# Insulin-Receptor Activity in Nondiabetic and Diabetic Urbanized South African Black Women

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**OBJECTIVE**— To evaluate insulin receptor binding characteristics of urbanized South African black women with normal glucose tolerance and of patients with newly diagnosed untreated non-insulin-dependent diabetes mellitus (NIDDM).

**RESEARCH DESIGN AND METHODS**— Four groups of 10 subjects each were selected by the following criteria: group A, young (20–39 yr) nonobese (body mass index [BMI] 19.0–24.9 kg/m$^2$) nondiabetic women; group B, middle-aged (40–60 yr) nonobese nondiabetic women; group C, middle-aged obese (BMI >30.0 kg/m$^2$) nondiabetic women; and group D, middle-aged obese newly diagnosed but untreated female patients with NIDDM. Insulin binding to monocyte receptors was determined by radioreceptor assay. Fasting plasma samples were analyzed for glucose, insulin, C-peptide, and nonesterified fatty acids.

**RESULTS**— In the four groups studied, maximum specific binding and receptor concentration were highest in group A, with a progressive and significant decrease in values through groups B and C to group D. Significant inverse correlations were obtained between maximum specific binding, 50% inhibition dose, and total receptor concentration on the one hand and glucose, insulin, and NEFA on the other.

**CONCLUSIONS**— Our study of urban South African black women showed decreasing insulin-receptor activity with obesity and glucose intolerance. In patients with NIDDM, hyperglycemia and β-cell dysfunction were associated with a reduction in receptor concentration. In this regard, our findings in South African blacks are consistent with results of similar studies of NIDDM in other communities.

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RECEIVED FOR PUBLICATION 11 FEBRUARY 1991 AND ACCEPTED IN REVISED FORM 25 SEPTEMBER 1991.

Studies of developing populations in different parts of the world show that the incidence of non-insulin-dependent diabetes mellitus (NIDDM) increases during the transition from a rural to an urban life-style (1,2). This phenomenon may also be happening in South Africa, where the rapidly urbanizing black population is at risk of acquiring the disease in growing numbers.

Available information indicates that insulin resistance in NIDDM and obesity is associated with decreased binding of insulin to cellular receptors (3,4). In South Africa, a study of young NIDDM patients of Asian Indian descent reported decreased insulin receptor binding (5), but whether this result applies to the South African black population has not been previously evaluated. Therefore, a study was undertaken to determine the insulin receptor binding characteristics of urbanized South African black patients with newly diagnosed NIDDM who were also obese. To observe the influence of age and obesity, we also studied groups of young and middle-aged nonobese and obese nondiabetic urbanized subjects. In addition, aspects of their carbohydrate metabolism were assessed, with particular emphasis on a possible relationship to insulin-receptor activity.

## RESEARCH DESIGN AND METHODS

— Before the start of the study, four groups of subjects were defined by the following criteria: group A, young (age 20–39 yr) nonobese (body mass index [BMI] 19.0–24.9 kg/m$^2$) nondiabetic women; group B, middle-aged (40–60 yr) nonobese nondiabetic women; group C, middle-aged obese (BMI >30.0 kg/m$^2$) nondiabetic women; and group D, middle-aged obese newly diagnosed but untreated female patients with NIDDM. Forty ambulatory normotensive (blood pressure [BP] <160/95 mmHg) black women were eventually recruited, so that 10 subjects

**Table 1**—*Clinical features and fasting biochemical values of 4 study groups*

| Groups ($N$ = 10) | Age (yr) | Body mass index (kg/m$^2$) | Plasma glucose (mM) | Plasma insulin (pM) | Plasma C-peptide (nM) | Plasma NEFA (g/L) |
|---|---|---|---|---|---|---|
| A Young nonobese | 31 ± 1.3* | 23.0 ± 0.6† | 4.1 ± 0.1 | 47 ± 5 | 0.30 ± 0.07 | 0.27 ± 0.01 |
| B Middle-aged nonobese | 49 ± 2.1 | 23.0 ± 0.6† | 4.1 ± 0.3 | 48 ± 5 | 0.30 ± 0.07 | 0.29 ± 0.01 |
| C Middle-aged obese | 51 ± 2.3 | 35.6 ± 1.1 | 4.4 ± 0.2 | 79 ± 8§ | 0.43 ± 0.07 | 0.38 ± 0.03** |
| D Middle-aged obese diabetic | 49 ± 2.6 | 37.2 ± 1.1 | 12.2 ± 0.8‡ | 106 ± 11‖ | 0.17 ± 0.07¶ | 0.49 ± 0.05†† |

NEFA, nonesterified fatty acids.
Values are means ± SE.
*$P < 0.001$ vs. B, C, D.
†$P < 0.001$ vs. C, D.
‡$P < 0.001$ vs. A, B, C.
§$P < 0.01$ vs. A, B.
‖$P < 0.001$ vs. A, B.
¶$P < 0.01$ vs. C, $P < 0.04$ vs. A, B.
**$P < 0.01$ vs. A.
††$P < 0.001$ vs. A, B.

could be allocated to each of the four defined groups. They had lived in the Johannesburg/Soweto metropolitan area for several years and had adopted a semi-Westernized diet and life-style. The study was confined to women because most of the black diabetic patients seen in our hospital are female. Fasting plasma glucose concentrations in group D ranged between 8 and 16 mM, and no group D subjects were ketonuric. Normal glucose tolerance in the other groups was established by oral glucose tolerance testing (administration of 75 g glucose). Clinical features and fasting biochemical values of the four study groups are shown in Table 1.

None of the women were taking oral contraceptives or any medication known to affect carbohydrate or lipid metabolism. Fifteen women were postmenopausal, and to minimize any effect of female sex steroids on insulin receptor binding, we studied menstruating females in the early follicular phase of the menstrual cycle (later confirmed by plasma progesterone measurements <1 μg/L). All subjects gave informed consent to participate in the study, which was approved by the ethics committee of the University of the Witwatersrand.

After the patients fasted for 10 h overnight, venous blood samples were taken for insulin receptor binding studies and the measurement of glucose, insulin, C-peptide, progesterone, and nonesterified fatty acids (NEFAS). The samples were centrifuged and the separated plasma aliquots stored at −20°C until analyzed. Plasma glucose was measured by the glucose oxidase method with a Beckman glucose analyzer (Fullerton, CA). Radioimmunoassay kits were used to determine plasma insulin (Pharmacia Diagnostics, Uppsala, Sweden), C-peptide (Bio Data, Rome, Italy), and progesterone (Medgenix, Fleuris, Belgium). Plasma NEFA were measured by a titrimetric technique (6). Intra-assay coefficient of variation for each of these assays was <5%.

Insulin receptor radiobinding was performed on freshly isolated monocytes according to the method of Bar et al. (7). Insulin binding was measured by incubating a constant amount (~30,000 counts · min$^{-1}$ · tube$^{-1}$) of human A14-Tyr-$^{125}$I–labeled insulin (sp act 300 μCi/μg) with a 400-μl cell suspension over a range of unlabeled insulin concentrations from 0.2 to $10^5$ ng/ml for 3 h at 22°C. The bound insulin fraction was separated by centrifugation and the radioactivity counted in a γ-counter. Data on binding of [$^{125}$I]insulin to receptors were used to determine maximum specific binding and affinity ($ID_{50}$) by competition-inhibition curves. Total receptor concentrations were obtained from Scatchard plots generated by a computer-assisted curve-fitting program (8), and the number of receptors per cell was calculated. Statistical comparisons were made by analysis of variance (ANOVA) and the Wilcoxon rank-sum test, where appropriate. Pearson's coefficient of correlation ($r$) was computed between the variables. $P < 0.05$ was considered significant. Results are expressed as means ± SE.

**RESULTS** — In the diabetic patients, mean fasting plasma glucose concentration was, as expected, significantly

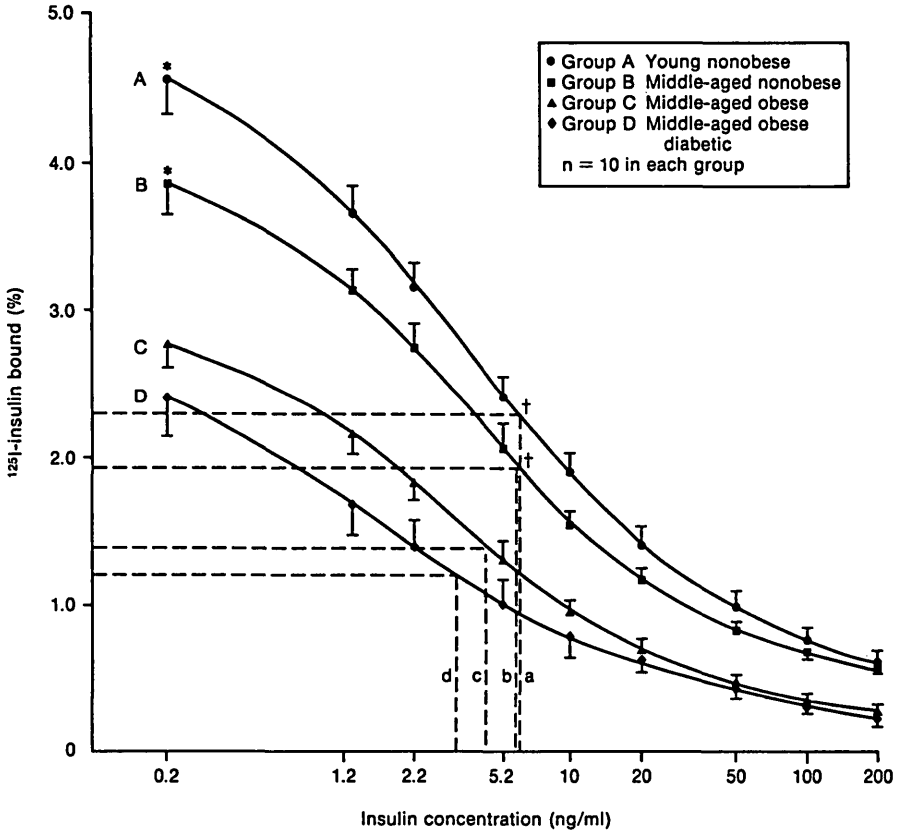


**Figure 1**—*Competition-inhibition curves of $^{125}$I-insulin binding to monocytes of nondiabetic subjects and diabetic patients studied in basal state. Values are means ± SE. A, B, C, D, maximum binding. a, b, c, d, $ID_{50}$. *$P < 0.001$ vs. C and D, †$P < 0.01$ vs. C and D.*

higher than in the nondiabetic groups. Fasting plasma insulin levels in diabetic group D and obese group C were significantly higher than in the nonobese groups A and B. By contrast, C-peptide concentration in group D was significantly lower than in groups A, B, and C (Table 1).

In the four groups studied, maximum specific binding was highest in group A, with a consistent decrease in values through groups B and C to group D, which had the lowest level (Fig. 1). Maximum binding in nonobese groups A and B was significantly higher than in obese groups C and D. $ID_{50}$ values of all four groups fell within the reference range determined in our laboratory (2–10 ng/ml). However, there was a decline in $ID_{50}$ values from groups A and B to groups C and D, indicating an apparent reciprocal increase in affinity (9). Scatchard analysis revealed that total receptor concentration also decreased significantly from group A through groups B and C to group D, which had the lowest level (Fig. 2). Insulin binding characteristics of the four groups are shown numerically in Table 2. Group A had the highest number of receptors per cell, with a progressive decrease through groups B and C to group D, which had the lowest number.

Maximum specific binding correlated inversely ($P < 0.01$) with BMI ($r = -0.75$), fasting plasma insulin ($r = -0.61$), glucose ($r = -0.58$), and NEFA ($r = -0.52$). Significant inverse correlations ($P < 0.01$) were also obtained between $ID_{50}$ values and BMI ($r = -0.52$), insulin ($r = -0.51$), glucose ($r = -0.43$), and NEFA ($r = -0.38$). Total receptor concentration correlated inversely ($P < 0.001$) with all the variables: BMI ($r = -0.63$), insulin ($r = -0.54$), glucose ($r = -0.45$), and NEFA ($r = -0.63$).

**CONCLUSIONS**— The group of South African black NIDDM patients that we studied has both β-cell dysfunction and decreased receptor concentration. In this regard, our findings are consistent with similar reports on NIDDM and obesity (4,5). Our data do not allow definite conclusions to be drawn about whether the changes in receptor activity are caused by a primary abnormality or are secondary to some of the underlying metabolic disturbances. However, the significant correlations between insulin receptor binding and fasting plasma glucose, insulin, and NEFA levels support the latter possibility.

In the diabetic group, impairment of β-cell function was confirmed by the lower plasma C-peptide concentration, and glucotoxicity from prolonged hyperglycemia may have contributed to the β-cells becoming increasingly unresponsive to glucose (10). The mean fasting plasma insulin level was inappropriately low in response to the prevailing glucose concentration, although it was higher than in the nonobese nondiabetic groups. This apparent hyperinsulinemia may have been accentuated by decreased hepatic metabolism of insulin caused by accompanying obesity (11), and increased secretion of proinsulin by failing β-cells (10). It is possible that excessive amounts of proinsulin may have

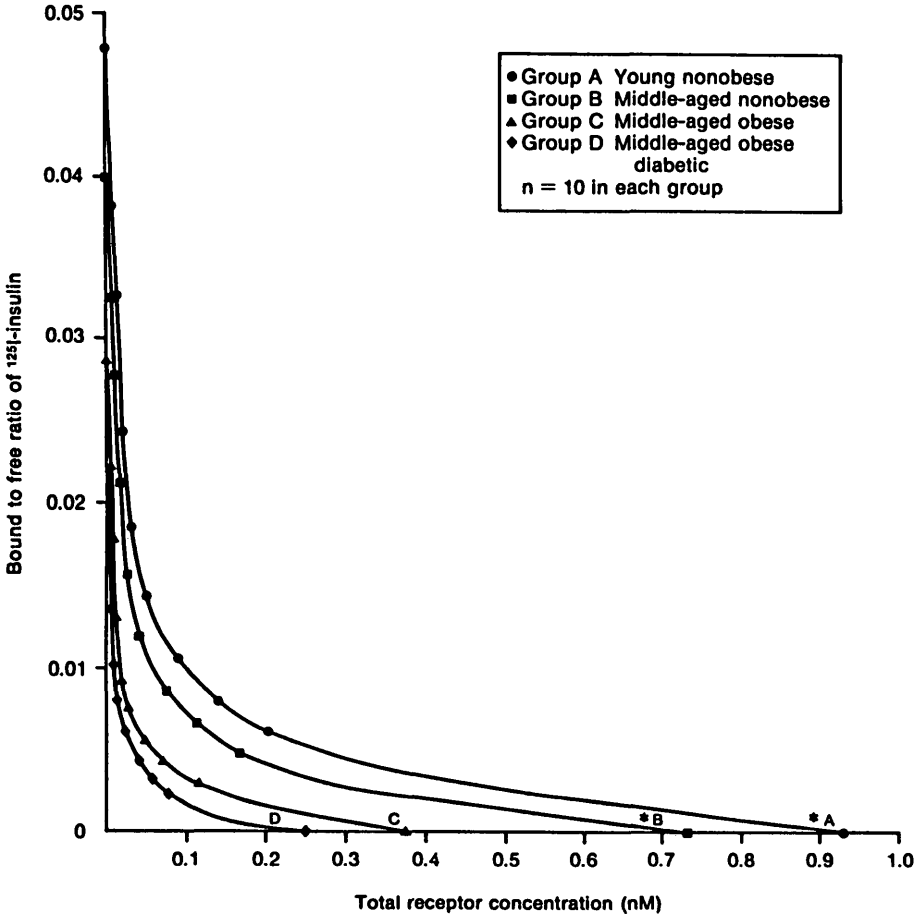


**Figure 2**—*Scatchard plots of [$^{125}$I]insulin binding to monocytes of nondiabetic subjects and diabetic patients studied in basal state. A, B, C, D, total receptor concentration. *P < 0.001 vs. C and D.*

cross-reacted somewhat with insulin in the conventional radioimmunoassay, artifactually raising the insulin levels (12). Thus, true insulin secretion may have been deficient in the diabetic patients, and this deficiency may explain the increase in receptor affinity in this group, because it is at low physiological concentrations of insulin that most receptors are unoccupied and in their highest state of affinity (9).

In our study, NIDDM appeared to be preceded by a progressive decline in receptor concentration in middle-aged obese nondiabetic individuals in whom fasting hyperinsulinemia may have led to the downregulation of insulin receptors. Although postreceptor abnormalities were not assessed in this study, the progressive changes in insulin binding nevertheless suggest that receptor phenomena remain important in the pathogenesis of NIDDM in developing communities.

**Table 2**—*Binding characteristics of [$^{125}$I]insulin to monocyte receptors of nondiabetic patients in groups A, B, and C and diabetic patients in group D*

| Groups | Percent maximum specific binding (0.2 ng/ml) | $ID_{50}$ (ng/ml) | Total receptor concentration (nM) | Receptors/cell (*N*) |
|---|---|---|---|---|
| A | 4.6 ± 0.2* | 6.2 ± 0.5† | 0.93 ± 0.13‡ | 27,900 ± 4000‡ |
| B | 3.8 ± 0.2* | 6.1 ± 0.5† | 0.73 ± 0.07‡ | 21,900 ± 2200‡ |
| C | 2.8 ± 0.2 | 4.4 ± 0.3 | 0.38 ± 0.04 | 11,300 ± 1200 |
| D | 2.4 ± 0.3 | 3.6 ± 0.5 | 0.25 ± 0.08 | 7400 ± 2300 |

Values are means ± SE. $ID_{50}$, 50% inhibition dose.
*$P < 0.001$ vs. C, D.
†$P < 0.01$ vs. C, D.
‡$P < 0.001$ vs. C, D.

**Acknowledgments**— This study was supported by the South African Medical Research Council and the Professor Grieve Foundation, University of the Witwatersrand, Johannesburg.

We thank J. Pieters and C. May for skillful assistance.

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