

## GLUCOSE INDUCED INSULIN RELEASE IN $\beta$ -THALASSEMIA HETEROZYGOTES

Sara T.O. Saad and Mario J.A. Saad

### ABSTRACT

The human  $\beta$ -globin gene cluster and the insulin gene have been assigned to the short arm of chromosome 11 with evidence for linkage (Lebo *et al.*, 1983). In order to investigate if heterozygosity for  $\beta$ -thalassemia ( $\beta$ -thal) is linked to an abnormality in insulin secretion, an intravenous glucose tolerance test (IVGTT) was performed on nine  $\beta$ -thal patients and on 15 healthy subjects. No significant differences were observed between the mean plasma glucose levels of the patients with  $\beta$ -thal and those of the control group at any time of the IVGTT, and the glucose disappearance rate was similar in the two groups. Serum insulin levels before and after glucose infusion in patients with  $\beta$ -thal were not significantly different from normals. All indexes of first phase glucose induced insulin release were also similar in the two groups. Previous reports have demonstrated that in sickle cell anemia and associated with the sickle cell trait there is abnormal insulin secretion. However, the results of the IVGTT in the present study suggest that there is no alteration in insulin release in  $\beta$ -thal patients, possibly because the decreased insulin secretion is an independent genetic abnormality in linkage disequilibrium with the B<sup>s</sup> gene but not with  $\beta$ -thal genes.

### INTRODUCTION

Several genes such as the human  $\beta$ -globin gene cluster and the insulin gene have been assigned to the short arm of chromosome 11. Both are of considerable interest because homozygosity for abnormal genes can result in diabetes (Craighead, 1978) or  $\beta$ -thalassemia and sickle cell anemia (Maniatis *et al.*, 1980). Pedigree analysis has shown that the recombination rate between the structural insulin and  $\beta$ -globin gene loci is 14% (Lebo *et al.*, 1983) and has a lod score of 3.57 (Antonarakis *et al.*,

1983), with evidence for linkage. We recently demonstrated that patients with sickle cell anemia and sickle cell trait have an inadequate insulin secretion during the intravenous glucose tolerance test (Saad *et al.*, in press). This defect could be an independent abnormality in linkage disequilibrium with the B<sup>s</sup> gene. In order to investigate if other hemoglobinopathies are also linked to this abnormality, we investigated insulin secretion in patients heterozygous for  $\beta$ -thalassemia.

## SUBJECTS AND METHODS

Nine patients (6M and 3F) heterozygous for  $\beta$ -thalassemia ( $\beta$ -thal) and fifteen (10M and 5F) healthy subjects (N) participated in the study. The controls had no abnormal hemoglobin and were carefully matched to the patients for race, age and body mass index (Table I). Diagnosis was based on clinical, familial and laboratory (Table I), including electrophoresis on cellulose acetate at pH 8.9 and on agar gel at pH 6.2 (Weatherall and Clegg, 1983), solubility test (Zago *et al.*, 1982), estimation of HbF (Pembrey *et al.*, 1972) and of Hb A<sub>2</sub> (Weatherall and Clegg, 1983). Both patients and controls had negative family histories for diabetes mellitus, were not receiving any medication, and were asked to take carbohydrate-rich meals for at least 3 days prior to the study. After an overnight fast of at least 12 h, glucose tolerance was tested by infusing dextrose intravenously (0.5 g/kg body weight), as a 20 to 25% solution over a period of 2-3 min. Blood samples were collected before (0 min.) and 1, 3, 5, 10, 20, 30, 40, 50, 60 min. after the end of the rapid glucose infusion, and they were used for glucose (Somogyi, 1945) and insulin (Soelner and Slone, 1965) assays. The sum of the 1 and 3 min. insulin values (Srikanta *et al.*, 1985) and the total area under the curve during the first ten min. (Smith *et al.*, 1988) were used as an index of the early phase insulin response. The glucose disappearance rate was determined according to Felig *et al.* (1986). The Student's t test was used to evaluate the statistical significance of the differences between the two groups.

## RESULTS AND DISCUSSION

No significant differences were observed between the mean plasma glucose levels of the patients with  $\beta$ -thal and those of the control group at any time during the intravenous glucose tolerance test (IVGTT), and the glucose disappearance rate was similar in the two groups (N =  $1.87 \pm 0.11\%/min.$ ;  $\beta$ -thal =  $1.93 \pm 0.18\%/min.$   $x \pm sem$ ). Serum insulin levels before and after glucose infusion in patients with  $\beta$ -thal were not significantly different from normals (Figure 1). All indexes of first phase glucose induced insulin release were also similar in the two groups (Table II). In general, IVGTT may be the test of choice to study insulin release because results are

less affected by differences in glucose absorption, variable incretin effect (Hampton *et al.*, 1986), and due to higher sensibility for the detection of early preclinical beta-cell dysfunction (Ganda *et al.*, 1984).

Table I - Physical characteristics and laboratory data for patients and controls ( $x \pm \text{sem}$ ).

	No.	Age (years)	Body mass index	Hb (g/dl)	VCM (fl)	HCM (pg)	HbA <sub>2</sub> (%)	HbF (%)
$\beta$ -thal	1	27	22.1	10.7	71	23.2	5.3	2.1
	2	24	23.2	10.2	63	20.5	4.4	0.3
	3	25	22.9	11.2	65	21.2	7.4	0.3
	4	33	24.2	12.3	70	22.8	3.6	0.3
	5	22	23.6	13.4	69	23.0	4.4	0.4
	6	26	22.8	11.8	61	19.8	5.1	1.3
	7	28	23.0	10.1	53	17.0	6.3	2.0
	8	40	24.7	13.1	55	17.8	3.8	0.2
	9	21	21.9	10.9	62	22.5	7.1	0.6
$x \pm \text{sem}$		$27.3 \pm 2.0$	$23.2 \pm 0.3$	$11.5 \pm 0.4$	$63.2 \pm 2.1$	$20.9 \pm 0.8$	$5.3 \pm 0.5$	$0.8 \pm 0.2$
Controls (n = 15)		$29.0 \pm 2.2$	$22.6 \pm 0.7$	$14.2 \pm 0.2$	$85.0 \pm 1.3$	$28.2 \pm 0.2$	$2.7 \pm 0.1$	$0.7 \pm 0.1$

Table II - Index of first phase insulin release and kg (glucose disappearance rate) in patients with  $\beta$ -thal and controls during IVGTT ( $\bar{x} \pm \text{sem}$ ).

	Sum 1 + 3 insulin levels ( $\mu\text{U/ml}$ )	Total area under the first 10 min. ( $\mu\text{U}\cdot\text{ml}^{-1}\cdot\text{min}^{-1}$ )	Kg (%/min.)
$\beta$ -thal	$226 \pm 28$	$925 \pm 118$	$1.93 \pm 0.18$
Controls	$228 \pm 15$	$893 \pm 63$	$1.87 \pm 0.11$

Previous reports have demonstrated that in sickle cell anemia (Saad *et al.*, 1989) and sickle cell trait (Saad *et al.*, in press) insulin secretion is abnormal. Moreover, sickle cell trait is significantly more frequent in diabetics than in healthy

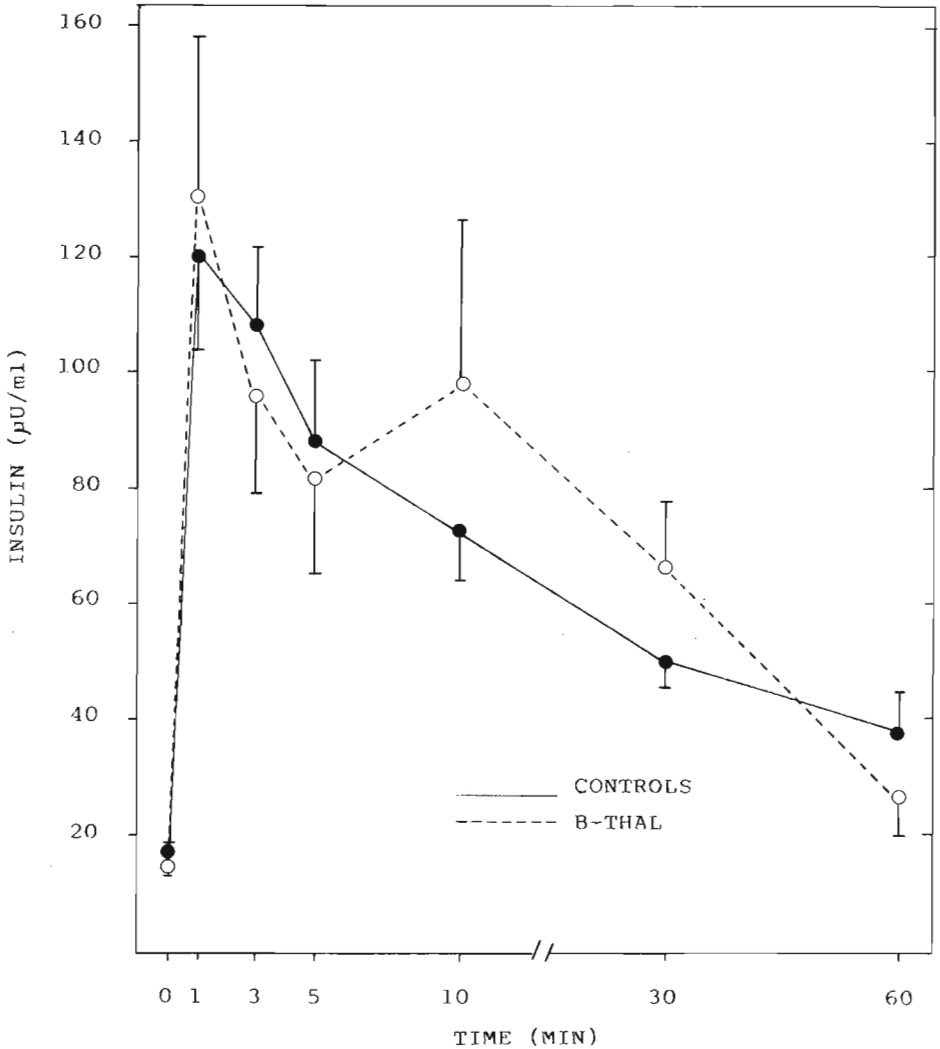


Figure 1 - Insulin levels before (0) and after intravenous glucose infusion (0.5 g/kg) in patients and controls.

subjects (Reid *et al.*, 1988). The decrease in first phase insulin release observed in sickle cell trait is probably a genetic abnormality, since these patients do not have any organ damage caused by sickling in normal conditions.

The molecular lesion in  $\beta$ -thalassemia is extremely heterogeneous; over 50 different mutations have been found in association with this phenotype. They include

deletions of the  $\beta$ -globin gene and nondeletional mutations that may affect the transcription, processing or translation of  $\beta$ -globin messenger RNA (Weatherall, 1989). In Brazil, the prevalent molecular defects in  $\beta$ -thalassemia are those found in populations from the Mediterranean, which include the mutation in codon 39 (C-T), the IVS1-110 (G-A) and the mutation IVS-1-6 (Costa *et al.*, 1990). Thus, the results of the IVGTT in the present study suggest that there is no alteration in insulin release in Brazilian  $\beta$ -thal heterozygotes, at least with respect to these three thalassemic mutations, possibly because the decreased insulin secretion is caused by an independent genetic abnormality in linkage disequilibrium with the B<sup>s</sup> gene but not with  $\beta$ -thal genes.

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### RESUMO

Os genes da  $\beta$ -globina e insulina estão localizados no braço curto do cromossomo 11, com evidências de ligação gênica entre os mesmos. Estudos recentes demonstraram que na anemia falciforme e no traço ciclêmico há uma anormalidade na secreção de insulina. Com o objetivo de investigar se a  $\beta$ -talassemia associa-se a uma anormalidade na secreção de insulina, foi realizado teste de tolerância à glicose endovenoso (GTT-EV) em 9 pacientes heterozigotos para  $\beta$ -talassemia e 15 controles normais. Não houve diferença significativa entre pacientes e controles quando os níveis de glicose plasmática, em cada tempo do GTT-EV, foram comparados; a velocidade de desaparecimento da glicose também foi semelhante nos dois grupos. Não houve diferença entre os dois grupos quando comparados os níveis de insulina antes e após a infusão de glicose. Todos os índices de avaliação da fase rápida de secreção de insulina induzida por glicose foram semelhantes nos dois grupos.

Os resultados deste trabalho sugerem que não há alteração na secreção de insulina em pacientes  $\beta$ -talassêmicos, provavelmente porque este defeito seja uma anormalidade independente em ligação gênica com o gene B<sup>s</sup>, mas não com genes da  $\beta$ -talassemia.

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