Glucose Intolerance in Thalassemia Major Is Related to Insulin Resistance and Hepatic Dysfunction

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Glucose intolerance is a common consequence of transfusion therapy in patients with thalassemia major (TM), but the relative contribution of pancreatic damage and insulin resistance to glucose intolerance is unclear. We have investigated oral (OGTT) and intravenous (IVGTT) glucose tolerance, insulin sensitivity, and fasting concentrations of insulin, proinsulin, and des 31,32 proinsulin in 12 patients with TM (seven hepatitis C virus [HCV] antibody–negative and five–positive), eight patients with hepatic cirrhosis, and nine healthy controls. Two-hour plasma glucose concentrations were marginally higher in anti-HCV–negative (median, 7.4 mmol/L; range, 4.0 to 8.2) and significantly so in anti-HCV–positive thalassemics (median, 8.5 mmol/L; range, 6.4 to to 23.0) and cirrhotics (median, 8.0 mmol/L; range, 4.7 to 17.6) than in controls (median, 5.5 mmol/L; range, 3.0 to 6.3). Insulin sensitivity was also reduced in the three patient groups ($P < .05$). Insulin resistance was the main determinant of oral glucose intolerance in all patient groups (partial $r^2 = .49$, $P < .0001$, n = 28). In turn, the main determinants of insulin insensitivity in TM patients were liver damage (albumin, $r = .67$, $P = .02$) and serum ferritin concentration ($r = -.62$, $P = .03$). There was no relationship of either 2-hour or incremental insulin concentrations with ferritin levels or with HCV status in TM subjects. Moreover, these patients showed no elevation of concentrations of proinsulin and des 31,32 proinsulin, markers of pancreatic β-cell damage, in excess of those observed in cirrhotic patients. In conclusion, the glucose intolerance of TM, like that of cirrhosis, is associated with insulin resistance, not insulin deficiency, and may be a direct or indirect consequence of hepatic damage.

DIABETES MELLITUS occurs in iron overload and is thought to be due to abnormal iron deposition in the pancreas.[1] However, previous studies in β-thalassemia major (TM) patients, in whom chronic blood transfusion treatment leads to iron overload, have shown a discrepancy between the degree of overload and the development of diabetes mellitus.[2] Furthermore, it has been shown that some TM patients demonstrate hyperinsulinemia in the fasting state and in response to a glucose load, suggesting that insulin resistance rather than deficiency may be the underlying defect.[2,3]

If insulin resistance is the primary abnormality leading to glucose intolerance in chronically transfused TM patients, it is possible that liver disease due either to iron overload or to hepatitis C virus (HCV) infection,[4-6] and not pancreatic damage, may be the major factor contributing to glucose intolerance. Liver disease has already been shown to be related to insulin resistance.[7] Both iron overload and chronic hepatitis due to transfusion-transmitted viral infection may contribute to liver disease in TM patients.[8]

We have investigated the relationship between glucose tolerance, insulin sensitivity, and specific insulin levels in a group of TM patients and correlated these with HCV status, liver function, and degree of iron overload. This group of patients was compared with a group of normal controls and with patients known to have liver cirrhosis not caused by transfusional iron overload. We have tested the hypotheses that (1) the degree of glucose intolerance in TM and cirrhosis is related to insulin resistance and not to insulin deficiency, and (2) the insulin resistance is related to liver damage, caused in TM patients by iron overload or HCV.

SUBJECTS AND METHODS

Whittington Hospital Ethics Committee approval and written consent from the subjects involved were obtained before commencing the study, in accordance with the Declaration of Helsinki.

Nine healthy volunteers (aged 35.2 ± 7.1 years [mean ± SD]; seven women and two men) took part in the study. They had no known illnesses or previous glucose intolerance, and none had any family history of glucose intolerance.

Twelve TM patients (aged 22.8 ± 12.8 years; four women and eight men) took part in the study. Seven were anti-HCV–negative and the other five were anti-HCV–positive. None were previously diagnosed as diabetic, but eight had shown impaired oral glucose tolerance on past testing (2-hour venous plasma glucose concentration 7.8 to 11.0 mmol/L following a 75-g glucose load[9] [Table 1]). TM patients had received 491 ± 135 U blood, amounts not differing significantly between anti-HCV–positive and –negative subgroups.

Eight patients with biopsy-proven cirrhosis (aged 55.3 ± 10.6 years; three women and five men) were also tested. Four patients had a Pugh grading[10] of A, three B, and one C. Four were anti-HCV–positive, but in only two was this thought to have been implicated in the development of cirrhosis, and five had serological evidence of hepatitis B (of whom two were also anti-HCV–positive). Interferon had been administered to four cirrhosis between 2 and 12 months before the study, two who were anti-HCV–positive and two who were carriers of hepatitis B. None of these patients were known to have preexisting glucose intolerance.

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Table 1. Characteristics of TM Subjects

No.	Sex/Age (yr)	Anti-HCV Status	Units Transfused	Serum Ferritin (μg/L)	AST (IU/L)	Previous Abnormal OGTT
1	F/22	Neg	450	2,553	26	Yes
2	F/24	Neg	550	1,014	46	Yes
3	M/23	Neg	300	950	25	Yes
4	M/20	Neg	520	688	51	Yes
5	M/23	Neg	600	940	36	No
6	F/28	Neg	600	755	35	Yes
7	F/24	Neg	700	1,648	25	No
8	M/19	Pos	430	1,152	43	Yes
9	M/26	Pos	600	1,472	135	No
10	M/18	Pos	300	1,310	198	Yes
11	M/23	Pos	300	4,000	124	Yes
12	M/23	Pos	540	7,650	75	No

Abbreviations: Neg, negative; Pos, positive.

Patients were weighed while wearing light clothing and no shoes, and skinfold measurements were performed using Holtain calipers (Holtain, Crosswell, Wales, UK), taking three measurements of subscapular and three of triceps skinfold thickness. Waist and hip girth measurements were taken at the umbilicus and anterior superior iliac spine, respectively, using a steel tape. Waist to hip ratio (WHR) and subscapular to triceps skinfold ratio (STR) were estimated, and body mass index (BMI) was calculated as weight divided by height squared.

Because of the possibility that hepatic damage may differentially affect oral and intravenous glucose tolerance,[11-13] glucose tolerance was assessed by two methods, an oral (OGTT) and an intravenous (IVGTT) glucose tolerance test. The OGTT and IVGTT and the insulin sensitivity test were all performed following an overnight fast with a period of at least 2 weeks between each of three tests. The venipunctures during each test were performed to determine plasma glucose, serum insulin, C-peptide, and intact and des 31,32 proinsulin levels.

OGTT. After a baseline blood sample, 75 g or 1.75 g/kg body weight glucose (whichever was the smaller amount) was administered to each subject by mouth. Further blood samples were obtained at 30-minute intervals up to 180 minutes.

IVGTT. Venipuncture was performed 10 minutes before and immediately before starting the test and at 3, 5, 7.5, 10, 15, 20, 25, and 30 minutes after an intravenous glucose bolus of 0.5 g/kg body weight as 20% glucose solution (1,111 mmol/L). The glucose fractional clearance rate (K_g) was determined from the half-life of decline in glucose concentration from 10 to 30 minutes.

An insulin sensitivity test was performed using the modified Harano technique[14] by administering glucose at a rate of 6 mg/kg body weight/min as 20% glucose solution (1,111 mmol/L) simultaneously with soluble insulin (Actrapid; Novo, Basingstoke, Hants) 50 mU/kg body weight/h over 150 minutes. Blood was taken from the other arm at 5-minute intervals from 120 to 150 minutes for estimation of steady-state plasma glucose, insulin, and C-peptide, and the metabolic clearance rate of glucose (MCR) was calculated from the steady-state plasma glucose.[14] There were no significant differences in steady-state plasma insulin concentrations between groups ($P = .19$ by ANOVA). Because of the variance of steady-state plasma insulin concentrations, MCR adjusted for these levels showed substantially weaker correlations with all variables than seen with unadjusted MCR. No correction has been made for steady-state plasma insulin levels in the data presented. Calculations were expressed as clearance per kilogram body weight, because expressing the data in terms of lean body mass[15] did not affect the significance of comparisons or correlations. Following the insulin sensitivity test, a 20% glucose infusion (1,111 mmol/L) was continued for a period of 10 minutes to avoid hypoglycemia.

Plasma glucose concentrations were analyzed using a glucose oxidase method (Beckman Analyzer; Beckman Instruments, Brea, CA), and hemoglobin A by electroendosmosis (Corning, Halstead, Essex, UK; normal range, 6.5% to 8.5%). C-peptide was assayed using a commercial kit (K6; Novo Nordisk Pharmaceuticals, Basingstoke, Hants, UK). Insulin assays were performed by an in-house immunoenzymometric modification[16] of a two-site immunoradiometric assay technique[17]; this method provides a detection limit of 3 pmol · L^{-1} and a coefficient of variation of 7.9% within assay and 14.3% between assay. Intact proinsulin and des 31,32 proinsulin were assayed using a microplate two-site monoclonal immunoradiometric assay,[18] with within- and between-assay coefficients of variation of 6.3% and 9.8% for intact proinsulin and 8.6% and 12.6% for des 31,32 proinsulin. Detection limits were 0.25

Table 2. Characteristics of the Study Groups

Group	No.	Sex	Age (yr) Median	Age (yr) Range	Anti-HCV– Positive	Previous Abnormal OGTT	BMI (kg/m²)†	WHR†	STR†	Ferritin (μg/L) Median	Ferritin (μg/L) Range	Serum Albumin (g/L) Median	Serum Albumin (g/L) Range	AST (IU/L) Median	AST (IU/L) Range
Control	9 7	2M/7F	35	23-42	0/9	0/9	23.8 ± 3.7	0.79 ± 0.08	0.77 ± 0.08	6	3-126	43	40-47	17	12-26
TM															
HCV–	7	3M/4F	23	20-28‡	0/7	5/7	21.3 ± 1.9	0.88 ± 0.07††	0.85 ± 0.10	950	688-2,553‡	47	41-48††	35	25-51‡
HCV+	5	5M/0F	22	18-26††	5/5	3/5	18.7 ± 1.8†††‡‡	0.89 ± 0.04††	0.95 ± 0.09‡	1,472	1,152-7,650‡	42	41-46	124	43-198¶#
Cirrhotic	8	5M/3F	55	38-71‡§‖	4/8	0/8	24.3 ± 1.8‖#	0.87 ± 0.06††	0.90 ± 0.13††	90	37-151‡§‖	31	24-42‡§**	58	37-185‡#
Significance by ANOVA*				.0001*			.003	.021	.014		<.0001*		.0005*		<.0001

*Kruskal-Wallis test.
†Mean ± SD.
‡$P \leq .001$ *v* controls.
§$P \leq .001$ *v* HCV-negative TMs.
‖$P \leq .001$ *v* HCV-positive TMs.
¶$P \leq .01$ *v* controls.
#$P \leq .01$ *v* HCV-negative TMs.
**$P \leq .01$ *v* HCV-positive TMs.
††$P \leq .05$ *v* controls.
‡‡$P \leq .05$ *v* HCV-negative TMs.

pmol · L^{-1} for intact proinsulin and 0.125 pmol · L^{-1} for des 31,32 proinsulin. Ferritin levels were measured by immunoradiometric assay (Becton Dickinson, Oxford, UK; normal range, 39 to 340 μg/L for males and 14 to 140 μg/L for females). Liver function tests were performed on a Technicon SMA2 autoanalyzer (Bayer Diagnostics, Basingstoke, UK). Antibodies to HCV were detected using first-generation enzyme-linked immunosorbent assays for the c-100 antigen (Ortho Diagnostics, Raritan, NJ).

Statistical Methods

Insulin, plasma glucose, MCR glucose, serum ferritin, K_g, and aspartate transaminase (AST) were converted to $\log_e$ values to improve normality. All statistical analyses were performed on SPSSPC+ (SPSS, Chicago, IL) and Minitab (Minitab Europe, Coventry, UK). Controls and TM and cirrhotic patients were compared using ANOVA, followed by multiple Student's *t* tests if there was heterogeneity of arithmetic or geometric means by ANOVA. Kruskal-Wallis tests, followed by multiple separate variance-estimate *t* tests, were performed when standard deviations were not homogeneous. Relationships between continuous variables were assessed by linear correlation with logarithmic transformation of skewed data (all variables except BMI, WHR, and STR). To control for the influence of confounding variables, ANOVA, analysis covariance, and regression were used. Results are expressed as the mean ± SD or median and range for skewed data. A *P* value of .05 was considered significant, but where multiple comparisons have been made using *t* tests, it might be considered appropriate to adjust the raw *P* values by multiplying them by 6, the number of comparisons made. Alternatively, results can be taken to be statistically significant for *P* less than or equal to .008.

RESULTS

Characteristics of the subjects are listed in Table 2. All patient groups had higher serum ferritin levels than controls, and the dry-weight liver iron measurement also varied between groups (median: anti-HCV–negative, 348 [range, 277 to 940]; anti-HCV–positive, 880 [range, 660 to 2,675] μg/100 mg dry weight liver). However, the liver iron data have not been included in further analyses, because of incomplete data and variable timing of biopsies.

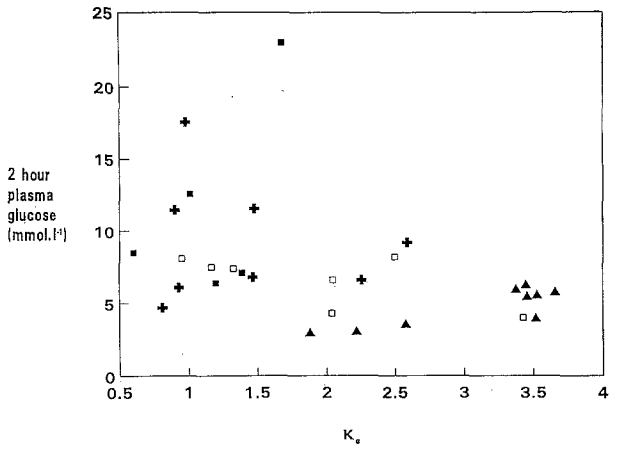

Fig 1. Relationship between K_g and 2-hour plasma glucose concentrations ($r = -.43$, n = 29, $P = .02$ for logarithmically transformed data). (▲) Control subjects; (□) TM subjects negative for HCV serology; (■) TM subjects positive for HCV serology; (+) Cirrhotic patients.

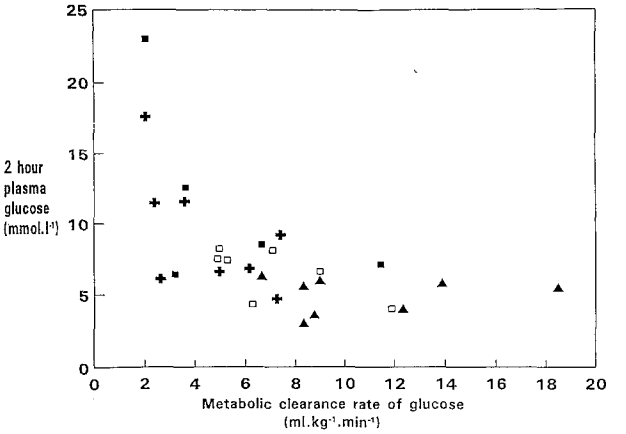

Fig 2. Relationship between MCR glucose and 2-hour plasma glucose concentration ($r = -.70$, n = 28, $P < .0001$ for logarithmically transformed data).

There was a significant negative correlation in all subjects between the concentrations of 2-hour plasma glucose and K_g (for logarithmically transformed variables, $r = -.43$, n = 29, $P = .02$; Fig 1). This relationship was not significantly different in slope or intercept between TM and cirrhotic patients, but there was a difference in slope between patients and controls ($P = .03$). For this reason, both 2-hour plasma glucose and K_g have been used as measures of glucose tolerance in further analyses. Using World Health Organization criteria,[14] glucose intolerance was found in none of the controls, two of seven anti-HCV–negative TM patients (both with impaired glucose tolerance [IGT]), three of five anti-HCV–positive TM patients (one IGT and two diabetic), and four of eight cirrhotics (one IGT and three diabetic). One subject (an anti-HCV–positive TM patient) had a fasting plasma glucose concen-

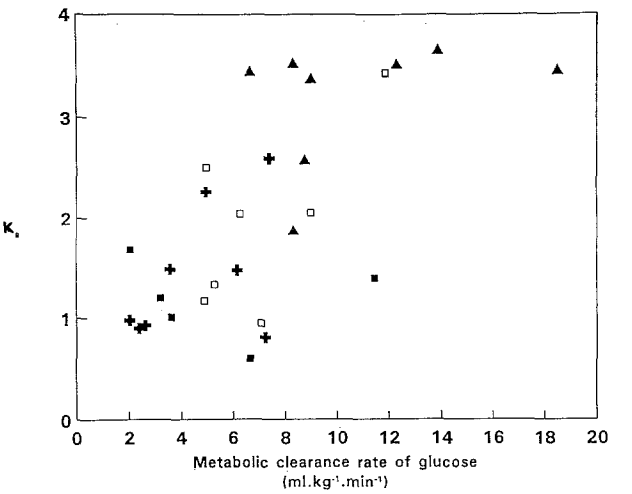

Fig 3. Relationship between MCR glucose and K_g ($r = .60$, n = 28, $P = .0007$).

Table 3. Glucose Tolerance, Insulin Sensitivity, and Insulin Levels in the Study Groups

Group	No.	Fasting Plasma Glucose (mmol/L)	2-Hour Plasma Glucose (mmol/L)	K_g	MCR Glucose (mL/kg/min)	Fasting Serum Insulin (pmol/L)	30-Minute Serum Insulin (pmol/L)	Insulin Increment (0 to 30 min)†	2-Hour Serum Insulin (pmol/L)
Controls	9	4.7 (3.7-5.1)	5.5 (3.0-6.3)	3.5 (1.9-3.7)	8.9 (6.7-18.5)	44 (14-140)	240 (95-1,694)	6.4 (1.6-11.1)	153 (34-740)
TM									
HCV−	7	5.0 (4.4-6.2)	7.4 (4.0-8.2)‖	2.0 (1.0-3.4)§	6.3 (4.9-11.9)‖	27 (15-67)	357 (81-572)	6.4 (4.4-20.2)	246 (25-464)
HCV+	5	4.7 (4.1-13.1)	8.5 (6.4-23.0)§	1.2 (0.6-1.7)‡	3.6 (2.1-11.5)‖	35 (25-91)	582 (68-1,828)	7.5 (0.6-66.7)	117 (61-692)
Cirrhotic	8	5.3 (4.2-5.8)‖	8.0 (4.7-17.6)§	1.2 (0.8-2.6)‡	4.3 (2.0-7.4)‡¶	61 (19-162)	379 (153-952)	4.9 (0.9-19.7)	306 (289-756)‖
Significance by ANOVA*		.235*	.003	.0002	.003	.402	.843	.832*	.247

NOTE. Values are shown as the median (range) for skewed variables.

*Kruskal-Wallis test.

†Insulin increment calculated as (insulin 30 insulin 0)/insulin 0.

‡$P \leq .001$ *v* controls.

§$P \leq .01$ *v* controls.

‖$P \leq .05$ *v* controls.

¶$P \leq .05$ *v* HCV-negative TMs.

tration of 13.1 mmol/L, and all other subjects had levels of 6.5 mmol/L or less.

Glucose tolerance was worse in all patient groups than in controls (Table 3). Insulin sensitivity, measured as MCR glucose, was also significantly lower in cirrhotic patients than in controls, whereas the difference between TM patients and controls was of borderline significance (anti-HCV–negative, $P = .032$; anti-HCV–positive, $P = .013$). Group differences were similar when controlling for BMI and WHR (data not shown).

There was a strong negative relationship between 2-hour plasma glucose and insulin sensitivity (MCR glucose), which was similar across all patient groups (on logarithmically transformed data, $r = -.70, P < .0001$, n = 28; Fig 2). Using regression analysis, 49% of the variance of 2-hour plasma glucose was explained on the basis of MCR glucose alone (n = 28, $P < .0001$). With K_g as the dependent variable, the contribution of MCR glucose was similar but positive (36% of variance explained, n = 28, $P = .0007$; Fig 3).

The determinants of insulin insensitivity were assessed by relating MCR glucose to indices of iron overload in TM patients and of liver dysfunction in both groups. In TM patients, MCR glucose correlated negatively with serum ferritin ($r = -.62, P = .03$) and positively with serum albumin ($r = .67, P = .02$), but the relationships with AST levels and with units of blood transfused were not significant. In cirrhotic patients, relationships of the Pugh score[15] and AST with MCR glucose were not significant. In a multiple regression model in TM patients, HCV status did not contribute significantly to the variance of MCR glucose.

Fasting serum insulin concentrations did not differ significantly between groups, although 2-hour serum insulin was significantly higher in cirrhotic patients than in controls (Table 3). β-Cell function, assessed as the insulin increment (0 to 30 minutes), was also not significantly different between groups. In TM patients, neither serum ferritin nor anti-HCV antibody status were related either to 2-hour serum insulin ($P = .79$ and $P = .97$, respectively) or to insulin increment ($P = .90$ and $P = .13$, respectively).

The contribution of insulin deficiency to glucose intolerance was assessed by correlating three measures of insulinemia (fasting insulin, 2-hour insulin, and the insulin increment from 0 to 30 minutes) with measures of glucose tolerance after excluding the anti-HCV–positive TM patient with severe fasting hyperglycemia. This subject had a substantially impaired insulin increment (0.62) and low 2-hour serum insulin concentration (61 pmol/L), and diabetes was attributed to insulin deficiency. MCR glucose was a significant determinant of 2-hour plasma glucose, but neither incremental insulin nor 2-hour insulin contributed to the model. In a similar model with K_g as the dependent variable, neither measure of insulin contributed significantly to glucose intolerance (Table 4). TM patients had significantly lower K_g values than the other groups, even after controlling for insulinemia.

Fasting C-peptide concentrations were related to fasting

Table 4. Multiple Regression Model for Determinants of 2-hour Plasma Glucose and K_g in All Subjects Excluding the Patient With Severe Fasting Hyperglycemia

Independent Variable	Regression Coefficient	SE of β	*P*
2-hour plasma glucose			
MCR*	−.450	.172	.017
Insulin increment*	.177	.095	.078
2-hour serum insulin*	−.032	.088	.722
Patient group	−.053	.171	.762
	Multiple $R^2 = .52$		
	Adjusted $R^2 = .42, P = .007$		
K_g			
MCR*	.487	.229	.048
Insulin increment*	−.063	.126	.622
2-hour serum insulin*	.144	.117	.236
Patient group	.570	.228	.022
	Multiple $R^2 = .60$		
	Adjusted $R^2 = .51, P = .002$		

*Variables logarithmically transformed for inclusion in the model.

Table 5. Concentrations of C-peptide and Proinsulin-Like Molecules in the Study Groups

Group	No.	Fasting C-peptide (nmol/L)‡	Fasting C-peptide to Insulin Ratio	Fasting Intact Proinsulin (pmol/L)	Fasting des 31,32 Proinsulin (pmol/L)	Fasting Proinsulin (%)†
Controls	9	0.96 ± 0.36	22.0 (17.1-57.1)	2.1 (1.2-2.9)	0.7 (0.5-2.5)	7.1 ± 3.3
TM						
HCV−	7	0.86 ± 0.44	29.8 (9.7-48.0)	3.9 (1.9-5.8)‡	1.8 (0.5-11.6)	16.0 ± 7.7§
HCV+	5	0.74 ± 0.26	28.6 (4.6-34.0)	2.5 (1.0-3.3)	1.3 (0.8-2.2)	9.2 ± 6.7
Cirrhotics	8	1.09 ± 0.43	13.3 (5.6-101.1)	4.3 (2.4-6.4)‖	2.6 (1.4-10.6)‖	13.7 ± 6.1‡
Significance by ANOVA*		.481	.622*	.008*	.028*	.028

NOTE. Values are shown as the median and range for skewed variables.

*Kruskal-Wallis test.

†Proinsulin percent = 100 [(proinsulin + des 31,32 proinsulin)/(proinsulin + des 31,32 proinsulin + insulin)].

‡$P < .05$ *v* controls.

§$P < .02$ *v* controls.

‖$P < .01$ *v* controls.

insulin concentrations as an index of hepatic extraction[19] (Table 5). There were no significant differences between C-peptide to insulin ratios in the four subject groups. Fasting proinsulin and des 31,32 proinsulin concentrations were significantly elevated in anti-HCV–negative TM patients and in cirrhotics, and the proportion of proinsulin-like molecules, expressed as a percentage of total insulin-like molecules, was significantly elevated in these subject groups (Table 5). However, this elevation was no longer significant after controlling for glucose intolerance.

DISCUSSION

Iron overload, whether due to hemochromatosis or to TM, produces both liver and pancreatic damage. Liver disease results in hyperinsulinemia as a consequence of increased insulin secretion, impaired hepatic removal, and porta-systemic shunting of insulin.[7,12,13,20,21] Elegant studies by Kruszynska et al[11,21,22] have shown that the oral glucose intolerance of cirrhosis is in part the result of a more rapid entry of glucose into the systemic circulation, but is mainly the consequence of diminished glucose disappearance, resulting both from a lower insulin sensitivity of peripheral tissues and, in diabetic cirrhotics, from reduced glucose effectiveness. The cause of the peripheral insulin resistance in cirrhosis is unclear,[7,23] but may represent abnormalities of insulin receptors or glucose transporters, perhaps related to increased production of cytokines such as tumor necrosis factor-α or of nonesterified fatty acids.

Previous studies on TM subjects with glucose intolerance have shown high circulating insulin concentrations,[3,8] but these data are confounded by the problems of interpreting conventional insulin assays.[24,25] Two groups have found an elevated basal C-peptide concentration and an increased C-peptide response to stimuli in TM subjects,[3,26] suggesting that β-cell function is not deficient in this condition.

We used the modified Harano method for estimating insulin sensitivity, a technique that does not use somatostatin to suppress endogenous insulin production.[14] The glucose and insulin infusion with somatostatin has been validated in cirrhotic patients,[27] but the present technique has not been so assessed. It is possible that diminished insulin clearance in patients with liver disease[21] might affect interpretation of any test of insulin sensitivity, including that using a euglycemic clamp.

The major determinant of glucose intolerance in both TM and cirrhotic patients is insulin insensitivity. In TM patients, insulin resistance relates to the degree of liver damage and serum ferritin levels, but not to HCV status. These findings confirm previous reports that the major component of glucose intolerance in TM patients without fasting hyperglycemia is related to liver and not to pancreatic damage, with this in turn being related to transfusional iron overload.[3,28] In comparison to cirrhotic patients, TM subjects had extremely mild abnormalities of liver function despite parallel impairment of insulin sensitivity and glucose tolerance. It is possible, then, that insulin resistance and liver damage in TM may both result from increased cytokine production[23] or tissue deposition of iron,[3] which affect peripheral insulin action. In TM subjects, there was no evidence for a role of either ferritin levels or HCV status in contributing to insulin concentrations, and we found no evidence for a contribution of insulin deficiency to glucose intolerance. However, it is likely that more severely glucose-intolerant subjects show greater degrees of insulin deficiency,[21,28] and the contribution of HCV to frank diabetes mellitus remains to be defined. We have found no evidence in this study that the proportions of proinsulin-like molecules differ in different subject groups independently of the degree of glucose intolerance, supporting our findings in a larger group of cirrhotic patients.[29]

In conclusion, we have shown that the glucose intolerance in TM is mainly determined by insulin resistance. The insulin resistance in turn parallels the degree of liver damage, which is predominantly related to iron overload. HCV may contribute further to the degree of glucose intolerance, perhaps by its effect on hepatic function or by the release of cytokines, but we have found no evidence of a contribution of insulin deficiency, either from iron overload or HCV infection, to the glucose intolerance of TM subjects.

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REFERENCES

1. Saudek CD, Hemm RM, Peterson CM: Abnormal glucose tolerance in β thalassaemia major. Metabolism 26:43-52, 1977
2. Dandona P, Hussain MAM, Varghese Z, et al: Insulin resistance and iron overload. Ann Clin Biochem 20:77-79, 1983
3. Merkel PA, Simonson DC, Amiel SA, et al: Insulin resistance and hyperinsulinemia in patients with thalassaemia major treated by hypertransfusion. N Engl J Med 318:809-814, 1988
4. Zurlo MG, De Stefano P, Borgna-Pignatti C, et al: Survival and major causes of death in thalassaemia major. Lancet 2:27-30, 1989
5. Alter HJ: Chronic consequences of non-A, non-B hepatitis, in Seeff LB, Lewis JH (eds): Current Perspectives in Hepatology. New York, NY, Plenum, 1989, pp 83-97
6. De Sanctis V, Zurlo MG, Senesi E, et al: Insulin dependent diabetes in thalassaemia. Arch Dis Child 63:58-62, 1988
7. Petrides AS, DeFronzo RA: Glucose metabolism in cirrhosis: A review with some perspectives for the future. Diabetes Metab Rev 5:691-709, 1989
8. De Sanctis V, Ascola GD, Wonke B: The development of diabetes mellitus and chronic liver disease in long term chelated β thalassaemic patients. Postgrad Med J 62:831-836, 1986
9. World Health Organization Study Group: Diabetes mellitus. WHO Tech Rep Ser 727: 1985, p 11
10. Pugh RNH, Murray-Lyon IM, Dawson JL, et al: Transection of the oesophagus for bleeding oesophageal varices. Br J Surg 60:646-649, 1973
11. Kruszynska YT, Meyer-Alber A, Darakhshan F, et al: Metabolic handling of orally administered glucose in cirrhosis. J Clin Invest 91:1057-1066, 1993
12. Megyesi C, Samols E, Marks V: Glucose tolerance and diabetes in chronic liver disease. Lancet 2:1051-1056, 1967
13. Conn HO, Schreiber W, Elkington SG: Cirrhosis and diabetes. II. Association of impaired glucose tolerance with portal-systemic shunting in Laennec's cirrhosis. Dig Dis 16:227-239, 1971
14. Heine RJ, Home PD, Poncher M, et al: A comparison of 3 methods for assessing insulin sensitivity in subjects with normal and abnormal glucose tolerance. Diabetes Res 2:113-120, 1985
15. Durnin JVGA, Womersley J: Body fat assessed from total body density and its estimation from skinfold thickness: Measurements on 481 men and women aged from 16 to 72 years. Br J Nutr 32:77-97, 1974
16. Mohamed-Ali V, Yudkin JS: An end-point amplified enzymoimmunoassay (IEMA) specific for human insulin. Clin Sci 82:4P, 1992 (suppl 27, abstr)
17. Sobey WJ, Beer SF, Carrington CA, et al: Sensitive and specific two-site immunoradiometric assays for human insulin, proinsulin, 65-66 split and 32,33 split proinsulins. Biochem J 260:535-541, 1989
18. Mohamed-Ali V, Yudkin JS: Simple and highly sensitive microplate immunoradiometric assays (IRMAs) for intact proinsulin and des 31,32 proinsulin. Diabetic Med 10:S30, 1993 (suppl 1, abstr)
19. Kasperska-Czyzykowa T, Heding LG, Czyzyk A: Serum levels of true insulin, C-peptide and proinsulin in peripheral blood of patients with cirrhosis. Diabetologia 25:506-509, 1983
20. Smith-Laing G, Sherlock S, Faber OK: Effects of spontaneous portal-systemic shunting on insulin metabolism. Gastroenterology 76:685-690, 1979
21. Kruszynska YT, Home PD, McIntyre N: Relationship between insulin sensitivity, insulin secretion and glucose tolerance in cirrhosis. Hepatology 14:103-111, 1991
22. Kruszynska YT, Harry DS, Bergman RN, et al: Insulin sensitivity, insulin secretion and glucose effectiveness in diabetic and non-diabetic cirrhotic patients. Diabetologia 36:121-128, 1993
23. Kruszynska YT, McIntyre N: Glucose intolerance and diabetes in liver disease. Diabetes Ann 7:55-82, 1993
24. Temple RC, Clark PMS, Nagi DK, et al: Radioimmunoassay may overestimate insulin in non–insulin-dependent diabetics. Clin Endocrinol (Oxf) 32:689-693, 1990
25. Nagi DK, Hendra TJ, Ryle AJ, et al: The relationships of concentration of insulin, intact proinsulin and 32-33 split proinsulin with cardiovascular risk factors in type 2 non–insulin-dependent diabetic subjects. Diabetologia 33:532-537, 1990
26. Livadas DP, Economou E, Sofroniadou K, et al: A study of beta-cell function after glucagon stimulation in thalassaemia major treated by high transfusion programme. Clin Endocrinol (Oxf) 27:485-490, 1987
27. Greco AV, Rebuzzi AG, Altomonte L, et al: Glucose, insulin and somatostatin infusion for the determination of insulin resistance in liver cirrhosis. Horm Metab Res 11:547-549, 1979
28. Dmochowski K, Finegood D, Francombe B, et al: Factors determining glucose tolerance in patients with thalassemia major. J Clin Endocrinol Metab 77:478-483, 1993
29. Kruszynska YT, Harry DS, Mohamed-Ali V, et al: The contribution of proinsulin and des 31,32 proinsulin to the hyperinsulinemia of diabetic and nondiabetic cirrhotic patients. Metabolism 44:254-260, 1995