and path analysis suggest an adverse effect of annual oxidant concentration on the proportion of total cover represented by native species of coastal sage scrub. Further tests suggest that oxidants are affecting community structure not only by reducing the total cover of native species, but by reducing species richness and equitability. These tests are relatively standard methods, and their results are consistent with other studies on the effects of pollution on community structure (*11, 16, 17*). It must be recognized, however, that such synecological methods, like epidemiological methods examining the effects of pollution on public health, can only suggest likely causal routes of damage. Actual demonstration of an effect of oxidants on particular coastal sage species must await laboratory experiments. In view of the importance of the natural functioning of coastal sage scrub to the environment and economy of southern California (*18*), these results suggest the value of initiating such laboratory experiments promptly.

WALTER E. WESTMAN
Department of Geography, University of California, Los Angeles 90024

References and Notes

1. Southern California Association of Governments and South Coast Air Quality Management District, *Preliminary Draft Air Quality Management Plan* (Los Angeles, 1978), p. I-19. [The federal standard has recently been changed to 12 pphm ozone (for 1 hour).]
2. P. R. Miller and J. R. McBride, in *Responses of Plants to Air Pollution* (Academic Press, New York, 1975), p. 195.
3. W. W. Heck and C. S. Brandt, in *Air Pollution* A. C. Stern, Ed. (Academic Press, New York, 1977), vol. 2, p. 157.
4. For reviews of the ecology of coastal sage scrub and chaparral, see articles by H. A. Mooney and by T. L. Hanes, in *Terrestrial Vegetation of California*, M. G. Barbour and J. Major, Eds. (Wiley, New York, 1977), pp. 471 and 417, respectively.
5. H. L. Bauer, *Ecology* **24**, 45 (1943).
6. Variables of community structure were standing crop of litter mass, light penetration to 10 cm aboveground, median canopy height; of topographic position, latitude, longitude, distance to coast, elevation, slope, aspect; of substrate, bulk density (8 cm depth), texture, field capacity, conductivity, exchangeable calcium, magnesium, potassium, ammonium, nitrate, base-extractable phosphate, total nitrogen, *p*H (15 cm depth) and nature of parent material; of climate, mean, mean minimum, and minimum temperature of the coldest month, and mean, mean maximum, and maximum temperature of the warmest month; annual precipitation, mean precipitation of the driest and wettest months (interpolated from 20-year records from nearest one, two, or three weather stations); of fire history, minimum time since last fire (from records and shrub stem wood rings); of grazing history, intensity on a five-point scale (from past aerial photos, local interviews, and field observations); of air pollution, oxidants, CO, SO_2, NO_2, NO, NO_x, total hydrocarbons, particulates, and synergistic indices for oxidants with SO_2 and NO_2 with SO_2. Indices are of the form: $2[c_i w_i + 2(c_j w_j)]$ where c_i = concentration of the pollutant with the larger absolute value, and w is 100 if the pollutants that it weights are oxidants and 20 if SO_2; for the NO_2–SO_2 index, w = 20 if SO_2 and 2.5 if NO_2. Index constants were derived by K. Preston from levels of enhanced plant damage noted in the literature, mostly for crop species, when the pollutant pairs co-occur.
7. R. J. Oshima, *J. Air Poll. Control Assoc.* **24**, 576 (1974).
8. J. S. Sandberg, M. J. Basso, B. A. Okin, *Science* **203**, 82 (1979).
9. K. G. Jöreskog and D. Sörbom, *Lisrel IV. Analysis of Linear Structural Relationships by the Method of Maximum Likelihood. User's Guide* (National Educational Resources, Chicago, 1978). For background on path analysis, see, for example, O. D. Duncan, *Introduction to Structural Equation Models* (Academic Press, New York, 1975); D. R. Heise, *Causal Analysis* (Wiley, New York, 1975).
10. J. Eldon, J. Trijonis, K. Yuan, *Statistical Oxidant Relationships for the Los Angeles Region*, contract A5-020-87 (California Air Resources Board, Sacramento, 1978).
11. G. M. Woodwell, *Science* **168**, 429 (1970); R. H. Whittaker and G. M. Woodwell, in *Ordination of Plant Communities*, R. H. Whittaker, Ed. (Junk, The Hague, 1978), p. 51.
12. R. H. Whittaker, *Taxon* **21**, 213 (1972).
13. E. H. Simpson, *Nature (London)* **163**, 688 (1949).
14. M. O. Hill, *J. Ecol.* **61**, 237 (1973).
15. F. W. Preston, *Ecology* **29**, 254 (1948).
16. R. Patrick, M. H. Hohn, J. H. Wallace, *Not. Nat. Acad. Nat. Sci. Philadelphia* (1954), p. 416.
17. A number of native herb and shrub species in the grassland-oak and conifer forests of Utah experienced visible injury from 2 hours' exposure to ozone at 15 to 30 pphm in field fumigation experiments [M. Treshow and D. Stewart, *Biol. Conserv.* **5**, 209 (1973)].
18. W. E. Westman, *Science* **197**, 960 (1977).
19. Supported by grant DEB 76-81712 from the National Science Foundation. I thank A. Yates for assistance with the path analysis program and K. Preston for comments.

21 February 1979; revised 14 May 1979

Insulin Receptor Binding in Obesity: A Reassessment

Abstract. *A defect in the binding of insulin to circulating monocytes occurs when obese patients are hospitalized and fed a liberal carbohydrate diet. Under ordinary circumstances, most obese patients have normal insulin binding despite very high concentrations of serum insulin. These results show that insulin does not necessarily regulate its own receptor in vivo—as it does in vitro.*

A decrease has been observed in the number of insulin receptors in lymphocytes and hepatocytes that have been grown in the presence of insulin (*1*). Thus, it has been suggested that "down regulation" of receptor binding by insulin itself explains the apparent insulin resistance noted in certain clinical states characterized by hyperinsulinemia (*2*). Obesity is commonly associated with an increase in the concentration of serum insulin and an apparent resistance to the action of exogenous insulin (*3*). Several studies (*4*) indicate that obese patients have decreased insulin binding to circulating monocytes and isolated fat cells, which suggests that a defect in the interaction between insulin and its receptor may account for the apparent insulin resistance of obese persons (*2*). In one study (*5*), however, no defect was found in the binding of insulin to fat cells from obese patients; their insulin resistance was attributed to a defect in intracellular glucose metabolism.

We investigated insulin binding to circulating monocytes in 27 massively obese patients prior to giving them intestinal bypass surgery (*6*). These patients had been observed in our clinic for 2 to 3 months before our investigation. They were instructed to maintain their usual diet and level of activity. Their average weight gain during this period of time was 0.7 ± 0.3 kg per month (mean ± standard error). Blood was obtained on the morning after the patients were admitted to the hospital and had fasted

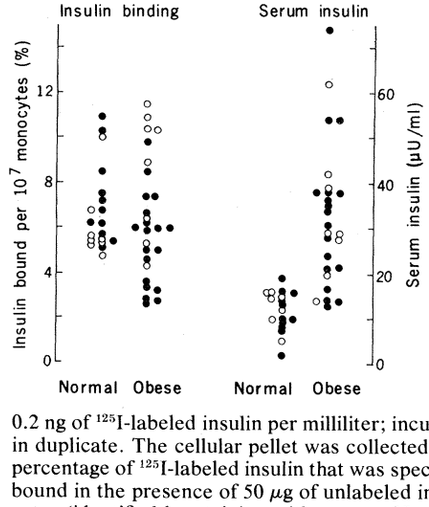

Fig. 1. Serum insulin concentrations during fasting and insulin binding in obese patients and in controls of normal weight. Males are denoted by ○ and females, by ●. Mean serum insulin was 33 ± 3 μU/ml (mean ± standard error) in obese patients and 12 ± 1 μU/ml in controls. Insulin binding was 6.2 ± 0.5 percent in obese patients and 6.8 ± 0.4 percent in controls. [Insulin was measured by immunoassay (*11*).] The method for determining the binding of insulin to circulating monocytes has been described by de Meyts (*12*). Mononuclear leukocytes were separated from whole blood by centrifugation on a Ficoll-Hypaque gradient. Approximately 25 million cells were incubated for 90 minutes at 25°C in Hepes buffer [4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; 0.5 ml] containing 0.2 ng of ^{125}I-labeled insulin per milliliter; incubations were performed in triplicate and sampled in duplicate. The cellular pellet was collected and washed in a Beckman microcentrifuge. The percentage of ^{125}I-labeled insulin that was specifically bound (percentage total minus percentage bound in the presence of 50 μg of unlabeled insulin per milliliter) was expressed per 10^7 monocytes (identified by staining with nonspecific esterase). Samples from obese patients were assayed at the same time as samples from control subjects. In nine controls, the data plotted are means of multiple determinations.

0036-8075/79/0907-1003$00.50/0

for 10 hours. Serum insulin and insulin binding to monocytes were compared to that of 19 control subjects of normal weight. As shown in Fig. 1, serum insulin concentrations during fasting were markedly elevated in the obese patients (33 ± 3 compared to 12 ± 1 μU/ml, $P < .001$, Student's t-test). All but five obese patients had serum insulin concentrations that were higher than the highest concentration among the controls. Mean insulin receptor binding did not differ between obese and normal subjects (6.2 ± 0.5 compared to 6.8 ± 0.4 percent, $P > .10$); however, eight of the 27 subjects showed less insulin binding than the control with the least amount of binding. The patients with reduced insulin receptor binding did not differ from the others in fasting serum insulin concentration (34 ± 6 compared to 32 ± 4 μU/ml), glucose (87 ± 3 compared to 91 ± 2 mg/100 ml), or in the severity or duration of their obesity.

Studies that report a defect in insulin binding in massive obesity (*4, 7*) have primarily concerned female patients who were hospitalized and given a liberal carbohydrate diet. The only study that failed to show this defect (*5*) resembled ours in that almost half of the subjects were male and their diets had not been restricted. We, therefore, considered the possibility that a liberal carbohydrate diet could in itself affect insulin binding in obese patients. Figure 2 shows data for six obese patients. Hospitalization on a 45 percent carbohydrate isocaloric diet resulted in a significant ($P < .01$) decrease in insulin receptor binding occurring at insulin concentrations in the incubation medium ranging from 0.2 to 100 ng/ml. Although serum insulin concentrations increased (from 40 ± 5 to 52 ± 9 μU/ml, $P < .10$), there was no correlation between insulin binding and serum insulin. In response to the 45 percent carbohydrate diet, insulin binding became abnormal in four of the six patients. Patients A, B, and C (Fig. 2) were then placed on a carbohydrate-restricted diet (10 percent carbohydrate, 40 percent protein, 50 percent fat) for 7 days. Insulin binding increased (34 ± 13 percent) in all three patients to within the normal range. Serum insulin concentrations showed no consistent changes and remained grossly elevated (59 ± 15 μU/ml).

Our results help to explain some of the apparent contradictions related to insulin receptor binding in massive obesity. Most obese patients have normal insulin binding under ordinary circumstances. But when they are hospitalized on a 45 percent carbohydrate diet, a defect in insulin binding is induced that is not present when their diet and activity are unrestricted and that does not occur in similarly treated subjects of normal weight (*8*).

The reduction in insulin binding in the obese patients whose data are shown in Fig. 2 could be explained had they been consuming, prior to hospitalization, a diet low in carbohydrates. Such a diet would be unusual in obese subjects and was not suggested by their dietary histories. A second factor to consider is a change in physical activity. Prior to hospitalization, these patients had been employed, or engaged in household duties. Although ambulation around the ward was encouraged, the obese patients were far less active than were thin subjects admitted for similar studies. Since exercise increases insulin binding (*9*), it is possible that decreased physical activity in the obese patients could have contributed to the reduction in insulin binding.

Decreased receptor binding in patients with insulin-secreting tumors (*10*) is the clinical correlate of the down regulation of insulin receptors that occurs when cells are cultured in the presence of a high concentration of insulin (*1*). Our data indicate that most obese patients have normal insulin binding despite hyperinsulinemia. Thus, hyperinsulinemia does not necessarily lead to down regulation of insulin receptors in vivo. Insulin resistance in most obese patients appears to be caused by a metabolic abnormality beyond the receptor level.

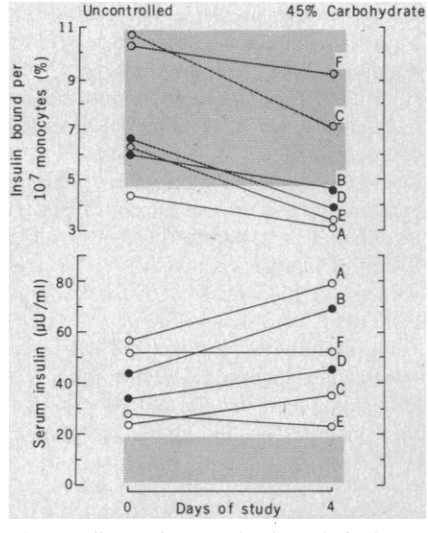

Fig. 2. Effects of a diet high in carbohydrates on insulin receptor binding. Four obese men (○) and two obese women (●) were studied at a clinical research center before and after 4 days of a controlled isocaloric diet (45 percent carbohydrate, 20 percent protein, and 35 percent fat). The caloric content for males was 30 kcal/kg for the first 100 kg of body weight and 12 kcal/kg beyond 100 kg. For females, the caloric content was 28 kcal/kg for the first 100 kg and 10 kcal/kg thereafter. No patient demonstrated a weight change during hospitalization. The shaded areas indicate the range of serum insulin and insulin binding in the controls shown in Fig. 1. For analytical methods used, see the legend to Fig. 1. To exclude the possibility that changes in binding were due to interassay variation, an internal control of IM-9 cultured human lymphocytes was included in each assay. The reduction in binding to the freshly isolated monocytes was associated with a 3.5 percent (not statistically significant) increase in binding to the cultured lymphocyte standard.

ROBERT I. MISBIN
J. PATRICK O'LEARY
ANDREA PULKKINEN

Division of Endocrinology, University of Florida School of Medicine, Gainesville 32610

References and Notes

1. J. R. Gavin, J. Roth, D. M. Neville, *Proc. Natl. Acad. Sci. U.S.A.* **71**, 84 (1974); W. G. Blackard, P. S. Guzelian, M. E. Small, *Endocrinology* **103**, 548 (1978); J. N. Livingston, P. J. Purvis, D. H. Lockwood, *Nature (London)* **273**, 394 (1978).
2. R. S. Bar, L. C. Harrison, M. Muggeo, P. Gorden, C. R. Kahn, J. Roth, *Adv. Intern. Med.* **23**, 24 (1979).
3. D. Rabinowitz, *Annu. Rev. Med.* **21**, 219 (1970).
4. J. A. Archer, P. Gorden, J. Roth, *J. Clin. Invest.* **55**, 166 (1975); R. S. Bar, P. Gorden, J. Roth, C. R. Kahn, P. de Meyts, *ibid.* **58**, 1123 (1976); L. C. Harrison, F. I. R. Martin, R. A. Melick, *ibid.*, p. 1435; R. A. Defronzo *et al.*, *ibid.* **62**, 204 (1978).
5. J. M. Amatruda, J. N. Livingston, D. H. Lockwood, *Science* **188**, 264 (1975).
6. Obese patients were 23 nondiabetic patients (plasma glucose during fasting < 110 mg/100 ml) who underwent intestinal bypass surgery at our hospital between 30 November 1977 and 30 May 1978. (Four more patients were added later.) Patients exceeded ideal body weight by at least 100 percent or 45.4 kg. The obese group consisted of 8 men and 19 women who ranged in age from 22 to 48 years (mean = 34). All had been unable to achieve or maintain weight loss by dieting. The control group consisted of 8 men and 11 women who ranged in age from 22 to 44 years (mean = 30) and who were within 10 percent of ideal body weight as determined by the Metropolitan Life Insurance Table. No control subject had a family history of diabetes or had had a recent change in weight or dietary habits. Blood was obtained after an overnight fast.
7. J. M. Olefsky reported decreased insulin binding to fat cells and monocytes in male patients on an unrestricted diet [*J. Clin. Invest.* **57**, 1165 (1976)]. These patients were older and considerably less obese than ours and than those cited above (*4, 5*).
8. To determine whether hospitalization on a 45 percent carbohydrate diet affected insulin binding in subjects of normal weight, we studied a second control group consisting of four men and two women from 23 to 29 years of age. On the freely accessible diet, insulin binding at concentrations of from 0.2 to 100 ng/ml in these controls did not differ from binding in the six obese subjects shown in Fig. 2. In contrast to the obese subjects, this control group did not show any significant change in insulin binding after 4 days of the controlled diet.
9. V. Koivisto, V. Soman, P. Conrad, R. Hendler, P. Felig, *Clin. Res.* **27**, 487A (1979).
10. R. S. Bar, P. Gorden, J. Roth, *J. Clin. Endocrinol. Metab.* **44**, 1210 (1977).
11. R. S. Yalow and S. H. Berson, *J. Clin. Invest.* **39**, 1157 (1960).
12. P. de Meyts, in *Methods in Receptor Research*, M. Blecher, Ed. (Dekker, New York, 1976), vol. 9, p. 301.

13. This research was supported by a NIAMDD special emphasis research career award in diabetes K01 AM00561-01, NIH grant RR-82, and grants from the Juvenile Diabetes Foundation and the dean of the University of Florida School of Medicine.

27 February 1979; revised 18 May 1979