"Staircase" Glucose Stimulation of Insulin Secretion in Obesity

Measure of Beta-cell Sensitivity and Capacity

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SUMMARY

A special glucose infusion test was used to provide successive steplike increments of glucose stimulation in six normal-weight and seven obese subjects. This "staircase" method of glucose infusion demonstrates that insulin responds in "spike" fashion despite maintenance of a continuous, fixed, submaximal glucose stimulation. Further, the "spike" response recurs as the glucose concentration is stepped up.

Obese subjects showed an exaggeration of both the first phase "spike" pattern and the more gradual second phase of insulin release. Mathematical analysis of early phase insulin release indicates that obese persons have greater *total* amounts of insulin available for release at all glucose concentrations than do normals, yet the *proportion* of totally available insulin released to a given glucose stimulus is not increased in obesity.

These findings imply that the early phase, hyperinsulin response to glucose in obesity is due to a greater quantity of insulin available for release rather than to an increased sensitivity of the beta cell to glucose. DIABETES *23*:763-70, September, 1974.

In obese patients abnormally high levels of circulating insulin have been found under basal conditions and after pancreatic stimulation with practically all known insulin stimulators, including glucose,[1] tolbutamide,[2] glucagon,[3] arginine,[4] and leucine.[5] Characterization of this hyperinsulinism suggests that it consists predominantly of biologically active insulin[6] of normal molecular size[7,8] whose elevated levels in obese patients represent hypersecretion rather than impaired clearance.[9,10] This hypersecretion of insulin in obese patients is a consequence of the obesity, since it becomes normal on reducing to normal weight[11] and can be acquired in normal subjects made obese after forced feeding.[12]

The mechanism underlying this hypersecretion of insulin has not yet been determined. It has been attributed to some unknown signal for beta-cell hypersecretion to compensate for the well demonstrated insensitivity to insulin action exhibited by peripheral tissues,[13] as well as the liver,[14] in obese subjects. Others suggest that a beta-cytotrophic effect of excessive caloric intake, particularly carbohydrate excess, may be the primary cause of hyperinsulinism, with the tissue insensitivity to insulin being a compensatory adaptation to prevent hypoglycemia.[15]

While both of these concepts are compatible with increased islet cell mass to explain the hypersecretion of insulin, they do not exclude the presence of beta cells that have adapted to the obese state by becoming more sensitive to stimulation due either to some qualitative change or to extrinsic factors. In the latter instance, the demonstration of excessive circulating amino acids in the plasma of obese patients[16] raises the possibility that their beta-cell hypersecretion might be a function of the well established potentiation of glucose-induced insulin release by amino acids.[17]

To evaluate the nature of this hyperinsulin response to glucose in obesity, a special glucose infusion test was designed to provide successive steplike increments of glucose stimulation. This "staircase" method of glucose infusion permits characterization of both the early and late phases of insulin release at various levels of glucose stimulation. This technic not only allows comparison of the total amount of insulin released after comparable glucose stimulations in normal-weight and obese human beings, but also provides a simultaneous estimate of the relative sensitivity of their beta cells to varying levels of glucose stimulation.

MATERIALS AND METHODS

Clinical characteristics of the subjects studied. The normal subjects, two women and four men, were volunteers with ages ranging from twenty-one to forty-five years (mean thirty-two) and whose weight was within 5 per cent of the ideal as measured by Life

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Accepted for publication June 10, 1974.

Extension Examiner Tables.[18] None of the women were taking oral contraceptive hormones.

The obese subjects selected had a decided excess of adipose tissue but no associated medical or endocrine disease. They were receiving no medications and consumed at least 200 gm. of carbohydrate daily while on a weight-maintaining diet for at least three days before testing. Carbohydrate tolerance was characterized by the subjects' response to a standard oral glucose tolerance test using the criteria of Conn and Fajans adapted for plasma glucose[19] (table 1). The four women and three men ranged in age from twenty-three to forty-seven years (mean thirty-one), and weighed from 63 to 129 per cent over the ideal. Not one of the subjects was glycosuric and all had normal fasting plasma glucose levels. However, three of the subjects showed slightly abnormal elevations of plasma glucose, according to the criteria of Conn and Fajans (table 1).

mg. glucose per minute was selected as the optimal rate to achieve the initial hyperglycemic plateau, with increases to 500 and 750 mg. per minute at each of the subsequent ten-minute periods to produce increasing steps. Solutions of 50 per cent glucose were used for both the rapid and constant glucose infusions; the constant infusions were regulated by a Harvard Pump. Multiple samples for plasma glucose and serum insulin were obtained during the baseline state and at one- to two-minute intervals during the first thirty to forty minutes and then every fifteen minutes for a total of ninety minutes. These samples were obtained from a scalp vein needle placed in the contralateral antecubital vein, whose patency was maintained by a slow drip of 0.9 per cent saline solution. The infusion was tolerated without discomfort and no phlebitic sequelae occurred.

Laboratory methods. Plasma glucose was assayed by a glucose oxidase method using a Beckman oxygen sensor to measure the rate of oxygen consumed,[20] and serum insulin was assayed by the method of Grodsky and Forsham.[21] Total insulin response to glucose during the early and late phases of insulin release was approximated indirectly by integration of the area under each serum insulin curve. Calculations were performed with a Gelman planimeter, and results expressed in microunits per milliliter times minutes. The first phase of insulin release was defined as that acute response to each steplike increase in glucose challenge. It is characterized by a *rapid rise* in insulin concentration above basal levels (and above the slowly developing second phase of insulin release) and a simi-

TABLE 1

Clinical characteristics of obese subjects

Subject	Age (yr.)	Sex	Ht. (in.)	Wt. (lb.)	% over ideal body wt.	Oral glucose tolerance test (100 gm.) Plasma glucose mg./100 ml. 0′	30′	60′	90′	120′	Serum insulin μU./ml. 0′	30′	60′	90′	120′
Normal glucose tolerance															
A.C.	33	F	64	300	129	68	117	130	132	140	49	170	132	174	158
F.K.	26	F	60	270	113	82	96	136	137	140	48	240	368	272	351
C.G.	23	F	67	250	76	91	141	111	108	96	22	200	109	103	65
P.K.	24	M	73	296	63	74	122	154	131	100	5	72	123	183	103
Mild glucose intolerance*															
J.P.	39	M	71	418	119	89	145	185	161	145	24	220	240	240	265
R.Y.	24	M	69	260	79	85	161	207	161	141	30	150	303	220	248
B.S.	47	F	61	210	70	78	162	203	214	176	50	203	343	544	705

*Criteria of Conn and Fajans: Carbohydrate intolerance is diagnosed if plasma glucose is above 185 mg. per 100 ml. at one hour, above 160 mg. per 100 ml. at one and one-half hours, and above 140 mg. per 100 ml. at two hours.

Procedures. Successive combinations of a small rapid bolus of glucose given over thirty seconds, followed immediately by a carefully regulated ten-minute glucose infusion, proved capable of producing several steplike increments of relatively constant hyperglycemia ("staircase" glucose effect). Glucose levels during the ten-minute infusion periods were monitored with an Ames Reflectometer and slight adjustments to the glucose infusion rate were occasionally required to maintain a steady hyperglycemic stimulus. A satisfactory "staircase" stimulation pattern was achieved by using 3 gm. glucose for the initial bolus and 5 gm. for the subsequent ones. A constant infusion of 190

lar *rapid decline* in a spikelike pattern. It was quantified (as diagrammed in figure 1) by adding the areas under the consecutive spike responses, after subtracting the extrapolated base of the spike for each separate ten-minute period. The total area of first phase insulin release for individual subjects was measured for each of three successive ten-minute periods.

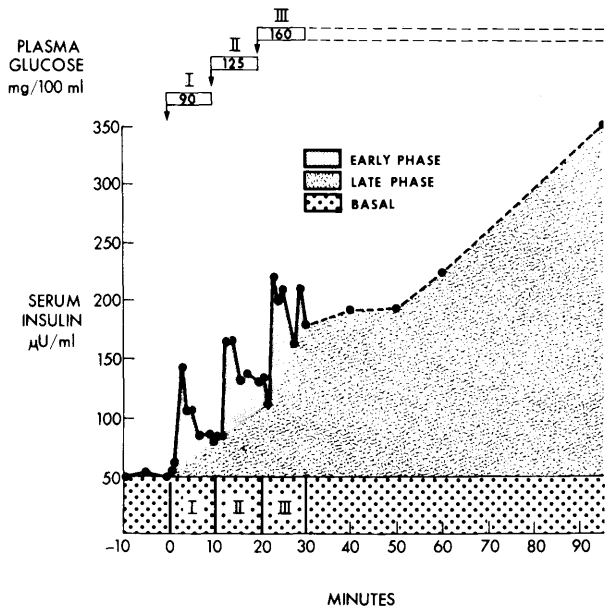

FIG. 1. Quantification of early and late phases of insulin release using areas under the curve. Roman numerals I, II, and III represent plasma glucose levels during "staircase" infusions of glucose in an obese patient (A.C.).

RESULTS

Effect of "Staircase" Glucose Stimulation in Normal Subjects (figure 2, table 2).

In six normal subjects baseline fasting plasma glucose levels ranged from 78 to 90 mg. per 100 ml. The initial bolus of 3 gm. of glucose produced a rapid increment in plasma glucose, ranging in various subjects from +10 to +40 mg. per 100 ml., which was maintained at this plateau for ten minutes by infusing glucose at a rate of 190 mg. per minute. During successive ten-minute periods, progressive increments of hyperglycemia were achieved in staircase fashion as shown in figure 1 to reach final plateaus ranging from 175 to 280 mg. per 100 ml. Individual variation in the specific level of hyperglycemia at each step was not unexpected and reflected differences among the individual subjects in the space in which the glucose was distributed and the effectiveness of the mechanism by which it was cleared.

Following increments in plasma glucose from only 9 to 40 mg. per 100 ml. above baseline (mean 27 mg.

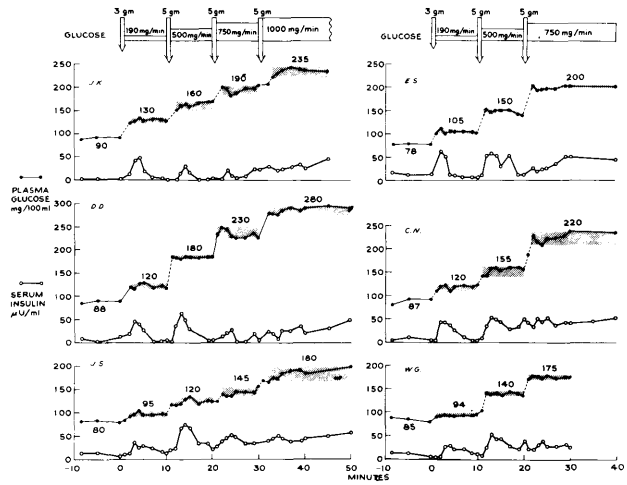

FIG. 2. Serum insulin response to "staircase" glucose stimulation in normal subjects.

per 100 ml.), an initial rapid rise in insulin concentration occurred in all normal subjects from basal levels below 15 μU. per milliliter to peak levels of 30 to 60 μU. per milliliter by the third to fourth minute after administration of the glucose. However, despite persistence of the glucose stimulus, insulin levels returned to the baseline. During the second stimulus the insulin concentration again rose immediately, but subsequently declined in spite of a fixed plateau of hyperglycemia. After the third and fourth steplike increments of glucose stimulation, the initial rapid phase of insulin release was less and associated with the appearance of a more gradual, slowly rising phase in response to the sustained glycemic stimulus.

Relative sizes of the first and second spikes of rapid insulin release varied among subjects and were related to the glucose levels achieved. For example, in most of the normal subjects the initial glucose stimulation did not exceed 120 mg. per 100 ml. and the proportionately greater glucose rise at the second step produced a second spike of rapid insulin release slightly larger than the first. In J. K., however, the first spike was larger than the second, since the initial glucose rise to 130 mg. per 100 ml. reflected a steplike glucose increment that was proportionately greater than the second glucose rise. In all cases, the area under the third spike of rapid insulin release was less than the others, regardless of the degree of rise in the hyperglycemic stimulus at this point, indicating that a maximum was being approached. By the end of twenty minutes of continuous glucose stimulation, a slowly rising, sustained second phase of insulin release developed,

TABLE 2

Staircase glucose stimulation and insulin responses in normal and obese subjects

		Mean plasma glucose mg./100 ml.					Early phase insulin release (area under spikes as) (μU./ml.-min.)				Late phase insulin release (area as) (μU./ml.-min.)
		0′	I	II	III	IV	I	II	III	I+II+III	I+II+III
Normal	J.K.	90	130	160	190	215	152	72	44	268	44
	D.D.	88	120	180	230	250	160	216	96	472	40
	J.S.	80	95	120	145	170	120	272	108	500	224
	E.S.	78	105	150	200		124	280	132	536	92
	C.N.	87	120	155	220		156	148	96	400	452
	W.G.	85	94	140	175		112	204	92	408	204
	Mean:	85	111	151	194	212			Mean ± S.E.M. =	431 ± 39	= 176 ± 70
Obese	A.C.	70	90	125	160		228	492	412	1,132	1,416
	F.K.	95	110	140	190		248	540	364	1,152	1,744
	C.G.	85	110	150	175		216	196	156	568	880
	P.K.	80	95	120	135		212	216	136	564	468
	J.P.	90	115	150	185		448	484	412	1,344	2,020
	R.Y.	95	120	150	200		728	884	860	2,464	1,976
	B.S.	92	110	155	200		224	300	144	668	840
	Mean:	87	107	141	175				Mean ± S.E.M. =	1,127 ± 240	= 1,335 ± 231

which contrasted decidedly with the "spike" patterns of insulin released during the initial two periods of glucose infusion.

Effect of "Staircase" Glucose Stimulation in Obese Subjects.

In four obese subjects with normal carbohydrate tolerance, plateaus of hyperglycemia similar to those of the normal subjects were achieved (figure 3, table 2). However, because of greater body mass their levels of blood glucose tended to be slightly lower than that obtained in normal subjects in response to identical amounts of infused glucose. In the three obese subjects who had mild carbohydrate intolerance, "staircase" glucose levels were comparable to, but not higher than, those of the normal subjects (figure 4, table 2).

Basal insulin levels were elevated in all but two of the obese subjects (C.G. and P.K.). The mean of three baseline measurements in each of the seven obese subjects (33 μU. per milliliter) was significantly higher than that in the normal group (7.1 μU. per milliliter; $p < .01$).

As in the normal subjects, their early insulin release showed a definite "spike" pattern characterized by an initial rapid rise followed by a decline in insulin concentration, despite maintenance of a constant stimulatory level of glucose. However, certain differences in their degree of insulin response were apparent when compared with normals.

In the obese subjects an exaggeration of both the early phase "spike" pattern and the more gradual secondary phase of insulin release was apparent at all glucose concentrations, especially in those with elevated basal levels of insulin. In fact, the secondary

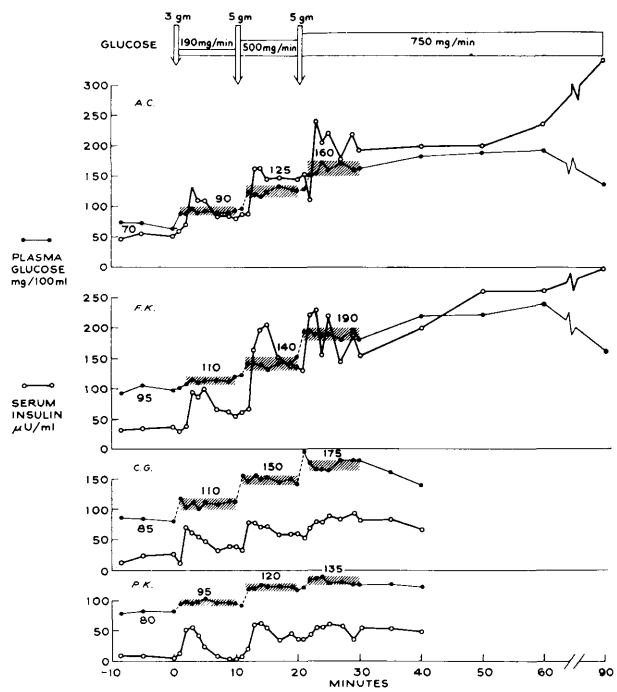

FIG. 3. Serum insulin response to "staircase" glucose stimulation in obesity.

phase of insulin release became evident within the first ten minutes of glucose stimulation in six of the seven obese subjects.

Table 2 compares the insulin response in the various subjects expressed as area under the insulin curves during the successive steplike increments of blood glucose. Summation of the insulin released rapidly in "spike" patterns during the first thirty minutes of staircase glucose stimulation (I + II + III) showed a significantly higher mean of early phase insulin release in the obese subjects than in the normal subjects (mean levels of 1,127 versus 431 μU. per milliliter times minutes; $p < .01$), despite a tendency toward lesser stimulatory levels of plasma glucose in the obese nondiabetic group during the infusion. The late phase insulin release was also much greater in the obese subjects (mean levels of 1,335 versus 176 μU. per milliliter times minutes; $p < .005$).

Cumulative Mean Plasma Insulin Concentration After "Staircase" Glucose Stimulation.

Each level of "staircase" glucose stimulation (three or four per subject) was plotted against total cumulative insulin released during the early phase at that glucose concentration plus the sum of early phase insulin released at any previously lower steps of glucose stimulation. In both the control and obese subjects it was found that in a certain intermediate range of plasma glucose (between 100 and 180 mg. per 100 ml.) small increments in the glycemic stimulus produced a striking response in early phase insulin release; during subsequent stimulation with higher concentrations of glucose, however, the per cent change of insulin release was substantially less in the normals and in certain of the obese subjects.

These data can be acceptably fitted to the sigmoidal type of curve for glucose-induced insulin release previously described in the rat pancreas[22,23] and in man.[24] The sigmoidal curve corresponds to the initial threshold distribution curve previously described in the perfused rat pancreas[23] and can be represented by the following formula:

$$I(G) = I_{MAX} \frac{G^K}{G_{50}{}^K + G^K}$$

where I(G) stands for the amount of insulin released in an early phase when constant glucose stimulation level is G; I_{MAX} is the maximal releasable amount of insulin in an early phase, when the glucose stimulation is very high ($G \rightarrow \infty$); K is an exponent that is directly

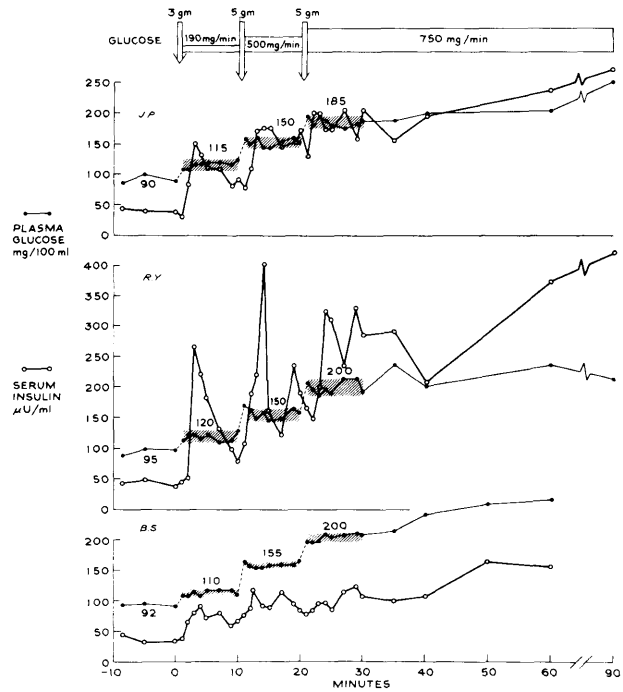

FIG. 4. Serum insulin response to "staircase" glucose stimulation in obese subjects with mild carbohydrate intolerance.

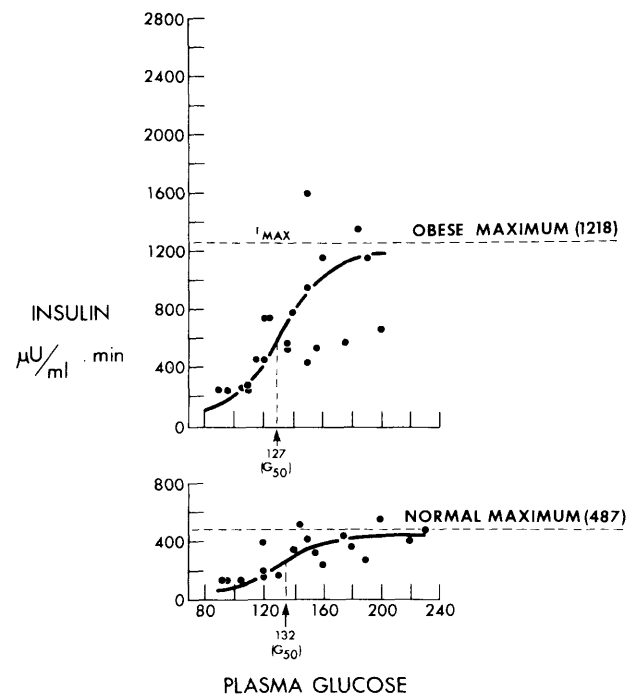

FIG. 5. Cumulative insulin concentration in obese and normal subjects during "staircase" glucose stimulation. The sigmoidal curve was selected by a computer to produce the "best fit" for the individual data.

proportional to the steepness of the sigmoidal curve and G_{50} is that level of glucose stimulation at which the release of insulin is half of the maximal (I [G_{50}] = IMAX/2). The mathematical basis for this formulation has been previously reported.[23]

Using the BMDX85, a nonlinear least squares fitting procedure,[25] the three parameters of the sigmoidal curve (i.e. IMAX, G_{50}, and K) were determined individually from the measurements on each subject and are listed in table 3. Statistical analysis reveals that, while G_{50} and K (the position of the half-maximal release and the steepness of the curve) are not significantly different in both groups, the mean value of IMAX differs significantly in obese subjects from that in normal ones ($p < .01$). In three obese subjects (A.C., J.P., R.Y.) the projected IMAX is similar to their observed, early phase insulin release, despite data indicating that maximization had not yet been reached. In these three cases it is possible to retain satisfactory sigmoidal features by increasing their IMAX by two asymptotic standard deviations as denoted in table 3.

The sigmoidal curve representing the "best fit" for these values was selected by a computer and is depicted in figure 5. It shows that even though the predicted mean of total insulin released under maximal glucose stimulation was two and one-half times greater in the obese than in the normal subjects, the pattern of their sigmoidal curves suggests that the proportion of insulin released, compared with the maximum amount releasable, was similar in both groups [e.g. 50 per cent of the mean maximal releasable insulin was discharged at practically identical glucose concentrations (127 and 132 mg. per 100 ml.)]. Similarly, when the least squares fitting procedure was used to provide the best fit for a *hyperbolic* type of curve as described in humans,[26] it was again demonstrated that the total insulin secretory capacity was greater in the obese group without measurable reduction in sensitivity to glucose.

TABLE 3

Calculated values* of maximum early phase insulin release and glucose sensitivity in individual subjects

Subject	I MAX (μU./ml.-min. ± S.D.†)	G 50 (mg./100 ml.)	K (slope)
Normal			
J.K.	371.6 ± 68.1	147.1	4.97
D.D.	585.2 ± 67.8	157.5	4.37
J.S.	556.0 ± 32.5	109.8	9.41
E.S.	568.0 ± 22.5	129.7	6.40
C.N.	406.8 ± 30.5	130.5	6.96
W.G.	438.8 ± 93.8	119.2	6.40
Mean	487.6	132.3	6.42
S.E.M.	38.0	7.2	0.72
Obese			
A.C.	1,368.4 ± 214.1	122.2	5.77
F.K.	1,180.8 ± 87.2	129.4	9.28
C.G.	700.4 ± 391.1	135.8	5.26
P.K.	628.4 ± 227.0	106.8	8.22
J.P.	1,334.0 ± 111.4	130.7	6.86
R.Y.	2,634.0 ± 297.8	139.8	7.39
B.S.	682.8 ± 121.7	127.1	6.99
Mean	1,218.4	127.4	7.11
S.E.M.	264.8	4.1	0.52

*Values satisfying the "least squares fit."
†Asymptotic standard deviation.

In three obese subjects (A.C., J.P., R.Y.), a satisfactory maximization of insulin response had not been achieved. However, these data showed an acceptable fit to the sigmoidal type of curve. While the IMAX measurement may well have been underestimated in these particular patients, the half-maximum would be even greater than estimated. Results in these patients would thus not detract from our conclusion that increased glucose sensitivity of the beta cells was an untenable explanation for the hyperinsulin response in obesity.

Thus, while both types of mathematical analysis provide the same conclusions, we selected the sigmoidal fit for the following reasons: (1) it represents a model based on a large body of accumulated in vitro data, some of which rely on experiments at low glucose concentrations, unachievable in vivo; (2) it produces an acceptable fit for the data in the obese as well as the normals, with asymptotic standard deviations of parameters averaging 17 per cent (range 4 to 50 per cent); and (3) it is based on a model described in humans[24] and does not require the sharp transition from nonresponse to response, a feature less prevalent in biologic systems.

DISCUSSION

The "staircase" type of glucose challenge provides important data that extend knowledge obtained from other forms of glucose testing in man. This method demonstrates that, during a sustained, fixed, submaximal glucose stimulation, insulin responds in spike fashion despite continuation of the glucose stimulus, and this type of spike response recurs as the glucose concentration is stepped up. This pattern of glucose-induced insulin release is similar to results observed in the perfused rat pancreas.[23] Furthermore, in the latter system, the sum of total insulin released as spikes in the "staircase" series leading to a given glucose con-

centration is the same as when that concentration was used as a single step.[23]

Among various alternative hypotheses that might be invoked to explain these unusual spike patterns of early insulin release despite a continuous stimulus are the following: (1) as each step increase of glucose occurs, the beta cell is exposed to an acute stimulus whose effectiveness rapidly diminishes as the interior of the cell accommodates to each new fixed glucose step; this accommodation may consist of a simple diminution of the gradient initially achieved by the acute increment in glucose stimulation (delta model) or of some metabolic feedback effect attempting to restore equilibrium (feedback inhibition); and (2) in pancreatic islets of man as well as the rat there may exist within a rapidly releasable insulin compartment individual packets that are distributed on the basis of increasing threshold susceptibilities to glucose stimulation.[23]

Regardless of underlying mechanisms, results indicate that hyperinsulinism in the obese person is due to greater amounts of insulin available for release in both the early and late phases of insulin secretion and that sensitivity of the pancreas to glucose remains normal in the early phase at least.

The convenience of using venous blood sampling in man limits the exact quantification of the threshold of glucose stimulation at which these packets are released; in contrast, in the perfused rat pancreas the response is directly related to the glucose level in the perfusate. However, Elrick et al.[27] have demonstrated that, with glucose infusions ranging from 250 to 740 mg. per minute for intervals of forty to 145 minutes, the arteriovenous glucose difference in man remained relatively constant and did not vary significantly with duration of the infusion. Thus, even though the absolute glucose threshold is indeterminate in man when venous blood sampling is used, the venous level is a useful indicator since it represents a fixed and constant fraction of the arterial level of glucose "seen" by the beta cell.

The "staircase" method of glucose stimulation extends the previous observations of excessive early and late phases of insulin release in obesity[28,29] to suggest that the exaggerated early phase of glucose-induced insulin release in obese subjects is more a function of increased insulin available for release than an altered threshold sensitivity to glucose. The most likely explanation for this increased content of insulin is an increase in pancreatic beta-cell mass, as suggested by the observation of islet cell hyperplasia in obese human beings.[30] A variety of factors would contribute to this and all could arise from the excessive ingestion of food in obese patients.[31] This would result in stimulation of pancreatic beta cells by exposure to excessive substrate (glucose, amino acids) after overeating, as well as the frequent neurotropic effect of vagal stimulation during both cephalic and gastric phases of food intake. In addition, provision of food to the gastrointestinal tract stimulates beta-cytotrophic factors of a humoral nature, which could contribute to islet hyperplasia after prolonged excesses of food ingestion. A final consequence of chronic overfeeding is the engorgement of storage depots with triglyceride and glycogen. This apparently initiates a form of feedback inhibition which reduces the rate of further uptake of substrate into the liver, muscle, and adipose tissue for a given amount of insulin. A chronic delay in clearing substrate after eating in obese subjects would prolong the stimulus to their beta cells and could contribute to their hyperplasia, producing a compensatory hyperinsulinism to rectify the tissue insensitivity to insulin. This latter factor of storage depot engorgement may be the most important, since persons with excessive muscle mass, who presumably also ingest excessive amounts of food (but expend it enough to keep their adiposity normal), do not show hyperinsulin responses to beta-cell stimulation.[32]

ACKNOWLEDGMENT

We are grateful to Dr. V. Licko for his assistance in developing the mathematical formulas and the computer applications for this project; Dr. Licko was assisted by a grant from the Academic Senate of the University of California, San Francisco.

Dr. Karen Burrill was recipient of a San Francisco Diabetes Association Diabetes Fellowship during this study.

Dr. K.-N. Ching held a Research Fellowship provided by the China Medical Board of New York, Inc., during his leave of absence from the National Defense Medical Center in Taipei, Taiwan.

This investigation was supported in part by USPHS Grant AM-12763(04), the Levi J. and Mary Skaggs Foundation of Oakland, California, and the Robert Kroc Foundation of Santa Ynez, California. The studies were carried out in the General Clinical Research Center, University of California, San Francisco, with funds provided by the Division of Research Resources, RR-79 of the USPHS.

We appreciate the help of Mr. Michael D. L. O'Connor for his assistance in reviewing the manuscript and for his valuable suggestions.

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