemic control or insulin secretion. *Diabetes* 42:544–49, 1993

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NIDDM, non-insulin-dependent diabetes mellitus; RIA, radioimmunoassay; HPLC, high-pressure liquid chromatography; BMI, body mass index.

Diabetes mellitus is a common and early manifestation of severe iron overload in individuals with primary, familial hemochromatosis (1–4). Reduction of body iron stores by repeated venesection results in improvement or normalization of carbohydrate metabolism in up to 40% of individuals with hemochromatosis, suggesting that diabetes in these individuals is a secondary and reversible manifestation of iron overload (4–6). Cutler reported a trial of iron chelation therapy in a group of 9 patients with elevated serum ferritin levels and NIDDM (7). Treatment of those patients with intravenous administration of the iron chelator deferoxamine normalized their serum ferritin levels, and 8 of 9 showed a dramatic clinical response characterized by cessation of insulin or oral hypoglycemic therapy, normalization of GHb values, and normalization or near-normalization of fasting glucose levels. Cutler postulated that disordered iron metabolism may be common in diabetic individuals and may represent a heretofore unrecognized and reversible form of secondary diabetes mellitus. However, he did not assess the possible mechanisms through which deferoxamine improved glucose control. In particular, no measurements of basal or glucose-stimulated insulin secretion were reported.

To confirm the results reported by Cutler and to examine the potential beneficial effects of deferoxamine on β-cell function, we conducted a trial of deferoxamine therapy in patients with NIDDM and elevated serum ferritin, following the same protocol described by Cutler (7).

RESEARCH DESIGN AND METHODS

Individuals with a clinical diagnosis of NIDDM were recruited from our clinic population and through advertisement in the local media. After giving informed

TABLE 1
Clinical characteristics of study subjects

Subject	Age (yr)	BMI (kg/m^2)	Duration of diabetes (yr)	Serum iron (μM)	Serum transferrin (g/L)	Iron saturation (%)	Serum aspartate aminotransferase (U/L)
1	57	49.9	3	14	2.7	24	20
2	52	32.8	5	13	3.0	20	44
3	66	30.0	6	7	2.4	13	20
4	64	28.7	11	12	2.6	20	22
5	69	26.5	11	25	2.3	47	19
6	63	23.1	12	14	2.3	27	9
7	70	34.2	12	13	2.7	22	24
8	66	35.2	13	20	3.2	27	32
9	60	29.0	16	16	3.0	24	28
Mean ± SE	63 ± 2	32.2 ± 2.5	10 ± 1	15 ± 2	2.7 ± 0.1	25 ± 3	24 ± 3
Normal range				10–34	2.0–4.0	20–55	0–50

consent, subjects underwent a complete history and physical examination; a fasting blood specimen was obtained for measurement of serum glucose, HbA_{1c}, hemoglobin, serum iron, total iron binding capacity, percent iron saturation, transferrin, ferritin, creatinine, aspartate aminotransferase, total bilirubin, and alkaline phosphatase. Individuals with elevated serum ferritin (>300 μg/L in men; >200 μg/L in women) on two separate determinations were candidates for deferoxamine therapy. Subjects were excluded if they had a history or current clinical evidence of hemochromatosis, liver or renal disease, malignancy, chronic inflammatory disease, history of drug or alcohol abuse within the past year, transfusion history of >10 units of blood, or current evidence of acute or chronic infection.

Deferoxamine treatment. Eligible subjects received deferoxamine, 10 mg/kg, given intravenously over 2 h, two times a week for a minimum of 6 wk. This period was elected because it exceeded the minimum treatment period of 5 wk reported by Cutler (7). Treatment was stopped after 6 wk (12 treatments) if serum ferritin had normalized to <300 μg/L; otherwise, treatments continued on a week-by-week basis until serum ferritin normalized. Subjects were monitored weekly for evidence of adverse effects of deferoxamine therapy on liver or renal function, or visual or auditory acuity. After completion of deferoxamine therapy, each subject underwent a repeat physical examination and laboratory studies.

Metabolic studies. Serum glucose, insulin, and C-peptide responses to intravenous arginine and glucose were measured, as described previously (8), pre– and post–deferoxamine therapy to determine whether treatment improved arginine and glucose-stimulated insulin release. All studies were conducted in the morning after an overnight fast. Subjects were instructed to withhold their insulin dose or oral hypoglycemic agent on the study day.

Serum insulin and C-peptide were measured by standard double-antibody RIA (9,10). Serum glucose was measured by glucose-oxidase method. Serum ferritin was measured by RIA (Baxter Travenol, Deerfield, IL). GHb was determined by automated HPLC. Other laboratory measurements were performed by the University of Minnesota Hospital Laboratory using standard methodology.

Fasting glucose, insulin, and C-peptide values were determined by averaging three baseline samples. Acute insulin responses and integrated C-peptide responses to arginine and glucose were calculated as described previously (8). Data are given as means ± SE. Pre– and post–deferoxamine therapy values were compared using paired Student's t test with two-tailed $P < 0.05$ considered statistically significant.

RESULTS

We screened 77 individuals with NIDDM initially by a random measurement of serum ferritin. Of the 77, 24 (31%) had an initial ferritin level >300 μg/L. Of the 24, 10 (all men), met the study criteria on further evaluation and agreed to participate. One of these 10 volunteers suffered a stroke after receiving only one deferoxamine treatment and was excluded from further treatments. The demographic and clinical characteristics of the 9 subjects who completed the study are shown in Table 1.

Response to deferoxamine treatment. Clinical and metabolic parameters of the 9 subjects before and after deferoxamine treatment are shown in Table 2. A decline in serum ferritin was observed in 8 subjects after deferoxamine treatment. In 7 of 8, serum ferritin was in the normal range at the end of the treatment period. Subject 3 had a marked decline in serum ferritin after 16 deferoxamine treatments; however, serum ferritin levels remained slightly elevated and subject 3 elected to stop treatment. Only subject 9 did not exhibit a decline in serum ferritin in response to deferoxamine.

The mean fasting glucose level was not significantly different pre– and post–deferoxamine therapy (11.6 ± 1.2 and 11.3 ± 1.5 mM, respectively, $P = 0.80$). Only subject 9 had a normal fasting glucose posttherapy. Paradoxically, this was the same subject whose serum ferritin did not decline after deferoxamine. The other 8

TABLE 2
Response to deferoxamine treatment

Subject	Total number of treatments	Serum ferritin (μg/L) Pre	Serum ferritin (μg/L) Post	Fasting glucose (mM) Pre	Fasting glucose (mM) Post	HbA_{1c} (%) Pre	HbA_{1c} (%) Post	Diabetes therapy (daily amount) Pre	Diabetes therapy (daily amount) Post	Follow-up: Mo post deferoxamine	Follow-up: Serum ferritin (μg/L)	Follow-up: HbA_{1c} (%)
1	12	436	150	11.6	14.3	10.1	9.7	Diet	Same	7.0	215	8.3
2	13	362	295	7.9	8.7	7.2	7.0	Insulin (66 U)	64 U	7.0	430	7.8
3	16	898	360	15.6	9.5	9.3	8.1	Glyburide (20 mg)	Same	8.0	542	10.4
4	18	327	271	8.8	8.6	7.1	6.5	Insulin (47 U)	42 U	1.5	226	6.9
5	16	391	261	15.2	15.8	11.0	10.9	Chlorpropamide (1 g)	Same	5.0	301	10.5
6	12	316	188	17.7	19.5	14.0	12.7	Glyburide (15 mg)	Same	3.0	149	8.2
7	28	503	297	8.8	9.0	7.2	7.5	Glipizide (7.5 mg)	Diet	1.5	510	7.6
8	12	374	282	11.1	11.5	8.3	8.0	Insulin (70 U)	Same	6.0	620	8.6
9	26	692	713	7.5	5.2	9.5	9.1	Glipizide (40 mg)	Same	6.0	1068	10.0
Mean ± SE	17 ± 2	478 ± 65	313 ± 54*	11.6 ± 1.2	11.3 ± 1.5	9.3 ± 0.7	8.8 ± 0.7*			5.0 ± 0.8	451 ± 94	8.7 ± 0.4
Normal range		29–300		4.0–5.9		4.3–6.0						

Pre, pre–deferoxamine treatment. Post, post–deferoxamine treatment.
*$P < 0.05$ vs. pretreatment.

subjects showed a slight decrease in posttherapy GHb relative to the baseline value. Overall, the mean GHb level for the group declined slightly, from 9.3 ± 0.7% pretherapy to 8.8 ± 0.7% posttherapy ($P < 0.05$). Subjects were encouraged not to alter their diabetic regimen during the study unless hypoglycemia occurred. Except for subject 7, who discontinued his oral agent after experiencing mild hypoglycemic symptoms, none of the subjects made any substantial alterations in their diabetic therapy (Table 2). Mean pre- and posttherapy weight did not differ significantly for the group (103 ± 8 vs. 104 ± 9 kg, $P > 0.05$). All subjects had normal hemoglobin values pretherapy, and mean hemoglobin levels pre- and posttherapy were not significantly different (14.9 ± 0.2 vs. 14.3 ± 0.4 g/dl, $P = 0.18$).

Intravenous arginine and glucose stimulation tests were performed pre– and post–deferoxamine therapy in 8 of 9 subjects (Figs. 1 and 2). Subject 7 was not available for posttherapy study. Fasting and stimulated glucose and C-peptide levels were measured in all 8 subjects after intravenous arginine or glucose injection (Fig. 1). Fasting and stimulated insulin levels also were measured in 5 subjects who were not receiving exogenous insulin (Fig. 2).

Serum glucose levels, both fasting and after arginine or glucose administration, were virtually identical pre– and post–deferoxamine therapy (Fig. 1*A* and 1*B*). Mean fasting C-peptide levels pre- and posttherapy were 0.74 ± 0.12 and 1.13 ± 0.18 nM, respectively ($P = 0.09$) (Fig. 1*C*). Stimulated C-peptide responses to arginine increased from 3.93 ± 1.36 nmol · min · L^{-1}, pretherapy, to 5.27 ± 1.37 nmol · min · L^{-1}, posttherapy ($P = 0.09$) (Fig. 1*C*). We noted little C-peptide response to intravenous glucose, either pre– or post–deferoxamine therapy (1.14 ± 1.39 and 2.47 ± 1.36 nmol · min · L^{-1}, respectively ($P = 0.40$) (Fig. 1*D*).

Fasting insulin levels in 5 subjects treated with either diet or oral agent were 125 ± 28 pM pretherapy and 175 ± 73 pM posttherapy ($P = 0.43$) (Fig. 2*C*). Acute insulin responses to intravenous glucose were absent both pre– and post–deferoxamine therapy (20 ± 14 and 24 ± 12 pM, respectively; $P = 0.85$) (Fig. 2*D*). Acute insulin responses to intravenous arginine increased from 95 ± 38 pM pretherapy to 260 ± 146 pM posttherapy, but this change was not statistically significant ($P = 0.25$; Fig. 2*C*).

All 9 subjects were reevaluated by measurement of HbA_{1c} and serum ferritin levels at 1.5–8 mo (5.0 ± 0.8 mo) after completion of deferoxamine therapy (Table 2). Mean HbA_{1c} for the group at follow-up was 8.7 ± 0.4%, essentially unchanged from the mean value immediately post–deferoxamine therapy (8.8 ± 0.7%). At follow-up, none of the subjects had achieved further reduction in dosage of oral hypoglycemic agent or insulin. Of the 8 subjects who had a decline in serum ferritin in response to deferoxamine, 6 had an increase over the immediate posttreatment serum ferritin value at follow-up. In 5 of these 6 subjects, the ferritin at follow-up was again >300 μg/L (Table 2).

DISCUSSION

These studies were performed in response to the report by Cutler, which found that short-term deferoxamine treatment in individuals with NIDDM and elevated serum ferritin levels resulted in substantially improved, and even normalized, glucose homeostasis—as evidenced by normal GHb values in the absence of exogenous insulin or oral hypoglycemic therapy. In these individuals, the elevated serum ferritin was thought to reflect increased body iron stores, although primary hemochromatosis was excluded based on clinical and laboratory assessment. We felt that such a dramatic response required confirmation. Furthermore, we wished to determine whether improved β-cell function might explain the reported improvement in glucose control. Preliminary sampling

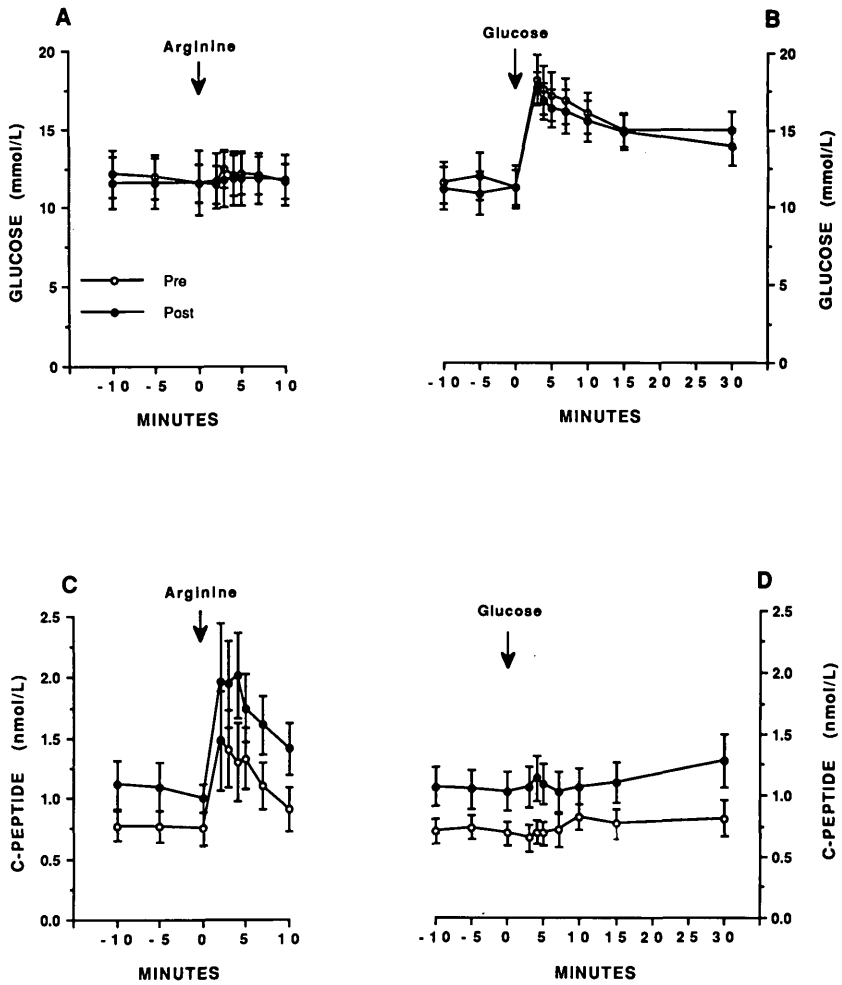

FIG. 1. Serum glucose and C-peptide responses to intravenous arginine and glucose in 8 subjects tested both pre- and post-deferoxamine therapy. *A*: Serum glucose levels during arginine stimulation test. Arginine (5 g) was injected intravenously at time 0. Fasting serum glucose levels, taken as the average of the −10, −5, and 0 min values were 11.9 ± 1.4 and 11.6 ± 1.6 mM pre- and posttherapy, respectively ($P = 0.78$). *B*: Serum glucose levels during intravenous glucose tolerance test. Glucose (20 g) was injected intravenously at time 0. *C*: C-peptide response to intravenous arginine. Incremental C-peptide responses over baseline for the 10 min after arginine were 3.93 ± 1.36 and 5.27 ± 1.37 nmol · min · L^{-1} pre- and posttherapy, respectively ($P = 0.09$). *D*: C-peptide response to intravenous glucose. Incremental C-peptide responses over baseline for the 30 min after glucose injection were 1.14 ± 1.39 and 2.47 ± 1.36 nmol · min · L^{-1} pre- and posttherapy, respectively ($P = 0.40$).

in our clinic population indicated that up to 30% of individuals with NIDDM may have serum ferritin levels on a single measurement >300 μg/L, a value typically quoted as an upper limit of normal for men (11), and the upper limit employed by our clinical laboratory. This finding reinforced the rationale for evaluating deferoxamine as a potentially important therapy for individuals with NIDDM.

We confirmed the previous observation that deferoxamine administration lowers serum ferritin levels in patients with NIDDM (7), presumably reflecting a reduction in body iron stores. This decrease in serum ferritin was not sustained after discontinuation of deferoxamine in most of our subjects. However, contrary to the report by Cutler, normalization of serum ferritin values in our subjects was not associated with improved fasting glucose levels or normalization of GHb values. Although GHb values were significantly lower after deferoxamine therapy, the magnitude of the change was small and of seemingly little clinical significance. With one exception, none of our subjects was able to discontinue or substantially decrease the dosage of agents used for diabetes therapy during the course of the study. Finally, deferoxamine therapy had no significant effect on pancreatic β-cell function, as assessed by insulin and C-peptide secretory responses to intravenous arginine and glucose.

It is possible that there were fundamental differences between our subjects and those reported on by Cutler. However, like Cutler, we selected diabetic individuals with persistently elevated serum ferritin levels who had no clinical evidence of hemochromatosis or other conditions known to elevate serum ferritin. Although the mean pretreatment serum ferritin was lower in our 9 subjects compared with the mean pretreatment ferritin in Cutler's high-ferritin group (478 ± 65 vs. 595 ± 49 μg/L, respectively), 7 of our 9 subjects had pretreatment ferritin levels that exceeded the lowest pretreatment ferritin level in Cutler's group of 8 respondants. Likewise, the mean posttherapy serum ferritin level in our 8 subjects with ferritin levels that declined in response to deferoxamine was almost identical to the mean posttreatment ferritin value in Cutler's group (263 ± 23 vs. 276 ± 17 μg/L, respectively). Therefore, we think it unlikely that our inability to demonstrate a beneficial effect of deferoxamine was the result of inadequate therapy or selection of subjects with insufficient elevation in initial ferritin levels.

Massive iron overload in familial hemochromatosis is associated with multiple factors that impair glucose tolerance, including initial insulin resistance and hyperinsu-

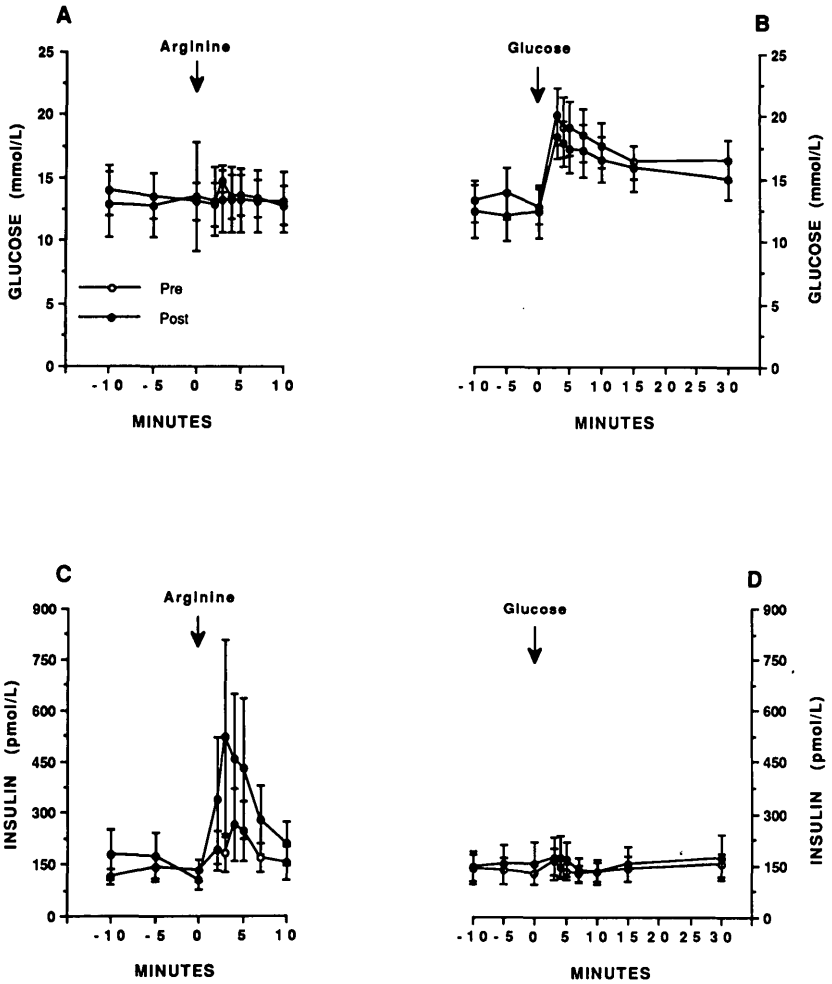

FIG. 2. Serum glucose and insulin responses to intravenous arginine and glucose pre- and immediately post-deferoxamine therapy in 5 subjects treated with diet or oral hypoglycemic agent. *A, B*: Serum glucose levels at baseline and after intravenous injection of arginine (5 g) or glucose (20 g), respectively. *C*: Serum insulin response to intravenous arginine. Acute insulin responses to arginine were 95 ± 38 and 260 ± 146 pM pre- and posttherapy, respectively ($P = 0.25$). *D*: Serum insulin responses to intravenous glucose. Pre- and posttherapy acute insulin responses to glucose were 20 ± 14 and 24 ± 12 pM, respectively ($P = 0.85$).

linemia, followed ultimately by pancreatic iron overload with selective β-cell loss and impaired insulin secretion (2,12–14). However, clinical expression of iron overload is thought to require body iron stores of 15–30 g, levels 5–10 times in excess of normal body stores (1,3,5). Moreover, studies of heterozygotes from families with familial hemochromatosis reveal that up to 30% exhibit mild degrees of iron overload without clinical manifestation of disease, even on long-term follow-up (15–17). Thus, there appears to be little evidence that mild iron overload is associated with impaired glucose metabolism.

In summary, although we confirmed Cutler's report that intravenous deferoxamine lowers, at least transiently, elevated serum ferritin levels in individuals with NIDDM, and presumed mild iron overload, we were unable to demonstrate substantial improvement in glycemic control or β-cell function in response to deferoxamine therapy. Thus, we cannot recommend deferoxamine as a therapy for hyperglycemia in patients with NIDDM.

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