Hyperinsulinemia in Offspring of Non–Insulin-Dependent Diabetes Mellitus Patients: The Role Played by Abnormal Clearance of Insulin

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Insulin resistance and hyperinsulinemia are often found in first-degree relatives of non–insulin-dependent diabetes mellitus (NIDDM) patients, and are currently considered a familial trait of this population at increased risk for diabetes. This study was undertaken to determine the role played by the metabolic clearance rate (MCR) of insulin (MCR-I) in the hyperinsulinism of these subjects. The proband population, consisting of 48 subjects aged 29.2 ± 4.4 (mean ± SD) years (18 men and 30 women; body mass index, 24.6 ± 0.8 kg/m^2; fasting plasma glucose, 4.54 ± 0.37 mmol/L), was assigned in random order to four groups (I, II, III, and IV), each receiving a double insulin/glucose infusion (I, 0.025/2.0; II, 0.050/3.5; III, 0.100/6.0; and IV, 0.200/8.0 U/kg · h and mg/kg · min, respectively) to calculate MCR-I and MCR of glucose (MCR-G). Forty (14 men and 26 women) age- and body mass index–matched healthy individuals served as controls. All subjects had a normal response to an oral glucose tolerance test (75 g) according to World Health Organization criteria. Basal plasma insulin and C-peptide levels in probands were significantly ($P < .05$) higher than in controls in each study group; similarly, MCR-I was significantly (at least $P < .05$) lower in probands than in controls in all groups. MCR-G was significantly (at least $P < .05$) decreased in probands as compared with controls of groups III and IV. These results suggest that a reduced plasma insulin removal by body tissues is operative in the offspring of NIDDM patients, and explain, at least in part, the hyperinsulinemia/insulin resistance in the early phase of the natural history of diabetes mellitus.

NON–INSULIN-DEPENDENT diabetes mellitus (NIDDM) is characterized by resistance of peripheral tissues to insulin and deficiency of insulin secretion.[1,2] The primary or earliest defect (determinant) that conveys a higher risk to develop diabetes to first-degree relatives of NIDDM patients is still being debated. Barnett et al[3] observed poor insulin responses to secretagogues in monozygotic twins of NIDDM parents, whereas Bonora et al[4] found physiological increments of β-cell secretory products (insulin and C-peptide) following an oral glucose challenge in relatives of NIDDM patients. Increased basal and stimulated insulin concentrations coupled with insulin resistance and reduced suppression of lipid oxidation have been reported to occur in offspring of couples with NIDDM.[5] Moreover, the insulin resistance of these individuals has been attributed to defects in the nonoxidative glucose pathway.[6]

In euglycemic conditions, fasting hyperinsulinemia is thought to indicate underlying insulin resistance and may represent a mechanism to maintain normal glucose homeostasis.[7] On the other hand, reduced hepatic extraction of insulin has been repeatedly reported in both obesity and hypertension, two conditions characterized by fasting hyperinsulinemia.[8,9] The present study aimed to verify whether an alteration of insulin metabolic clearance may be present in offspring of NIDDM patients.

SUBJECTS AND METHODS

This study was performed in 48 subjects (18 men and 30 women) selected from a large population of first-degree relatives of NIDDM parents attending the outpatient department of our institution. To take part in the study, all subjects had to meet the following criteria: (1) both parents affected by NIDDM, or only one NIDDM parent with a strong family history (ie, father or mother plus another relative of the same gender line among grandparents or siblings); (2) normal glucose tolerance according to World Health Organization criteria[10]; (3) normal body weight, ie, body mass index less than 27 kg/m^2 for men and less than 26 for women; (4) absence of any pharmacological treatment; and (5) normal blood pressure and absence of endocrinological or metabolic diseases. Forty normal subjects (14 men and 26 women) without a family history of diabetes and matched for age, sex, and weight served as a control group. All subjects gave informed written consent to participate in the study after a clear explanation of its nature. The study protocol was approved by the Ethics Committee of our department. Each subject was invited to consume during the 3 days before the study a standard (50% carbohydrate, 35% fat, and 15% protein) weight-maintaining diet without salt restriction. A preliminary oral glucose tolerance test (75 g) was performed in all subjects. Afterward, the 48 relatives were randomly assigned to four groups, each consisting of 12 subjects. Each group was submitted for 120 minutes to a continuous infusion of different doses of insulin (Humulin-R; Eli Lilly, Indianapolis, IN) dissolved in 0.9% NaCl containing 0.6% serum albumin (group I, 0.025; II, 0.05; III, 0.1; and IV, 0.2 U/kg · h) and glucose (as 20% glucose solution; group I, 2; II, 3.5; III, 6; and IV, 8 mg/kg · min). We used this dose-response protocol because the metabolic clearance rate (MCR) of insulin (MCR-I) is known to vary along with its plasma concentrations.[11]

The tests were begun at 8 AM after an overnight (10 to 12 hours) fast, with subjects lying supine. One polyethylene cannula was inserted into an antecubital vein (kept patent with 0.15 mol/L saline solution) for double infusion, which was performed by variable programmable-rate pumps. A second cannula was inserted retrogradely into a dorsal vein on the other hand (surrounded by a 60°C heated box to ensure arterialization of venous blood) for blood samplings.

Blood was obtained for measurements of plasma glucose and insulin at −30, 0, 30, 60, 90, 100, 110, and 120 minutes, and for plasma C-peptide at −30, 0, and 120 minutes.

The same procedures were applied to control subjects equally and randomly subdivided into four groups. Clinical characteristics of the subjects are summarized in Table 1.

Plasma glucose was assayed by the glucose oxidase method

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Table 1. Clinical and Metabolic Characteristics of Probands and Controls Divided Into Four Groups

Group	Sex (M/F)	Age (yr)	BMI (kg/m²)	Glucose (mmol/L)	IRI (pmol/L)	C-peptide (pmol/L)
I						
P	4/8	30.1 ± 4.2	24.7 ± 0.7	4.46 ± 0.38	62.42 ± 6.46†	400.51 ± 129.09*
C	4/6	29.7 ± 3.9	25.1 ± 0.9	4.37 ± 0.31	36.60 ± 8.61	334.31 ± 135.71
II						
P	5/7	28.4 ± 6.1	23.9 ± 0.9	4.43 ± 0.39	65.29 ± 7.17†	377.34 ± 122.47*
C	3/7	29.2 ± 4.7	24.8 ± 0.6	4.45 ± 0.33	38.74 ± 10.04	301.21 ± 109.23
III						
P	4/8	27.9 ± 5.3	24.8 ± 1.4	4.59 ± 0.34	58.12 ± 5.74†	344.24 ± 99.30*
C	4/6	28.6 ± 6.4	25.0 ± 0.8	4.41 ± 0.32	35.16 ± 7.89	281.35 ± 95.99
IV						
P	5/7	30.4 ± 3.9	25.1 ± 0.6	4.67 ± 0.39	58.83 ± 9.33†	354.17 ± 115.85*
C	3/7	30.6 ± 4.4	24.6 ± 0.9	4.49 ± 0.30	33.72 ± 6.46	287.97 ± 112.54

Abbreviations: P, probands; C, controls; BMI, body mass index; IRI, immunoreactive insulin.
*$P < .05$ *v* controls.
†$P < .005$ *v* controls.

(Beckman Glucose Analyzer; Beckman Instruments, Fullerton, CA). Plasma insulin and C-peptide levels were measured by radioimmunoassay (Sorin, Milan, Italy). Steady-state plasma glucose and steady-state plasma insulin concentrations (SS-IRI) were calculated as the mean of values obtained between minutes 90 and 120 of the infusion test. The MCR of glucose (MCR-G) during the steady state of the insulin-glucose infusion was calculated by dividing the glucose infusion rate (milligrams per minute) by steady-state plasma glucose (grams per liter). MCR-I was calculated according to the method reported by Ferrannini et al[12] as the ratio of insulin infusion rate to steady-state plasma exogenous immunoreactive insulin concentration (the last represents the difference between steady-state plasma insulin concentration and plasma endogenous insulin level) multiplied by the ratio of C-peptide at 120 minutes to basal C-peptide concentrations. The formula is as follows:

$$\text{MCR-I (mL/min} \cdot \text{kg)} = \frac{\text{exogenous insulin infusion rate } (\mu\text{U/min} \cdot \text{kg})}{\text{SS-IRI } (\mu\text{U/mL}) - \text{basal IRI } (\mu\text{U/mL})} \times \frac{\text{C-peptide at 120 minutes (ng/mL)}}{\text{basal C-peptide (ng/mL)}}.$$

This computation is based on the equimolecular pancreatic secretion of insulin and C-peptide, and requires that the MCRs of the two hormones are constant. MCR-I so calculated represents the plasma clearance of exogenous hormone by all body tissues, including liver.

Results are expressed as the mean ± SD. ANOVA was used to test the significance of differences between group means. Unpaired Student's *t* test was used to compare responses in each infusion group between relatives and controls. Multiple *t* test analysis was confirmed and corrected by the Bonferroni test. Correlations were tested by linear regression analyses.

RESULTS

Basal metabolic parameters found in probands and controls are shown in Table 1. In all groups, probands had basal plasma insulin and C-peptide concentrations that were significantly higher ($P < .005$ and $P < .05$, respectively) than in respective controls. The basal C-peptide to insulin molar ratio was 6.03 ± 1.01 in probands and 8.36 ± 3.10 in the whole control group ($P < .01$). Plasma glucose levels were not significantly different.

Plasma glucose concentrations following the oral glucose challenge were not significantly different between probands and controls (Fig 1), nor was there any significant difference in glucose area under the plasma glucose curve (222 ± 19 mmol/L/min in probands v 214 ± 22 in controls, P = NS). At all points of the curve, insulin levels following oral glucose were significantly (at least $P < .05$) higher in probands than in controls. Insulin area was 39,300 ± 740 in probands and 29,205 ± 570 pmol/L/min in controls ($P < .01$).

Table 2 shows the metabolic parameters during insulin-glucose infusions. In all groups, steady-state plasma insulin and C-peptide levels were significantly (at least $P < .05$) higher in probands than in controls, whereas steady-state plasma glucose concentrations were significantly higher only in groups III and IV.

Calculation of MCR-G produced progressively increasing values with increasing amounts of insulin-glucose infused per minute both in probands and in controls (Table 3). However, MCR-G was significantly (at least $P < .05$) lower in probands of the last two groups as compared with respective control groups.

MCR-I increased progressively throughout the study groups in both probands and controls (Table 3). At each infusion rate of the exogenous hormone, MCR-I was significantly (at least $P < .05$) lower in probands.

A strong significant relationship between MCR-G and MCR-I was found in both proband and control subjects ($r = .91$ and .92, respectively) independently of the insulin-glucose infusion rate (Figs 2 and 3, respectively).

DISCUSSION

Results of the present study show that normotolerant, non-obese offspring, who had at least one parent affected by NIDDM, as a group are hyperinsulinemic both in the fasting state and after an oral glucose challenge as compared with another group of carefully matched normal individuals whose parents had no diabetes or impaired

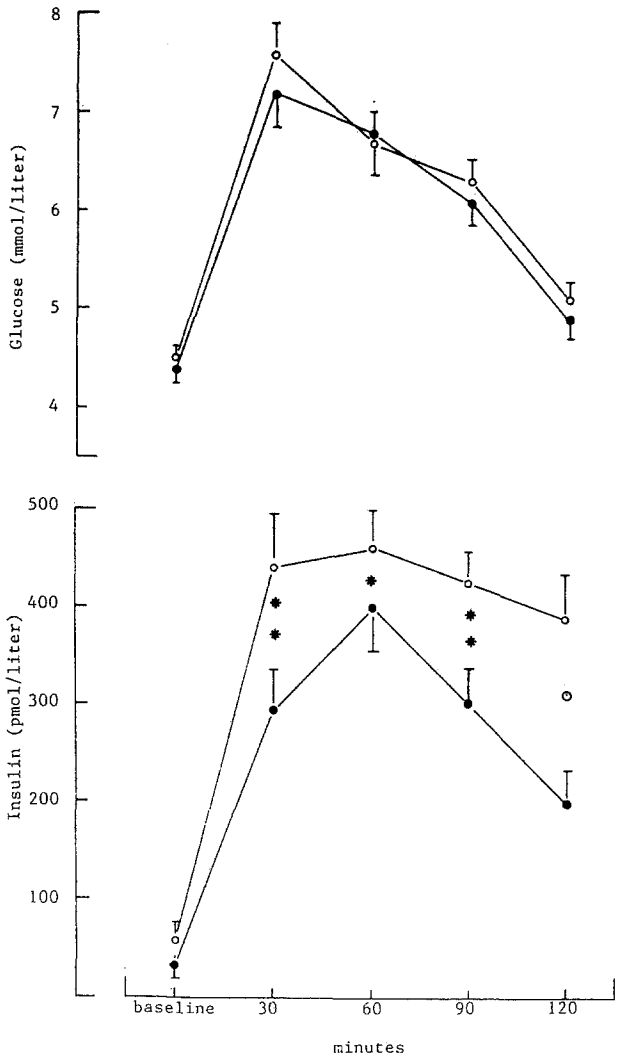

Fig 1. Glucose and insulin responses to a glucose (75 g) challenge in probands (○) and controls (●). *$P < .05$; **$P < .01$; °$P < .005$.

Table 2. Steady-State Metabolic Parameters in Probands and Controls

Group	Glucose (mmol/L)	IRI (pmol/L)	C-peptide (pmol/L)
I			
P	3.91 ± 0.39	359.47 ± 72.47†	178.74 ± 62.89*
C	3.84 ± 0.27	172.92 ± 64.57	95.99 ± 33.10
II			
P	4.22 ± 0.33	410.41 ± 53.09†	218.46 ± 89.37†
C	4.02 ± 0.36	189.42 ± 58.12	102.61 ± 56.27
III			
P	4.52 ± 0.34*	504.40 ± 71.03†	195.29 ± 69.51†
C	3.92 ± 0.38	195.88 ± 70.31	95.99 ± 36.41
IV			
P	5.39 ± 0.49*	675.88 ± 99.73†	228.39 ± 99.30†
C	4.45 ± 0.39	367.36 ± 81.79	122.47 ± 49.65

NOTE. Abbreviations are as in Table 1.
*$P < .05$ *v* controls.
†$P < .005$ *v* controls.

Table 3. MCR-G and MCR-I (mL/min · kg) in All Groups

Group	MCR	Probands	Controls	*P*
I	Glucose	2.80 ± 0.28	2.89 ± 0.21	NS
	Insulin	4.54 ± 1.12	6.40 ± 1.91	.05
II	Glucose	4.60 ± 0.60	4.83 ± 0.47	NS
	Insulin	10.09 ± 2.20	13.49 ± 2.63	.05
III	Glucose	7.37 ± 0.64	8.49 ± 0.69	.05
	Insulin	15.35 ± 5.46	25.30 ± 9.64	.005
IV	Glucose	8.23 ± 0.85	9.98 ± 0.79	.01
	Insulin	25.19 ± 11.42	30.11 ± 16.78	.05

glucose tolerance. Since hyperinsulinemia is currently viewed as a marker of insulin resistance,[13] first-degree relatives of NIDDM subjects are characterized by reduced insulin sensitivity. The higher fasting C-peptide levels found in probands indicate enhanced β-cell pancreatic secretion; however, the significantly lower C-peptide to insulin molar ratio observed in probands suggests that a decreased insulin removal may also contribute to their basal hyperinsulinism.

We used a double insulin-glucose infusion at different prefixed rates. This simple technique is a suitable methodology to determine insulin sensitivity, as validated by Heine et al,[14] who found a good correlation with the euglycemic clamp. Although steady-state metabolic parameters are usually measured by inhibiting endogenous insulin secretion with either epinephrine and propranolol[15] or somatostatin,[16] the need for these procedures has never been validated, and a highly significant correlation was found between insulin sensitivity assessed with and without somatostatin infusion.[17]

With this methodology, we found that probands had higher steady-state plasma glucose levels and lower MRC-G

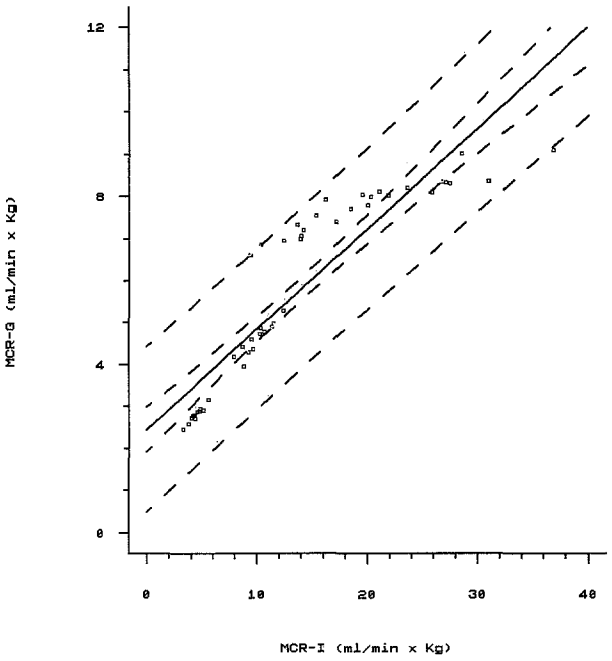

Fig 2. Correlation between MCR-G and MCR-I in all probands (r = .91).

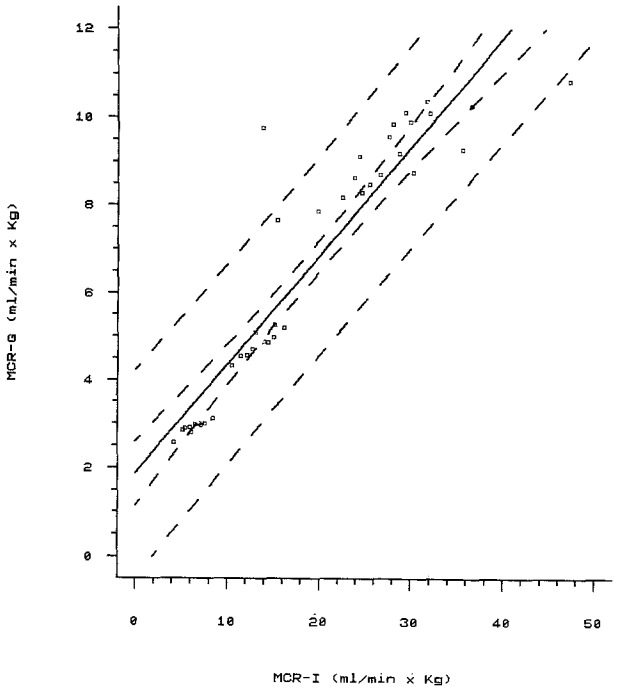

Fig 3. Correlation between MCR-G and MCR-I in all controls (r = .92).

than control subjects. This occurred despite equal infusion rates of exogenous insulin and glucose and despite higher steady-state plasma insulin concentrations in probands. Insulin-stimulated glucose uptake was significantly reduced at the two highest infusion rates that produced plasma insulin concentrations above the physiological range (~ 500 and 700 pmol/L).

The respective roles of insulin secretion and action as risk factors for the development of NIDDM have long been disputed.[18,19] Since insulin concentrations increase and decrease in the natural history of diabetes,[20] it is hard to interpret the significance of high and low insulin levels in most cross-sectional studies. These obstacles may be avoided by studying the normotolerant offspring of NIDDM parents, who are at increased risk of developing diabetes later in life. Even in these individuals, evidence for both reduced insulin sensitivity[5,6] and impaired insulin secretion[3,21] has been provided. On the other hand, with cross-sectional studies, determination of prediabetes is attempted by inference, whereas it can only be a retrospective diagnosis, a limitation of the current study. Longitudinal studies provide more secure information. Martin et al[22] evaluated for 6 to 25 years 155 normoglycemic first-degree relatives of couples who both had NIDDM and found that the development of diabetes was preceded by and predicted by defects in both insulin-dependent and insulin-independent glucose uptake. In most cases, the defects were detectable more than a decade before diagnosis of disease. Moreover, insulin secretion, especially the first phase, tended to be increased rather than decreased in this prediabetic phase.

In both probands and normal controls, there was a progressive increase of MCR-I from the lowest to highest insulin infusion, which seems compatible with the concept that insulin clearance occurs via a saturable receptor-mediated mechanism, as previously suggested.[11,23] Apart from the similar behavior of MCR-I in both types of populations, an interesting and novel finding of this study was the decreased MCR of exogenous insulin at all hormonal infusion rates in probands as compared with controls.

However, NIDDM is a heterogeneous disease, and therefore we cannot exclude the possibility that the abnormalities of insulin clearance found herein are restricted to the population studied and may not be extrapolated to other potential diabetics.

Moreover, the optimal correlations between MCR-G and MCR-I found in first-degree relatives of NIDDM patients suggested parallel alterations in all body insulin actions and insulin clearances at each insulin-glucose infusion rate.

In conclusion, normoglycemic, non-obese offspring of NIDDM parents present reduced insulin sensitivity both in the fasting state and in response to different rates of exogenous insulin infusion; moreover, the reduction of MCR-I indicates the presence of an alteration of insulin catabolism, which may contribute to the hyperinsulinism found in first-degree relatives of NIDDM parents. On the other hand, the available data do not allow us to clarify the issue of whether the changes in MCR-I are secondary to the development of insulin resistance or whether they represent a primary process in NIDDM.

REFERENCES

1. DeFronzo RA, Simonson D, Ferrannini E: Hepatic and peripheral insulin resistance: A common feature in non–insulin-dependent and insulin-dependent diabetes. Diabetologia 23:313-319, 1982

2. DeFronzo RA: The triumvirate: β-Cell, muscle, liver. A collusion responsible for NIDDM. Diabetes 37:667-687, 1988

3. Barnett AH, Spiliopoulos AJ, Pyke DA, et al: Metabolic studies in unaffected co-twins of non–insulin-dependent diabetics. Br Med J 282:1656-1658, 1981

4. Bonora E, Zavaroni I, Bruschi F, et al: Secretion and hepatic removal of insulin in the healthy offspring of type 2 (non–insulin-dependent) diabetic subjects. Diabete Metab 10:250-254, 1984

5. Gulli G, Ferrannini E, Stern M, et al: The metabolic profile of NIDDM is fully established in glucose-tolerant offspring of two Mexican-American NIDDM parents. Diabetes 41:1575-1585, 1992

6. Eriksson J, Franssila-Kallunki A, Ekstrand A, et al: Early metabolic defects in persons at increased risk for non–insulin-dependent diabetes mellitus. N Engl J Med 321:337-343, 1989

7. Haffner SM, Stern MP, Hazuda HP, et al: Increased insulin concentrations in nondiabetic offspring of diabetic parents. N Engl J Med 319:1297-1302, 1988

8. Davidson MS, Harris MD, Rosenberg CS: Inverse relationship of metabolic clearance rate of insulin to body mass index. Metabolism 245:E155-E159, 1983

9. Salvatore T, Cozzolino D, Giunta R, et al: Decreased insulin

clearance as a feature of essential hypertension. J Clin Endocrinol Metab 74:144-149, 1990

10. World Health Organization: Diabetes mellitus: Report of a WHO study group. WHO Tech Rep Ser 727. Geneva, Switzerland, WHO, 1985

11. Tillil H, Shapiro ET, Rubenstein AH, et al: Reduction of insulin clearance during hyperglycemic clamp. Dose-response study in normal humans. Diabetes 37:1351-1357, 1988

12. Ferrannini E, Wahren J, Faber OK, et al: Splanchnic and renal metabolism of insulin in human subjects: A dose-response study. Am J Physiol 244:E517-E527, 1983

13. Reaven GM: Banting Lecture 1988. Role of insulin resistance in human disease. Diabetes 37:1595-1607, 1988

14. Heine RJ, Home PD, Ponchner M, et al: A comparison of 3 methods for assessing insulin sensitivity in subjects with normal and abnormal tolerance. Diabetes Res 2:113-120, 1985

15. Shen SW, Reaven GM, Farquhar JW: Comparison of impedance to insulin mediated glucose uptake in normal subjects and in subjects with latent diabetes. J Clin Invest 49:2151-2160, 1970

16. Harano Y, Ohgaku S, Hiadaka H, et al: Glucose, insulin and somatostatin infusion for the determination of insulin sensitivity. J Clin Endocrinol Metab 45:1124-1127, 1977

17. Heine RJ, Bilo HJG, Van Der Meer J, et al: Sequential infusions of glucose and insulin at prefixed rates: A simple method for assessing insulin sensitivity and insulin responsiveness. Diabetes Res 3:453-461, 1986

18. Efendic S, Grill V, Luft R, et al: Low insulin response: A marker of pre-diabetes. Adv Exp Med Biol 246:167-174, 1988

19. Campbell PJ, Mandarino LJ, Gerich JE: Quantification of the relative impairment in actions of insulin on hepatic glucose uptake in non–insulin-dependent diabetes mellitus. Metabolism 37:15-21, 1988

20. Ratzmann KP, Schulz B, Heinke P, et al: Quantitative and qualitative changes in the early insulin response to glucose in subjects with impaired carbohydrate tolerance. Diabetes Care 4:85-91, 1981

21. Colwell JA, Lein A: Diminished insulin response to hyperglycaemia in prediabetes and diabetes. Diabetes 16:560-565, 1967

22. Martin BC, Warram JH, Krolewski AS, et al: Role of glucose and insulin resistance in development of type-2 diabetes mellitus: Results of a 25-year follow-up study. Lancet 340:925-929, 1992

23. Eaton RP, Friedman N, Allen RC, et al: Insulin removal in man: In vivo evidence for a receptor-mediated process. J Clin Endocrinol Metab 58:555-559, 1984