Diabetic Insulin Antagonism

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SUMMARY

A capacity of freshly-drawn, normal serum to antagonize the action of insulin in the epididymal fat pad system has been found to be increased in the serum of untreated diabetic patients. A comparable increase has also been found in the serum of very obese subjects who are not apparently diabetic.

The current status of insulin antagonism in diabetes has been briefly reviewed. Although a search for *the* diabetogenic insulin antagonist has been under way for several decades now, failure thus far to convincingly demonstrate its presence appears to have carried little conviction of its absence. To the several candidates currently in nomination, another has been added. DIABETES *16*:483-86, July, 1967.

Previous measurements of the concentration of insulin-like activity (ILA) in separated serum proteins have revealed values that are tenfold the ILA of intact serum;[1-3] and indicate that more than 90 per cent of the potential ILA of serum is masked or otherwise neutralized in the intact state. As a test of the possibility that an antagonist is present and at least partly responsible for this behavior, known amounts of crystalline insulin were added to freshly-drawn fasting serum from normal subjects and untreated diabetic patients. Recovery of the added insulin activity was then attempted in the rat epididymal fat insulin assay system. Recovery was found to be incomplete in both populations. Serum from untreated diabetic patients, however, caused a greater loss of insulin activity than did normal serum.

MATERIALS AND METHODS

Fasting venous blood was drawn from sixty-four healthy ambulant individuals with normal carbohydrate tolerance and without family histories of diabetes mellitus. All were within 15 per cent of their ideal body weight as calculated for "medium frame" individuals from Metropolitan Life Height-Weight Tables (1964). Specimens of blood were allowed to clot thirty to sixty minutes at room temperature and serum separated by centrifugation at 2° C. The serum was then refrigerated at 4° C. for one to three hours while preparations were made for its assay.

The diabetic population studied consisted of twenty-nine patients, all of whom had never been treated. They ranged in age from fifteen to fifty-eight years, and fifteen had a family history of diabetes. Five were within the range of their ideal body weight while the remainder were up to 20 per cent overweight. All had abnormal glucose tolerance tests compatible with those of diabetes,[4] or persistent fasting hyperglycemia in excess of 140 mg. per 100 ml. None had been in ketoacidosis. Blood for the studies was drawn after an overnight fast and processed as described for the normal population.

In addition to the normal and diabetic subjects, a population of twenty-seven very obese nondiabetic individuals was also investigated. They ranged in age from thirteen to sixty-four years, and eighteen of the twenty-seven were females. Four had a family history of diabetes mellitus. All were more than 25 per cent above their ideal body weight as calculated from the tables cited above, the majority weighing more than 300 lb. each. They had normal glucose tolerance tests and, except for their obesity, were free of overt disease.

Undilute aliquots of each specimen were prepared for assay with and without the addition of a standard amount of dry crystalline insulin. Glucose concentrations of all serum specimens were adjusted to 300 mg. per 100 ml. As a measure of the insulin actually added to serum, identical amounts were also added to a solution of gelatin-enriched bicarbonate buffer.[5] Each assay thus determined: 1. the activity of the serum, 2. the activity of the serum with its added complement of insulin, and 3. the activity of the added insulin. The assay method is a modification of the rat epididymal fat system and has been previously described.[6]

RESULTS

The findings in the normal, diabetic and obese subjects are given in table 1. Recovered activity is signi-

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TABLE 1

Failed recovery of insulin activity added to human serum

	Normal	Diabetic	Very obese
Serum ILA	123± 4*	151± 7	202±12
Added insulin	306±10	325±15	315±14
Expected activity	429±16	476±18	517±22
Assayed activity (Serum + insulin)	370± 5	363±16	402±23
Antagonized activity	59	113	115
Antagonism	19%	32%	33%

*Mean and standard error of the mean in micro-units per ml.

ficantly less than expected from arithmetic addition of the individual activities of the mixed components ($p < .01$).

Some degree of insulin antagonism characterizes the serum of all population groups, but the excess capacity of both abnormal populations is readily seen in figure 1. These results are in disagreement with those of Lyngsoe, who reported complete recovery in comparable circumstances.[7] Examination of his data, however, reveals that antagonism did occur but was not statistically significant, perhaps because only six determinations were performed. Assay variability is such that conclusions on double recovery studies cannot be drawn from so few observations.

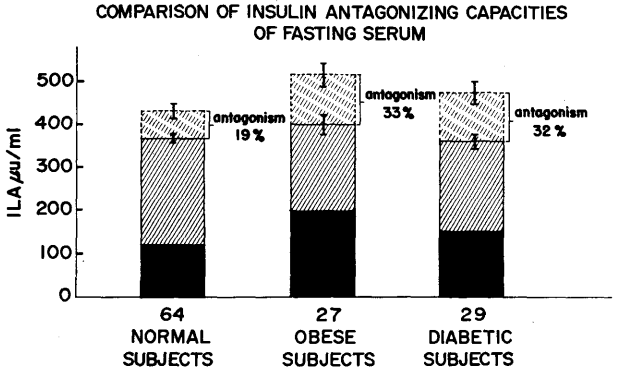

FIG. 1. Comparison of the insulin-inhibiting capacities of serum from a population of normal, diabetic and obese subjects.

When insulin suppression is calculated as a percentage for each individual, the mean percent suppression of the normal group is significantly less than both the obese and diabetic groups ($p < .01$). Neither of the latter groups, obese or diabetic, is statistically different from each other.

DISCUSSION

The search for insulin antagonism in human diabetes has been under way from early in the insulin era. Banting demonstrated that serum from insulin resistant patients was capable of protecting the rabbit against heavy doses of insulin,[8] an effect subsequently explained by the demonstration of insulin antibodies produced in response to insulin therapy,[9-12] and therefore not a cause of the disease.

Mirsky has proposed that an element of increased insulin degradation is critical,[13] while Bornstein and Park found antagonism to insulin in a lipoprotein fraction of alloxan diabetic rats.[14,15] Field has also demonstrated an antagonistic property in the serum of diabetics in ketoacidosis which becomes undetectable within hours of recovery.[16,17] And more recently the synalbumin antagonist of Vallance-Owen[18,19] and the basic protein of Antoniades[20,21] have been put forward as diabetogenic insulin antagonists.

The synalbumin antagonist requires appropriate extraction technics for its demonstration[22] and its role in intact plasma remains uncertain. Whether it is in fact the B chain of the insulin molecule, as suggested by Ensinck and Vallance-Owen,[23] is also at present uncertain.[24]

Antoniades has postulated two circulating insulin forms—one, a tissue active or "free" form and the other an inactive or "bound" form complexed with a circulating serum protein.[25] Diabetics, in certain circumstances, are said to bind endogenous insulin more avidly than normal.[21] Experimental confirmation of this hypothesis has been slow in coming, indeed attempts to demonstrate an insulin core in the "bound" form have been impressively negative.[26,27]

It seems a fair conclusion at the present time, however, that failure to demonstrate convincingly the presence of a diabetogenic insulin antagonist has carried little conviction of its absence.[28] Observations such as the refractoriness of the blood sugar fall in diabetics and prediabetics during routine insulin tolerance tests,[29,30] have gone some way toward keeping the conviction alive. Of the several candidates in nomination, each is supported by a reasonable modicum of scientific evidence, but confirmatory testing remains inconclusive. A similar outcome quite possibly awaits the serum property revealed in this report.

Studies of forearm metabolism in the obese have previously demonstrated the existence of resistance to several of the metabolic effects of insulin.[31] Glucose and potassium uptake by muscle as well as glucose uptake

by adipose tissue are resistant to standard doses of insulin in the obese forearm. These findings were interpreted to be a consequence of the obesity rather than a primary defect, as a result of experience with a patient whose insulin resistance was lost following weight reduction.[31] Whether or not the link between diabetes and obesity is production of a common insulin antagonist remains to be established.

In three respects now, blood from obese but apparently nondiabetic subjects behaves similarly to blood from recognizable diabetics. When compared to normal, 1. their plasma immuno-reactive insulin is higher,[32] 2. their insulin-like activity is higher,[33] and 3. their capacity for insulin antagonism on adipose tissue has now been found to be increased. As obesity and diabetes continue to converge in the laboratory, maintaining their present separation in the clinic may become difficult.

ACKNOWLEDGMENT

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Diarrhea Caused by Disaccharidase Deficiency

(*Continued from page* 482)

following resection of the small bowel, and there is historical evidence to suggest that surgery only brought out an hereditary disaccharide intolerance (F. Kern, Jr., J. Struthers, Jr., and W. L. Attwood, Gastroenterology, *45*:477, 1963).

Careful study of this patient was carried out to prove that her diarrhea was caused by disaccharidase deficiency, and to establish the mechanism responsible for the fat and lactic acid in the feces. The patient was a twenty-two-year old woman who underwent subtotal resection of the small bowel for obstruction caused by an incarcerated internal hernia. Her postoperative phase was unremarkable until she developed diarrhea soon after she began to eat. The stools were characteristic of malabsorption, and she lost twenty pounds.

The patient complained that she had had a long history (antedating her operation) of milk intolerance manifested by cramps, bloating, nausea, occasional vomiting, and diarrhea after drinking milk. She had two younger brothers with similar intolerance to milk. Physical examination and laboratory studies were not remarkable except for unexplained retention of bromsulphalein and slight elevation of the serum alkaline phosphatase and glutamic-oxalacetic transaminase. Liver biopsy was normal. Earlier reports have commented on unspecified liver impairment associated with disaccharidase deficiency.

Studies to establish disaccharide intolerance were striking, in that maltose and sucrose administered by mouth or by intraduodenal tube caused significant elevation of the blood glucose, whereas lactose administration was followed by no such alteration of the glucose level. When lactose was fed with exogenous lactase the changes in blood sugar were of the same magnitude as those found when galactose and glucose, the hydrolysis products of lactose, were fed. Thus it was shown that the defect lay in an inability to split lactose because the endogenous enzyme was absent rather than inactivated.

Milk products were fed systematically to evaluate the patient's history of milk intolerance. During successive six to nine-day periods the patient was fed various diets which incorporated whole milk or its components. Only when lactose was included did she have exceptional fecal fat and lactic acid excretion. Lactalbumin and milk fat did not impair absorption.

Jejunal biopsy was histologically normal. Examination of the tissue for disaccharidase activity revealed that while invertase and maltase activities were normal, lactase activity was essentially absent, but the significance of this observation is obscured by the fact that the enzymatic activity for counterpart regions of normal jejunum was also thought to be very low.

The fermentative diarrhea which patients with disaccharide malabsorption have is usually ascribed to bacterial fermentation. The authors attempted to establish this by culturing the small intestinal contents before and after a course of antibiotics. *Escherichia coli* were grown from the first jejunal aspiration but subsequent study was interrupted by small bowel perforation at the time of biopsy. Resumption of the study after operative intervention and postoperative parenteral antibiotic treatment gave no conclusive results, although it was noted that the lactic acid excretion was diminished. Steatorrhea persisted, and the authors comment that the mechanism for fat malabsorption in this disease remains unknown.

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