Metabolic Clearance of Insulin in Man

Saul M. Genuth, M.D., Cleveland

SUMMARY

Metabolic clearance of insulin was measured, under circumstances of acutely and chronically altered endogenous plasma insulin, employing I-131 bovine insulin (5 mc./mg., Abbott Laboratories) as tracer and the technic of constant infusion to equilibrium. Compared to normal subjects, clearance of radioiodinated insulin was unaffected by diabetes, was increased 25 per cent in obese subjects with hyperinsulinemia and did not change when their plasma insulin was reduced by seven days of fasting. Acute four- to fifteenfold elevation of endogenous insulin by glucose or tolbutamide injections to normal and obese subjects also failed to change clearance of radioiodinated insulin. These data support the assumption that alterations of plasma insulin in those circumstances largely reflect alterations in insulin secretion. However, in both normal and diabetic subjects, simultaneous clearances of unlabeled porcine insulin and radioiodinated insulin were also performed and disparate results obtained. Mean values were: unlabeled 861 ml./min. (Normals), 788 ml./min. (Diabetics); radioiodinated 227 ml./min. (Normals); 202 ml./min. (Diabetics). Ratio of unlabeled to radioiodinated clearance 3.8 (Normals), 4.1 (Diabetics). Because of this discrepancy, it is uncertain which clearance value affords the more accurate estimate of peripheral insulin delivery rate. DIABETES *21*:1003-12, October, 1972.

Plasma insulin determinations have formed the basis for evaluation of beta cell function since the introduction of radioimmunoassay.[1] Basal plasma insulin has been interpreted to indicate basal rates of secretion. Following an acute beta cell stimulus, the changing plasma insulin levels have been integrated with respect to time, and this insulin area interpreted as indicative of the extra insulin delivered to the circulation.[2,3] Underlying these interpretations is the assumption that peripheral metabolism of insulin is not appreciably altered by the conditions or agents under study. In a previous report by Stern et al.,[4] measurement of metabolic clearance of insulin following single injections of I-131 bovine insulin has supported the validity of this assumption in diabetes and during acute hyperglycemia and hyperinsulinemia in normal subjects. In the present report we further examine these assumptions by simultaneous measurements of endogenous plasma insulin levels with metabolic clearances of I-131 bovine insulin using the method of constant infusion to equilibrium. Diseases and conditions studied included diabetes, obesity, hyperthyroidism, prolonged fasting, glucose injections, and tolbutamide injections. In addition, clearances of unlabeled porcine insulin were also measured simultaneously with that of radioiodinated insulin in order to determine the quantitative accuracy of the latter.

Presented in part at the Thirtieth Annual Meeting of the American Diabetes Association, June 14, 1970.

From the Saltzman Institute for Clinical Investigation, The Mt. Sinai Hospital of Cleveland, and the Department of Medicine, Case Western Reserve University, Cleveland, Ohio 44106.

METHODS

Subjects

Thirteen normal subjects (six male, seven female) were hospital employees and physicians, twenty-one to forty-eight years old. All had normal oral glucose tolerance tests with cola-flavored 75 gm. carbohydrate load. All were within 116 per cent of ideal weight. Ten obese subjects (three male, seven female) were eighteen to fifty-four years old and without significant endocrine disease. Eight had normal oral glucose tolerance tests; plasma glucose in the other two subjects was 148 and 151 mg./100 ml. at two hours. Body weights ranged from 178 to 246 per cent of ideal weight. Eight diabetic subjects (four male, four female) were twenty-three to eighty-seven years old. All had fasting plasma glucose of 200 mg./100 ml. or greater and seven had ketonuria. In seven of these subjects, plasma insulin responses to oral glucose and intravenous tolbutamide were determined and found to be absent or markedly diminished. Three of these diabetic subjects were seen for the first time in diabetic acidosis. They were studied within four days of their presentation and after withholding Regular insulin for twenty-four hours. Four others had previously been treated ineffectively with oral hypoglycemic agents. None of these diabetic subjects had ever received long-term insulin treatment. Body weights ranged from 90 to 149 per cent of ideal weight. Eight hyperthyroid subjects (two male, six fe-

male) were twenty-five to eighty years old. Their diagnoses were confirmed by elevated radioactive iodine uptake, T3 resin uptake, or serum thyroxine measurements. Four were restudied after hyperthyroidism had been abolished by radioactive iodine therapy or surgery.

A second group of normal subjects (four male, four female) and diabetic subjects (one male, seven female) subsequently had combined radioactive and nonradioactive insulin clearance studies. Seven of the eight diabetics had fasting plasma glucose of 200 mg./100 ml. or greater but only one had ketonuria. None had received insulin treatment prior to study. All of the glucose tolerance results, the plasma insulin responses of the diabetic subjects, and the thyroid function tests in the hyperthyroid subjects have been filed with the American Society for Information Science and are available on request.

Procedures

Metabolic clearance of I-131 insulin was carried out by the technic of constant infusion to equilibrium[5] after an overnight fast. Indwelling venous needles were placed in both arms and the needle used for blood sampling was kept patent with heparinized saline. The I-131 bovine insulin was obtained from Abbott Laboratories at a specific activity of 5 mc./mg. Seventy-five to 150 μc. was added to 250 ml. of a solution of 0.4 per cent human serum albumin in 0.9 per cent saline. Fifty milliliters was injected intravenously as a priming dose. Delivery was then continued by constant infusion pump at 1 ml./min. for 180 minutes. After 120 minutes of infusion, 25 gm. of glucose (50 ml. of 50 per cent solution) or 1 gm. of tolbutamide (sodium salt in 20 ml. diluent) was injected intravenously in the opposite arm over one to two minutes. After preliminary studies had suggested that constant levels of specific immunoreactive insulin radioactivity were achieved by sixty to ninety minutes (see below, figure 1), heparinized blood samples were routinely obtained at 100, 110, 120, 125, 130, 140, 150, 160, 170, and 180 minutes as well as prior to the onset of infusion. Thus, there were three infusion samples obtained prior to injection of glucose or tolbutamide and seven obtained following the injection.

In the case of studies with unlabeled insulin, porcine insulin (Lilly) and 75 to 150 μc. of radioiodinated insulin were added to the albumin saline mixture. In those studies carried out in normal individuals, the infusate also contained 15 per cent glucose. Fifty milliliters was injected as a priming dose followed by continuous delivery at 1 ml./min. Sampling was carried out every fifteen minutes throughout the infusion and plasma glucose determined immediately on a Beckman Glucose Analyzer to avoid serious hypoglycemia. In normals, mean fasting plasma glucose was 88 and varied from 52 to 62 throughout the three hours. The lowest observed value was 36. No normal subject exhibited any signs of cerebral dysfunction though all had transient diaphoresis, flushing, and hunger during the first hour. In seven diabetics with fasting glucose greater than 200 mg./100 ml., plasma glucose at the end of the infusion ranged from 53 to 143 mg./100 ml. In the remaining diabetic subject with a fasting

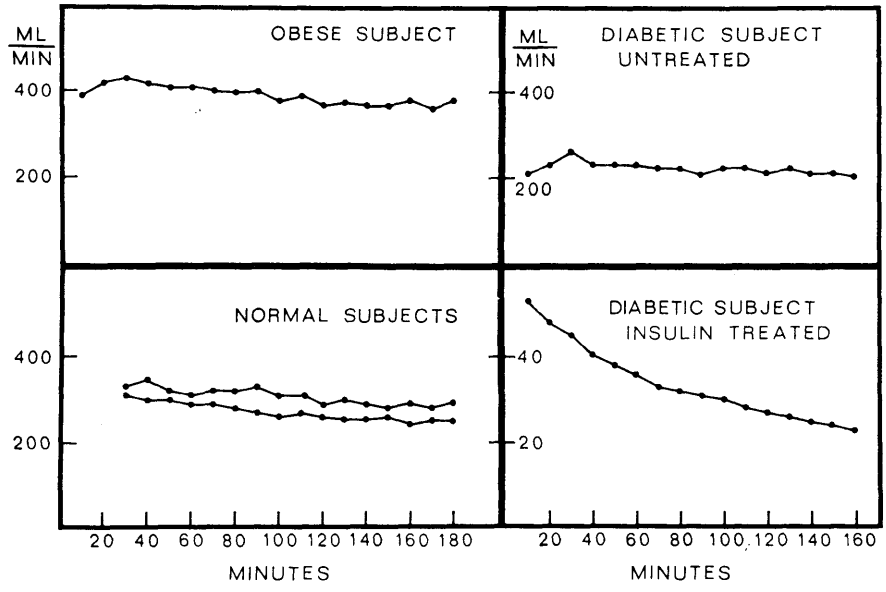

FIGURE 1

Metabolic clearance of radioiodinated insulin (MCI) plotted at ten-minute intervals during a three-hour infusion of I-131 bovine insulin in two normal subjects, and in one obese, one untreated diabetic, and one insulin-treated diabetic subject.

plasma glucose of only 122 mg./100 ml., the infusion had to be terminated at 105 minutes because of symptoms at a plasma glucose of 42 mg./100 ml.

Analyses

All heparinized venous blood samples were centrifuged promptly in the cold. Analysis of radioactivity specifically attributable to immunoreactive insulin was carried out in duplicate and begun on the same day. Aliquots of 0.8 ml. plasma were incubated with 0.2 ml. of a 1 to 100 dilution of potent guinea pig antiserum to insulin in phosphosaline buffer, at 4° for eighteen to twenty-four hours. To this was added 0.4 ml. of 50 per cent rabbit antiguinea-pig globulin (Arnell Products) in phosphosaline buffer containing 0.02 molar ethylenediamine tetraacetic acid and incubation continued another twenty-four hours. The resultant immunoprecipitates were centrifuged in the cold, washed with cold phosphosaline buffer containing 0.1 per cent human serum albumin, and recentrifuged. They were then counted in an Auto-Gamma counter for sufficient time to accumulate at least 3000 counts. Aliquots of the infusate obtained from the infusion tubing were added to 0.8 ml. of each patient's preinfusion plasma and similarly analyzed. In all instances, at least 90 per cent of the infusate radioactivity could be precipitated immunologically with insulin antiserum in the presence of the patient's plasma while less than 2 per cent could be so precipitated when control guinea-pig serum was substituted for the insulin antiserum. These procedures insured the absence of nonspecific interference by the patient's plasma with the double antibody procedure of analysis.

Nonradioactive immunoreactive insulin levels were determined in the plasma by routine double antibody radioimmunoassay employing I-125 insulin as tracer.[6] With lapse of time and proper discriminator settings, there was no interference from the I-131 in the patient's plasma. Human insulin standards were employed for measurement of endogenous plasma insulin. Exogenous insulin levels in the plasma and infusate were measured in the same immunoassay against porcine insulin standards. The infusate was appropriately diluted in phosphosaline buffer containing 0.5 per cent human serum albumin. Pipettes were preconditioned with infusate and subsequently rinsed several times with diluting fluid.

Calculations

Metabolic clearance of I-131 bovine insulin (MCI) was calculated[5] from infusion rate divided by plasma level (CPM/min. ÷ CPM/ml. = ml./min.). The plasma level was the mean of the three equilibrium samples at 100, 110, and 120 minutes of infusion, none of which individually differed from the mean by more than 10 per cent. Only radioactivity reactive with insulin antibody was used in these calculations. Basal insulin delivery rates were calculated by multiplying the endogenous fasting plasma insulin level by the MCI and converting to units per day (μU./ml. x ml./min. x $1440/10^6$ = units per day). Following glucose injection, the increments in endogenous plasma insulin were integrated with respect to time to yield the sixty-minute insulin area above baseline in μU./ml. • min. The insulin delivered during the sixty-minute postglucose period was then calculated by multiplication of this endogenous insulin area by the simultaneously determined MCI, (μU./ml. • min. x ml./min. ÷ 10^6 = units). The plasma level of I-131 bovine insulin for calculating the postglucose MCI was the mean of the seven samples obtained following glucose injection. Metabolic clearance of unlabeled porcine insulin was also calculated from the equation, infusion rate divided by plasma level (μU./min. ÷ μU./ml. = ml./min.).

RESULTS

Measurement of Metabolic Clearance of I-131 Bovine Insulin (MCI)

Figure 1 shows examples of MCI determined at ten-minute intervals for three hours. From ninety to 180 minutes, stable values were observed in the normal, obese, and previously untreated diabetic subjects. Thus, 120 minutes was selected as an appropriate time to introduce perturbations in endogenous plasma insulin levels. In contrast, a study carried out in a chronically insulin-treated diabetic subject shows failure to obtain equilibrium of plasma levels of I-131 bovine insulin and, therefore, failure to obtain a stable value for MCI. In addition, the apparent values of MCI are much lower. Circulating antibody to insulin in the plasma of such patients which binds the I-131 bovine insulin[7] appears a likely explanation for this phenomenon.

Figure 2 shows that MCI in normal subjects correlated positively with body surface area ($r = 0.70$, $p < .01$). This confirms a preliminary report by Hollobaugh et al.[8] Correlation has also been noted between metabolic clearance of iodinated growth hormone and body surface area.[9]

In figure 3 are presented values of MCI in normal subjects and diabetic subjects. No difference was found,

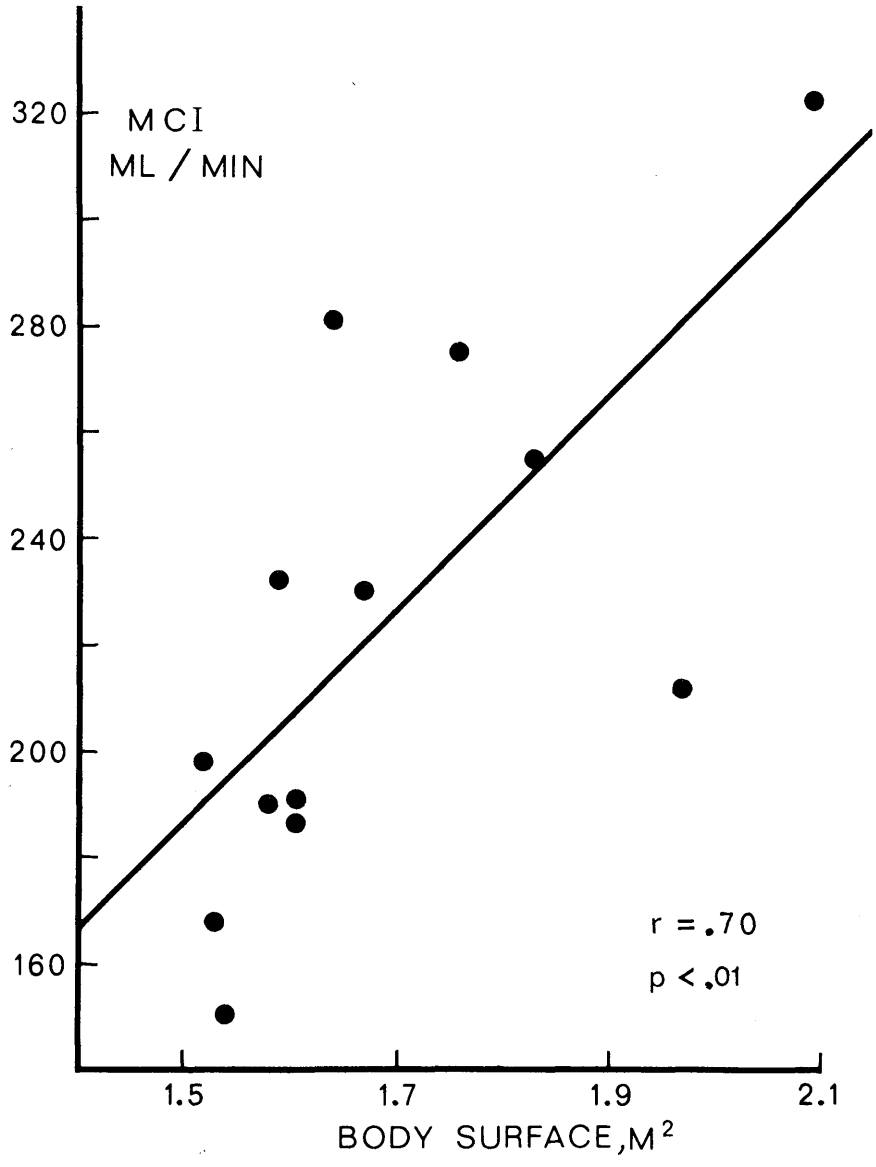

FIGURE 2

Metabolic clearance of radioiodinated insulin (MCI) in ml./min. plotted against body surface area in square meters for twelve normal subjects.

223 ml./min. vs. 231 ml./min. Obese subjects had a 25 per cent increase in mean MCI to 278 ml./min. ($p < .05$), whereas fasting plasma insulin levels were increased 150 per cent over those of normal weight subjects (25 μU./ml. vs. 9 μU./ml., $p < .001$). Following a seven-day period of total caloric deprivation, mean MCI was essentially unchanged in the obese subjects though plasma insulin had dropped significantly from 25 to 15 μU./ml., $p < .05$. Thus, the basal hyperinsulinemia of obesity was not accompanied by a reduction in MCI nor was the fall in plasma insulin produced by fasting accompanied by an increase in MCI.

Figure 4 shows the effects of intravenous administration of a glucose pulse on plasma insulin and MCI. The latter is plotted as percentage of the control value determined during the thirty-minute period preceding glucose injection. In normal and obese subjects, though plasma insulin rose acutely to five minute peak levels four- to fifteenfold higher than basal levels and was still elevated two to threefold at sixty minutes, MCI varied less than 10 per cent from pre-injection values. Thus, hyperinsulinemia stimulated by glucose did not effect a significant decrease in MCI. In severely diabetic subjects, no significant rise in plasma insulin was observed after glucose injection. This failure could not be

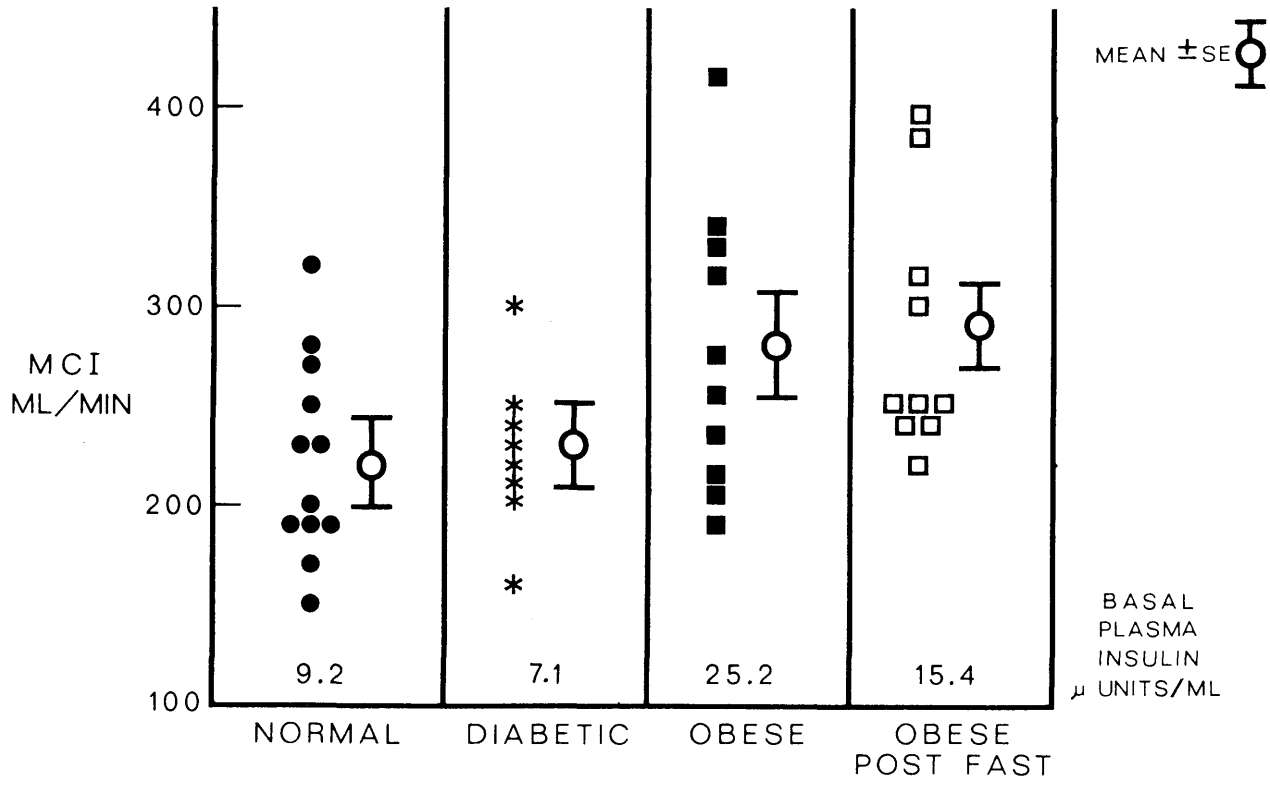

FIG. 3. Individual values of radioiodinated insulin clearance (MCI), Mean ± S.E.M., plotted for normal, diabetic, and obese subjects before and after fasting.

attributed to any sudden increase in MCI which again remained within 10 per cent of control values.

In figure 5 are shown studies of plasma insulin and MCI following injection of tolbutamide. In three of the studies, rises in plasma insulin provoked by tolbutamide were accompanied by insignificant changes in MCI from control values. In the obese subject, an abrupt 15 to 20 per cent fall in MCI was observed; however, this change could not account for the extreme elevation in plasma insulin observed.

Since thyroxine may affect the metabolism of other hormones, for example cortisol,[10] it was of interest to examine MCI in hyperthyroid subjects. The mean MCI of five nonobese hyperthyroid individuals was 254 ml./min. and of three obese hyperthyroid individuals, 283 ml./min. These values did not differ significantly from those of normal weight or obese subjects. Furthermore, four subjects were restudied after hyperthyroidism had been abolished and their mean euthyroid MCI of 261 ml./min. did not differ from their mean hyperthyroid MCI of 277 ml./min. A similar lack of effect of hyperthyroidism on iodinated growth hormone clearance has also been reported.[9,11]

Comparison of Unlabeled Insulin Clearance with Radioiodinated Insulin Clearance

The above studies demonstrated that short- or long-term alterations in plasma insulin were not accompanied by any substantial changes in the measured radioiodinated insulin clearances. However, any estimates of endogenous insulin delivery rates based on such measurements would depend on the knowledge that radioiodinated and native insulin are cleared from human plasma at the same rate. To obtain a direct comparison between labeled and unlabeled insulin clearances, simultaneous infusion to equilibrium of the I-131 bovine insulin and unlabeled porcine insulin was performed. The results of these studies are shown in table 1. An average unlabeled insulin clearance of 861 ml./min. was observed in normal subjects and a value of 788 ml./min. was observed in diabetic subjects. The average ratio of unlabeled insulin clearance to radioiodinated insulin clearance was 3.8 to 1 in normal and 4.1 to 1 in diabetic subjects. These differences were not statistically significant. When normal and diabetic results were analyzed together, unlabeled and

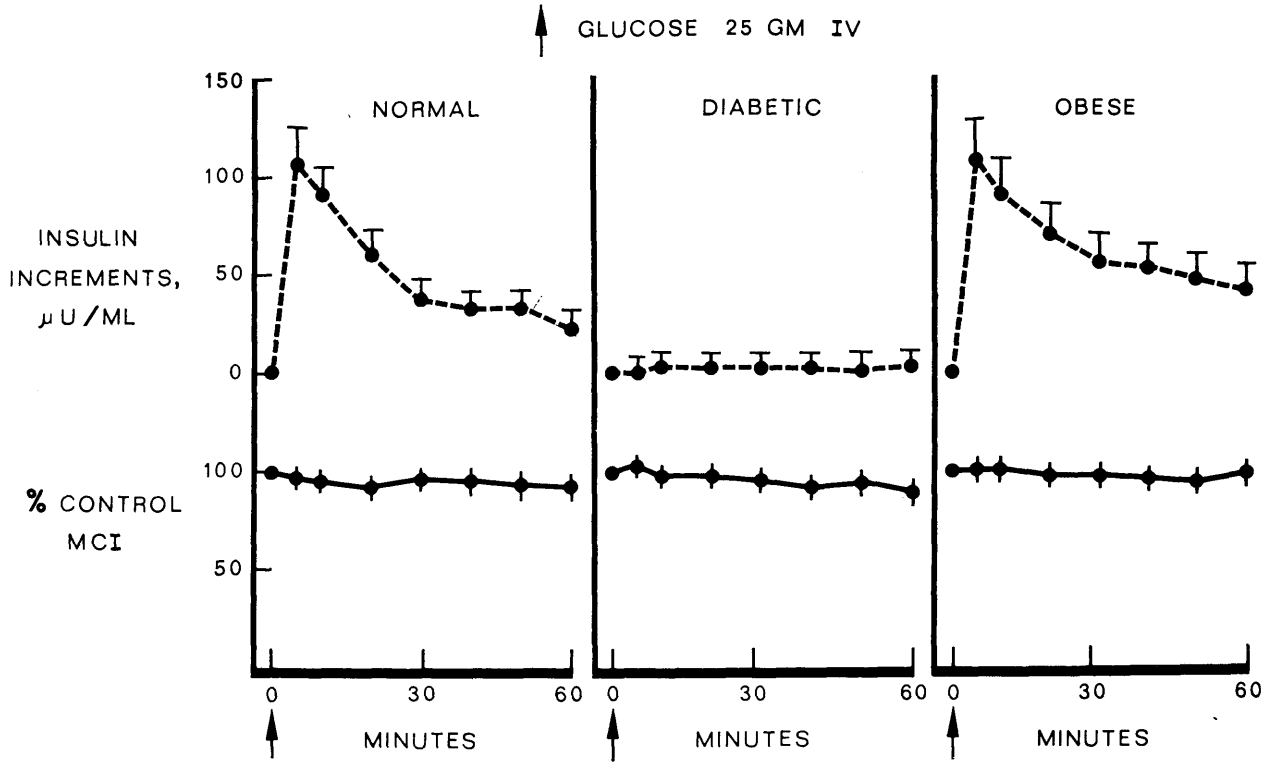

FIG. 4. Increments in plasma insulin following a pulse of 25 gm. glucose intravenously are plotted against time in the upper portion of the figure. Simultaneously determined radioiodinated insulin clearance (MCI) is plotted in the lower portion as a percentage of control values obtained just prior to glucose. Values are Mean ± S.E.M. of eleven normal, eight diabetic, and ten obese subjects.

radioiodinated insulin clearances showed a significant positive correlation ($r = .74$, $p < .005$).

Estimates of Insulin Delivery Rates

Based on the simultaneously determined radioiodinated insulin clearance and endogenous plasma insulin levels, estimates of basal insulin delivery rates (fasting plasma insulin x MCI) and postglucose insulin delivery (60 min. insulin area x MCI) could be made. Basal delivery based on MCI was 3.0, 2.4, and 9.9 U./day in normal, diabetic and obese subjects, respectively. If clearance of endogenous insulin more nearly approximated that of unlabeled porcine insulin, these values would be raised fourfold to 12, 10, and 40 U./day. Sixty minute postglucose delivery based on MCI was .63 U., .02 U., and .96 U. in the normal, diabetic and obese subjects. If similarly multiplied by a factor of 4, these would become 2.5, .08, and 3.8 U., respectively. When postglucose insulin delivery was plotted against the basal insulin delivery in normal and obese subjects (figure 6), a statistically positive correlation was noted ($r = .68$, $p < .001$). This supports the previous observations of Bagdade et al.[12] based on measurements of plasma insulin alone.

DISCUSSION

The present study of metabolic clearance of insulin was directed toward several questions. 1. Do plasma insulin levels accurately reflect differences in insulin secretion among various groups of patients? 2. Do acute rises in plasma insulin accurately reflect the magnitude of increased insulin secretion when the beta cells are suddenly stimulated? 3. Can peripheral insulin delivery rates be determined from insulin clearance measurements employing a commonly available tracer, radioiodinated bovine insulin?

The general method utilized was that of constant infusion to stable plasma levels of infusand, at which point the rate of removal of the hormone equals its rate of administration. This approach obviates the necessity for analyzing the multiexponential plasma decay curves which result after single injections of insulin.[4] Radioiodinated insulin was used in the majority of our studies, as it was in numerous previous reports, for

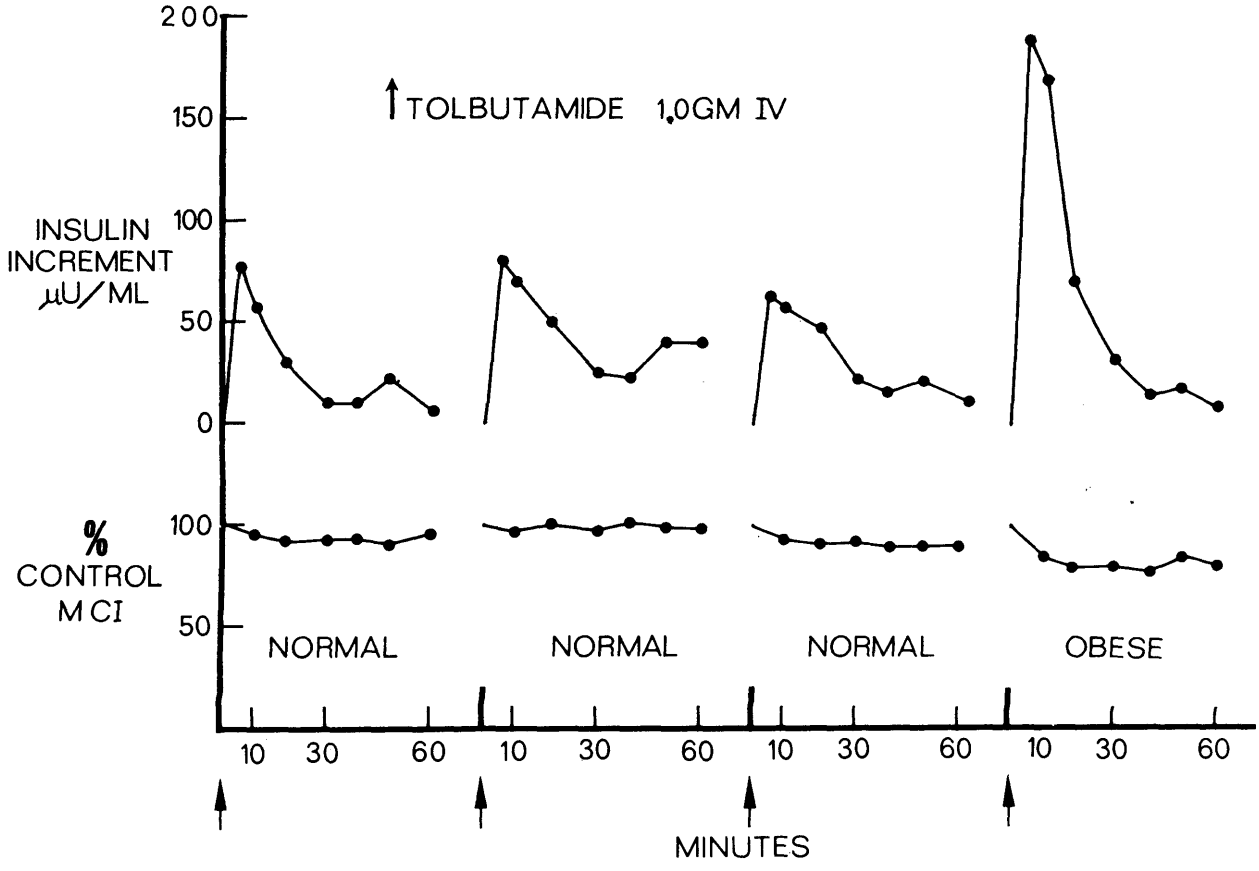

FIG. 5. Increments in plasma insulin following a tolbutamide pulse are plotted against time in the upper portion of the figure. Simultaneously determined radioiodinated insulin clearance (MCI) is plotted in the lower portion of the figure as a percentage of control values obtained just prior to tolbutamide.

several reasons. First, it is readily and specifically measurable at plasma concentrations which do not lower plasma glucose. Second, it may be independently measured while endogenous plasma insulin levels are simultaneously altered by beta cell stimulation or inhibition. Third, there is good evidence that radioiodinated insulin traverses the same metabolic routes and processes as native insulin: the kidney is a major common site of removal of both radioiodinated and endogenous insulin;[13] radioiodinated insulin is degraded by hepatic insulinase;[14] the rate of removal of radioiodinated insulin is diminished about 50 per cent when plasma insulin levels are raised 200-fold by pharmacological loading with unlabeled crystalline insulin.[15]

The results we obtained demonstrate that metabolic clearance of radioiodinated insulin is similar in normal and severely diabetic subjects, confirming the report of Stern et al.[4] who applied curvilinear analysis of plasma decay curves after single injections. We have further

TABLE 1

Simultaneous determinations of I-131 bovine insulin clearance and unlabeled porcine insulin clearance

	Unlabeled Clearance	Labeled Clearance	Ratio A/B
Normals	A	B	
1	739	241	3.1
2	1,026	276	3.7
3	1,056	236	4.5
4	644	193	3.3
5	937	227	4.1
6	763	220	3.5
7	743	217	3.4
8	977	204	4.8
Mean ± SEM	861 ± 55	227 ± 9	3.8 ± .2
Diabetics			
1	590	208	2.8
2	495	162	3.1
3	562	138	4.1
4	920	186	4.9
5	736	246	3.0
6	1,180	340	3.5
7	752	178	4.6
8	1,060	155	6.9
Mean ± SEM	788 ± 87	202 ± 23	4.1 ± .5

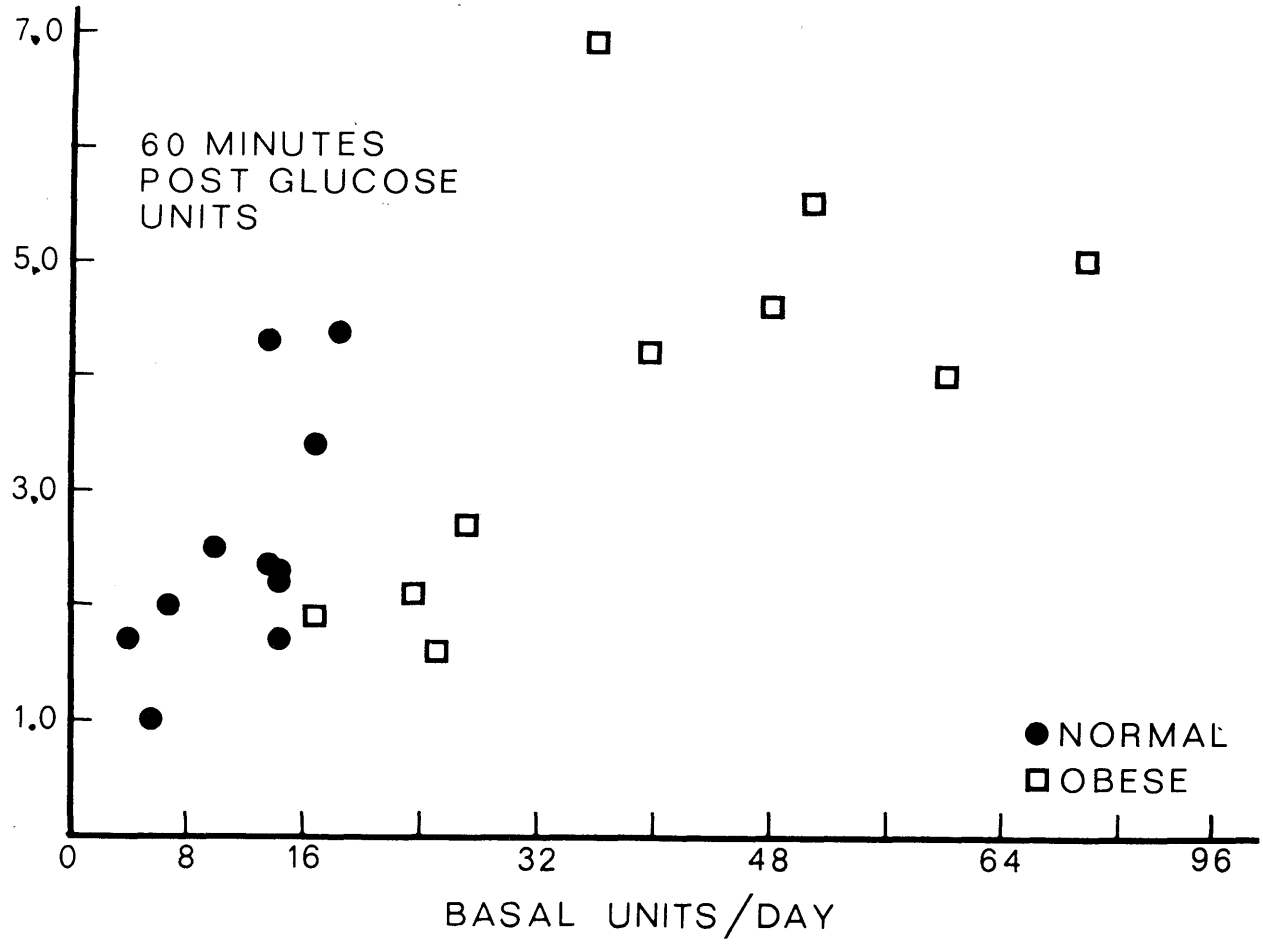

FIG. 6. Postglucose insulin delivery plotted against basal insulin delivery for normal and obese subjects. Calculations were performed as indicated in the text.

shown that despite their hyperinsulinemia, obese subjects have modestly increased rather than decreased clearances of radioiodinated insulin and that these clearances do not change significantly when their plasma insulin level is reduced by prolonged fasting. In both normal and obese subjects, sudden elevation of endogenous plasma insulin four- to fifteenfold by glucose or tolbutamide stimulation of the beta cells caused no appreciable change in the simultaneously measured radioiodinated insulin clearances. The latter was also unaltered by acute hyperglycemia which was not accompanied by significant endogenous insulin response in diabetic subjects. Therefore, insofar as radioiodinated insulin can be considered a valid tracer for native insulin, these results are in agreement with and extend previous conclusions[4,16] that acute and chronic alterations in plasma insulin do largely reflect alterations in insulin secretion rather than alterations in insulin disposal. They support the premises that obesity is characterized by insulin hypersecretion[12] and that diabetes[17] and prolonged fasting[18] are characterized by insulin hyposecretion. They imply that glucose and tolbutamide increase plasma insulin only by increasing insulin delivery. It must be stressed, however, that because the insulin tracer used was not intrinsically labeled in one of its native atoms but incorporated a foreign iodine atom, the above results still cannot constitute final proof of these conclusions.

Several observations prompt further examination of the appropriateness of radioiodinated insulin as a tracer for calculations of insulin delivery. There is evidence that radioiodination can alter biological and immunological properties of insulin[19] and, therefore, could reduce its relative affinity for sites of insulin disposal. In canine studies,[15] none of the twelve preparations of insulin iodinated electrolytically with I-131 or I-125 were cleared from plasma as rapidly as unlabeled crystalline insulin. With the least iodinated tracers, clear-

ances were 70 per cent of that observed for crystalline insulin. Our values of metabolic clearance of radioiodinated insulin in normal subjects (225 ml./min.) are similar to those which can be calculated from the studies of Stern et al.[4] using a similar tracer and curvilinear analysis after single injection (150 ml./min.). Compared to results obtained from studies of renal extraction of endogenous insulin,[20,21] ours and Stern's values are close to the reported estimates for renal insulin clearance (200 ml./min.). This would be anticipated if the kidneys were responsible for removing the bulk of circulating insulin as observed in the dog.[22] On the other hand, when we measured unlabeled porcine insulin clearance we found average values of 861 ml./min. and 788 ml./min. in normal and diabetic subjects, respectively. These did not differ significantly, confirming the physiological conclusion reached from radioiodinated insulin clearances that diabetes does not alter insulin disposal. However, all individuals studied had greater clearances of unlabeled than labeled insulin when these were determined simultaneously. The mean ratio was 3.8 to 1 in normals and 4.1 to 1 in diabetics. This disparity is not unprecedented. Shen et al.[23] have reported equilibrium plasma insulin levels of about 100 μU./ml. in both normals and diabetics when unlabeled insulin was infused at a constant rate of 50,000 μU./min. This would lead to a clearance value of 500 ml./min. for unlabeled insulin from the same laboratory that previously observed[4] a radioiodinated insulin clearance of 150 ml./min.

At the present time we cannot resolve this discrepancy on methodological or physiological grounds. Therefore, definitive measurement of endogenous insulin clearance and secretion probably awaits the availability of a highly labeled H-3 or C-14 human insulin preparation identical to that appearing in the pancreatic vein. Recently Turner et al.[24] calculated insulin delivery rates from continuous infusions as well as curvilinear analysis of single injections of monocomponent human insulin. They found in normals an average basal delivery rate of 14 U./day and following intravenous injection of 35 gm. of glucose, a sixty-minute insulin delivery of 3.2 U. These values are similar to those we computed using porcine insulin clearance figures. Thus, exogenous human insulin and exogenous porcine insulin may be similarly cleared by man.

ACKNOWLEDGMENT

All studies were carried out with the skillful cooperation of the entire staff of the Saltzman Institute for Clinical Investigation and the technical assistance of Mrs. Lea Renerts, Mrs. Naomi Gawiser, and Mrs. Leah Garron. The manuscript was prepared by Mrs. Sandra Ostler. Dr. Bernard Landau and Dr. Leonard Berman provided helpful criticism of the manuscript.

The work was supported by a grant from the Diabetes Association of Greater Cleveland and USPHS Grant No. 5-SOL-FRO5658-04.

REFERENCES

[1] Yalow, R. S., and Berson, S. A.: Immunoassay of endogenous plasma insulin in man. J. Clin. Invest. *39*:1157-75, 1960.

[2] Seltzer, H. A., Allen, E. W., Herron, A. L., and Brennan, M. T.: Insulin secretion in response to glycemic stimulus: Relation of delayed initial release to carbohydrate intolerance in mild diabetes mellitus. J. Clin. Invest. *46*:323-35, 1967.

[3] Perley, M. J., and Kipnis, D. M.: Plasma insulin responses to oral and intravenous glucose: Studies in normal and diabetic subjects. J. Clin. Invest. *46*:1954-62, 1967.

[4] Stern, M. P., Farquhar, J. W., Silbers, A., and Reaven, G. M.: Insulin delivery rate into plasma in normal and diabetic subjects. J. Clin. Invest. *47*:1947-57, 1968.

[5] Tait, J. F.: The use of isotopic steroids for the measurement of production rates in vivo. J. Clin. Endocrinol. Metab. *23*:1285-97, 1963.

[6] Morgan, C. R., and Lazarow, A.: Immunoassay of insulin: Two antibody system. Plasma insulin levels of normal, subdiabetic, and diabetic rats. Diabetes *12*:115-26, 1963.

[7] Berson, S. A., Yalow, R. S., Bauman, A., Rothschild, A., and Newerly, K.: Insulin I-131 metabolism in human subjects: Demonstration of insulin binding globulin in the circulation of insulin-treated subjects. J. Clin. Invest. *35*:170.

[8] Hollobaugh, S. L., Boshell, B. R., Chandalia, H. B., and Siegal, A. M.: Body size and insulin turnover rates in man. Clin. Res. *18*:53, 1970.

[9] MacGillivray, M. H., Frohman, L. A., and Doe, J.: Metabolic clearance and production rates of human growth hormone in subjects with normal and abnormal growth. J. Clin. Endocrinol. Metab. *30*:632, 1970.

[10] Hellman, L., Bradlow, H. L., Zumoff, B., and Gallagher, T. F.: The influence of thyroid hormone on hydrocortisone production and metabolism. J. Clin. Endocrinol. Metab. *21*: 1231, 1961.

[11] Taylor, A. L., Finster, J. L., and Mintz, D. H.: Metabolic clearance and production rates of human growth hormone. J. Clin. Invest. *48*:12, 1969.

[12] Bagdade, J. D., Bierman, E. L., and Porte, D.: The significance of basal insulin levels in the evaluation of the insulin response to glucose in diabetic and nondiabetic subjects. J. Clin. Invest. *46*:1549, 1967.

[13] Rubenstein, A. H., and Spitz, I.: Role of the kidney in insulin metabolism and excretion. Diabetes *17*:161, 1968.

[14] Tomizawa, H. H., Nutley, M. L., Narahara, H. T., and Williams, R. H.: The mode of inactivation of insulin by rat liver extracts. J. Biol. Chem. *214*:285, 1955.

[15] Ooms, H. A., Arnould, Y., Rosa, U., Pennisi, G. F.; and Franckson, J. R. M.: Total metabolic clearance of crystalline insulin and radioiodine substituted insulin. Pathol. Biol. (Paris) *16*:241, 1968.

[16] Yalow, R. S., and Berson, S. A.: Dynamics of insulin secretion in hypoglycemia. Diabetes *14*:341, 1965.

[17] Parker, M. L., Pildes, R. S., Chao, K. L., Cornblath, M., and Kipnis, D. M.: Juvenile diabetes mellitus, a deficiency in insulin. Diabetes *17*:27, 1968.

[18] Cahill, G. F., Herrera, M. G., Morgan, A. P., Soeldner, J. S., Steinke, J., Levy, P. L., Reichard, G. A., and Kipnis, D. M.: Hormone-fuel interrelationships during fasting. J. Clin. Invest. *45*:1751, 1966.

[19] Arquilla, E. R., Ooms, H., and Mercola, K.: Immunological and biological properties of iodoinsulin labeled with one or less atoms of iodine per molecule. J. Clin Invest. *47*: 474, 1968.

[20] Chamberlain, M. J., and Stimmler, L.: The renal handling of insulin. J. Clin. Invest. *46*:911, 1967.

[21] Rabkin, R., Simon, N. M., Steiner, S., and Colwell, J. A.: Effect of renal disease on renal uptake and excretion of insulin in man. N. Engl. J. Med. *282*:182, 1970.

[22] Swenson, R. S., Silvers, A., Peterson, D. T., Kohatsu, S., and Reaven, G. M.: Effect of nephrectomy and acute uremia on plasma insulin-I-125 removal rate. J. Lab. Clin. Med. 77:829-37, 1971.

[23] Shen, S. W., Reaven, G. M., and Farquhar, J. W:. Comparison of impedance to insulin-mediated glucose uptake in normal subjects and in subjects with latent diabetes. J. Clin. Invest. *49*:2151, 1970.

[24] Turner, R. C., Grayburn, J. A., Newman, G. B., and Nabarro, J. D. N.: Measurement of insulin delivery rate in man. J. Clin. Endocrinol. Metab. *33*:279, 1971.

APPENDIX

TABLE 1A

Oral glucose tolerance tests

	Plasma glucose, mg./100 ml.				
Normal subjects	**Fasting**	**30′**	**60′**	**120′**	**180′**
1	80	175	96	68	69
2	88	163	116	109	61
3	84	157	139	112	88
4	84	108	115	85	80
5	73	121	137	72	73
6	83	122	110	92	90
7	80	111	116	72	68
8	70	145	137	94	60
9	87	100	126	94	71
10	83	90	110	65	66
11	81	138	139	113	101
12	92	103	143	126	122
Diabetic subjects					
1	265	292	318	365	382
2	279	336	386	441	393
3	304	399	470	551	497
4	200	251	289	366	365
5	234	331	399	510	402
6	257	310	375	459	349
7	247	272	310	342	319
Obese subjects					
1	79	113	110	81	65
2	91	132	159	131	98
3	89	128	147	113	95
4	83	130	126	75	93
5	100	119	136	148	102
6	72	100	103	102	92
7	100	160	206	125	93
8	88	128	147	122	97
9	87	140	144	107	77
10	86	140	173	151	83

TABLE 2A

Plasma insulin responses (μU./ml.) in diabetic subjects

	Following oral glucose				
Subject	Fast	30′	60′	120′	180′
1	6	10	7	9	9
2	7	4	0	4	0
3	10	16	16	18	8
4	7	12	16	8	8
5	0	0	0	0	0
6	12	14	10	10	10
7	0	0	0	0	0
	Following intravenous tolbutamide				
Subject	Fast	2′	5′	15′	30′	60′
1	7	6	7	8	9	14
2	4	10	10	10	10	2
3	12	26	38	24	22	18
4	6	21	21	14	10	11
5	0	0	0	0	0	0
6	12	14	10	12	10	10
7	0	10	11	14	9	7

TABLE 3A

Thyroid function tests in hyperthyroid subjects

Subject	24 hr. radioactive iodine uptake %	T3 Resin uptake % normal	Serum thyroxine μgm. %
1	81	163	26.6
2	36	134	16.1
3	47	160	20.6
4	61	—	30.0
5	60	152	18.9
6	13*	124	15.6
7	54	132	17.7
8	52	141	13.7
Normal	10-40	80-120	6-13

* This subject had been treated two years previously with RAI and had a clear-cut symptomatic recurrence with elevated serum thyroxine and T3 resin uptake measurements. No explanation for the low RAI uptake is available. Her hyperthyroid state was further confirmed by prompt response to propylthiouracil and repeat RAI therapy.

ADDENDUM

Following submission of this manuscript, Sönksen et al. (Lancet 2:155-59, 1972) have published results of steady state plasma insulin concentrations in normal human subjects infused at constant rates with monocomponent human insulin. Calculation of metabolic clearance from these data yields values in the range of 900 ml. per minute. The similarity to our results with unlabeled porcine insulin further supports the interpretation that those results are closer to the correct value for endogenous human insulin.