# Insulin Resistance in Malnutrition-Related Diabetes Mellitus is not Mediated by Insulin Antibodies

R. HARSHA RAO, MD, MRCP

Malnutrition-related diabetes mellitus (MRDM) comprises at least two different clinical patterns termed *protein deficient (type J) diabetes* and *fibrocalculous pancreatic diabetes*, which are both seen in young individuals and are distinguished by the exocrine pancreatic failure characteristically seen in the latter (1). Certain unique clinical and biochemical characteristics common to both, such as ketosis resistance, insulinopenia, and insulin resistance, in addition to the common denominator of moderate-to-severe malnutrition, justify their inclusion under the umbrella term MRDM (1). Thus far, no attempt has been made to identify the cause of the peculiar insulin resistance that results in an insulin requirement that exceeds 2.5 $U \cdot kg^{-1} \cdot day^{-1}$. There is reason to suspect that insulin antibodies may contribute, at least in part, to the insulin resistance, because patients with type J diabetes, who usually belong to the poorest sections of society, and are unable to afford the cost of continuous therapy with insulin, characteristically stop taking insulin for periods of weeks or months. This pattern of intermittent therapy is very conducive to antibody formation with the highly immunogenic, mixed beef–pork insulin mixtures that are taken for their lower cost and greater availability (2,3). The purpose of this study was specifically to determine whether circulating antibodies to insulin contribute to insulin resistance in MRDM, as a first attempt to identify the pathophysiological basis for the insulin resistance seen in MRDM.

**PATIENTS**— Twelve insulin-dependent patients with type J diabetes who met all the criteria suggested by Ahuja (4), were studied (fasting plasma glucose level >11 mM, diabetes onset before age 25 yr, body mass index (BMI) <18 kg/$m^2$, an absence of ketosis on withdrawal of insulin for a period of 10 days, and an insulin requirement >2.5 $U \cdot kg^{-1} \cdot day^{-1}$). Six patients with type I diabetes belonging to the same socioeconomic group were studied for comparison. All patients had been taking mixed beef–pork insulin preparations for >1 yr. Because of the unreliable nature of the treatment, all patients were hospitalized and treated with insulin as part of the study protocol. The dose was adjusted to achieve near euglycemia (~150–200 mg/dl), and a 24-h urine that was free of glucose during a week's observation in the hospital. Thus, relatively good short-term glycemic control was established in these patients, which resulted in normal fasting levels of free fatty acids (FFAs) on the morning before study. Insulin requirements were calculated on this basis rather than from the stated dose being taken by the patient outside of the hospital. Insulin binding was assayed in plasma after an overnight fast, 36 h after the last dose of insulin. $^{125}$I-labeled insulin, prepared by the chloramine-T method and purified by gel chromatography on the morning of the assay (5), was incubated (20 femtomoles) with 0.1 ml plasma in barbital buffer containing 5% bovine serum albumin, pH 7.4, in a total volume of 1 ml at 4°C for 48 h, with and without an excess ($10^{-5}$ M) of cold insulin. At the end of the incubation, 1 ml of ice-cold 25% polyethylene glycol was added to precipitate immunoglobulins (6), the tubes were mixed on a vortex mixer and centrifuged at 2000 g for 20 min. The supernatant was decanted, the tubes were allowed to drain, and the pellet was counted. After subtraction of nonspecific binding, insulin binding (%) was calculated as specific counts in the pellet/total counts added to the tube × 100. In 10 control plasma samples, insulin binding averaged 1.2% with a standard deviation of 1.07% (range 0–2.8%). The upper limit of normal was taken as 3.5% (mean + 2SD).

**RESULTS**— Clinical, biochemical, and hormonal data have been reported in detail in an earlier article (7). The mean ages were not significantly different (16.8 ± 1 yr in type J and 16.2 ± 1.4 yr in type I, mean ± SE). Similarly, BMI and skin-fold thicknesses (ST) were not different in type J (BMI 16 ± 1 kg/$m^2$, ST 8.9 ± 1.1 mm) and type I patients (BMI 14 ± 1 kg/$m^2$, ST 6.5 ± 0.4 mm). The FFA level in plasma was also virtually

FROM THE DIVISION OF ENDOCRINOLOGY AND METABOLISM, NATIONAL INSTITUTE OF NUTRITION, HYDERABAD, INDIA.

ADDRESS CORRESPONDENCE AND REPRINT REQUESTS TO THE DEPARTMENT OF MEDICINE, MONTEFIORE UNIVERSITY HOSPITAL, UNIVERSITY OF PITTSBURGH SCHOOL OF MEDICINE, 3459 FIFTH AVENUE, PITTSBURGH, PA 15213.

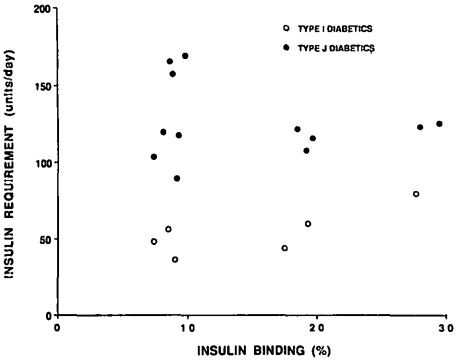


**Figure 1**—*Insulin requirement and percentage insulin binding plotted to show the absence of any correlation between the two parameters in type J diabetic subjects with significant insulin resistance (solid circles) and insulin-dependent diabetic subjects without insulin resistance (open circles).*

identical in the two groups prior to study (type J 636 ± 38 μM, type I 674 ± 54). Insulin responses to glucose (1.75 g/kg) were almost completely absent in both groups, with a maximum increment in free insulin levels of 48 ± 13 pM in type J patients and by 40 ± 12 pM in the type I patients.

Insulin was significantly bound (>5%) in the plasma of all subjects, but there was no difference between mean insulin binding in the two groups of diabetic patients (type J 14.7 ± 2.3% vs. type I 14.9 ± 3.3%). The proportion of diabetic patients who had significant amounts of binding in serum (>20%) was not different in the two groups (2 of 12 type J, 1 of 6 type I patients) and no subject had a percent insulin binding >30%. Despite this similarity in insulin binding, type J diabetic subjects had insulin requirements that were twice as high as those seen in type I diabetic subjects (125 ± 7 U/day in type J vs. 54 ± 6 U/day in type I, $P < 0.001$). There was no correlation between insulin binding and dose of insulin required for glycemic control in the type J patients (Fig. 1). In fact, in the three patients with the highest insulin requirement (>130 U/day, >3 U/kg/day) insulin binding was <10%.

**CONCLUSIONS**— The preliminary suspicion that the insulin resistance seen in MRDM could be due to anti-insulin antibodies that are induced by the intermittent therapy with impure insulins, has not been borne out by the results of this study. MRDM was shown to be associated with a 2.5 times greater insulin requirement compared with patients with type I diabetes who had the same degree of near-total β-cell failure. The marked insulin resistance of these insulinoprival, type J diabetic patients becomes evident when viewed in the proper perspective: a need for 3 U insulin · $kg^{-1}$ · $day^{-1}$, is greater than the needs of all but the most markedly resistant, hyperinsulinemic, non-insulin-dependent (type II) diabetic patients (300 U/day in a patient weighing 100 kg). Insulin tolerance tests confirm this: insulin sensitivities in MRDM are similar to those in type II patients, rather than type I patients (8). Because type J patients share features of insulinopenia and leanness with type I patients, this degree of insulin resistance in an insulinoprival form of diabetes is puzzling. Starvation can be excluded as a cause for this phenomenon because patients in this study were not starving to begin with and received a standard 2000 kcal hospital diet for at least 10 days before the study, which is adequate to reverse the insulin resistance of starvation (9). It is also unlikely that poorer glycemic control was responsible for the resistance because it has been shown that patients with MRDM are more resistant than even obese type II diabetic patients, despite comparable degrees of glycemic control, as measured by $HbA_1$ levels (10). It is possible that prolonged periods of glycemic control may be required to reverse insulin resistance in MRDM, but studies in type I diabetes (with which MRDM is comparable in terms of β-cell failure and bodily habitus) show that even a week can make a substantial difference (11). However, the role of glycemic control in this phenomenon cannot be decided from this study because the aims were limited to ruling out the involvement of antibodies.

In conclusion, the cause of the insulin resistance in MRDM is still unknown. However, based on the data reported here, it is reasonable to conclude that antibodies to insulin, acquired as a consequence of the treatment of these individuals, play no role in mediating the insulin resistance of MRDM.

## References


1. World Health Organization: *Diabetes Mellitus: Report of a WHO Study Group*. Geneva, World Health Org., 1985 (Tech. Rep. Ser. no. 727)
2. Rao RH: Diabetes in the undernourished: coincidence or consequence? *Endocr Rev* 9:67–85, 1988
3. Vigg BL, Rao RH: The problem of diabetes in a rural population. *J Diabetic Assoc India* 21 (Suppl 1.):17–22, 1981
4. Ahuja MMS: Diabetes—Special problems in developing countries. *Bull Deliv Health Care Diabetics Devel Countries* 1:5–6, 1980
5. Freychet P: Insulin receptors. In *Methods of Receptor Research*. Part II, Blecher M, Ed. New York, Marcel Dekker, p. 385–482, 1976
6. Desbuquois B, Aurbach GD: Use of polyethylene glycol to separate free and antibody-bound peptide hormones in radioimmunoassays. *J Clin Endocrinol & Metab* 33:732–38, 1971
7. Rao RH, Vigg BL, Rao KSJ: Suppressible glucagon secretion in young, ketosis-resistant, type J diabetic patients in India. *Diabetes* 32:1168–71, 1983
8. Ahuja MMS, Vishwanadham K: Differential mobilization of non-esterified fatty acids and insulin reserve in various clinical types of diabetes mellitus in India. *Indian J Med Res* 55:870–83, 1967
9. DeFronzo RA, Soman V, Sherwin RS, Hendler R: Insulin binding to monocytes

and insulin action in human obesity, starvation, and refeeding. *J Clin Invest* 62:204–13, 1978
10. Mohan V, Ramachandran G, Kumar GV, Snehlatha C, Viswanathan M: Insulin resistance in fibrocalculous pancreatic diabetes. *Horm Metab Res* 20:746–48, 1988
11. Lager I, Lonnroth P, Von Schenck H, Smith U: Reversal of insulin resistance in type 1 diabetes after treatment with continuous subcutaneous insulin infusion. *Br Med J* 287:1661–64, 1983

# Hyperproinsulinemia in Type II Diabetes

ROBERT M. COHEN, MD
DAVID M. NATHAN, MD
REX S. CLEMENTS, JR, MD

For quite some time, there has been concern that hyperinsulinemia may be a risk factor for accelerated vascular disease in patients with non-insulin-dependent diabetes mellitus (type II) (1–4). More recently it has become apparent that proinsulin makes up an increased proportion of the measured immunoreactive insulin (IRI) in the sera of patients with type II diabetes (5–8). In nondiabetic subjects, the proinsulin-IRI ratio was in the range of 10–20%, whereas in patients with type II diabetes this ratio can exceed 50% (5–9). The possibility has been raised that proinsulin and its split products (rather than insulin itself) may be associated with the recognized cardiovascular risk factors in type II diabetes (9). In addition, in a single study, an excess of myocardial infarctions was observed in patients with type II diabetes treated for 2 yr with exogenous intact human proinsulin (10).

Although the precise mechanisms responsible for hyperproinsulinemia in type II diabetes are poorly understood, it is predicted that the proinsulin-IRI ratio would be raised in patients with type II diabetes treated with sulfonylureas, but would be normalized if the β-cell was "put at rest" by the administration of exogenous insulin (6). To address this issue, we measured the fasting IRI, proinsulin, and proinsulin-IRI molar ratio in previously described patients with type II diabetes who were randomized to receive either an oral sulfonylurea (glyburide) or insulin injections (NPH insulin, once daily) (11). As indicated by their $HbA_{1c}$ percentages (Table 1), these two groups of patients achieved equivalent glycemic control within 1 mo of randomization.

Proinsulin measurements were performed using a modification (12) of our previously described assay using antiserum 11E (13). In this assay, des 31–32 proinsulin cross-reacts ~38% as well as intact proinsulin. Because des 64–65 proinsulin crossreactivity is only ~10% and because this product makes up a small fraction of the circulating proinsulin constituents (14–16), it provides a negligible contribution in this assay. In fasting, nondiabetic subjects, this assay gives a proinsulin-IRI molar ratio of 18.6 ± 3.5%, whereas the mean proinsulin-IRI ratio was raised in our study subjects before randomization (35.7 ± 1.9%, $P < 0.001$ vs. control subjects).

**Table 1—*Glycosylated hemoglobin, fasting immunoreactive insulin (IRI), proinsulin, and proinsulin-IRI molar ratio in patients with non-insulin-dependent diabetes treated for 1 mo with either sulfonylurea or insulin***

| TREATMENT GROUP | $HbA_{1c}$ (%) | IRI (pM) | PROINSULIN (pM) | PROINSULIN-IRI RATIO (%) |
|---|---|---|---|---|
| SULFONYLUREA-TREATED ($N = 14$) | 8.3 ± 1.4 | 124 ± 20 | 41.4 ± 8.3 | 42.3 ± 6.2 |
| INSULIN-TREATED ($N = 7$) | 8.1 ± 1.0 | 104 ± 12 | 19.5 ± 3.2 | 22.3 ± 2.4* |

Values are means ± SE.
*$P < 0.01$ vs. sulfonylurea-treated group by a 2-sample $t$ test.

FROM THE DEPARTMENT OF INTERNAL MEDICINE, UNIVERSITY OF CINCINNATI COLLEGE OF MEDICINE, CINCINNATI, OHIO; DEPARTMENT OF MEDICINE, MASSACHUSETTS GENERAL HOSPITAL AND HARVARD MEDICAL SCHOOL, BOSTON, MASSACHUSETTS; AND THE DEPARTMENT OF MEDICINE, UNIVERSITY OF PENNSYLVANIA SCHOOL OF MEDICINE, PHILADELPHIA, PENNSYLVANIA.

ADDRESS CORRESPONDENCE AND REPRINT REQUESTS TO REX S. CLEMENTS, JR, MD, NOVO NORDISK PHARMACEUTICALS INC., 100 OVERLOOK CENTER, PRINCETON, NJ 08540.