Human Serum Insulin-like Activity as Determined by a Rat Adipose Tissue Bio-assay Method

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It has been demonstrated that a very sensitive insulin assay may be obtained by measuring glucose uptake of rat epididymal adipose tissue.[1-3] This method is characterized by an increase of glucose uptake directly proportional to insulin concentration in the range of 1 to 100 micro-units of insulin/ml.[4]

The present report is concerned with studies of sera from forty-seven nondiabetic humans. Particular emphasis is given to the effects of dilution and to changes in serum insulin-like activity (ILA) after eating. The present investigation indicates that this assay method is sufficiently sensitive to detect readily serum ILA in specimens diluted 1/4 to 1/10. However, this assay, at present, lacks precision.

METHODS

The technic of insulin bio-assay employing glucose uptake by rat epididymal adipose tissue has been described in some detail.[4] The method is somewhat improved by placing each rat in an individual cage with unrestricted access to food.

Blood specimens were secured either from subjects in the fasting state and at various intervals following ingestion of 100 gm. of glucose, or at random, particular attention being given to the time intervening since the subject last ate. All subjects were in presumably good health, and were mainly males in the twenty to forty age group. After venesection, the blood was permitted to clot, the serum was separated, and the assays were usually performed within the following one to two days.

Results have been calculated as the difference between the glucose uptake (mg. glucose/gm. adipose tissue), computed as log 10, of any individual serum and the corresponding mean glucose uptake of nonserum controls incubated with adipose tissue from the same rat. A mean value of multiple determinations for each serum specimen is presented, with significance ("P") calculated by the Student "t" test.

RESULTS

Random specimens of normal human blood were collected, the sera pooled, and varying dilutions of the pooled sera assayed. Insulin-like activity diminished in rough proportion to dilution (figure 1). Marked activity was demonstrated for undiluted serum ($P<.001$) and serum diluted 1/4 ($P<.01$). There was equivocal activity at 1/10 dilution ($P>.05,<.1$) and insulin-like activity of serum diluted 1/100 was significantly lower than that of corresponding nonserum controls. Previous studies in this laboratory have demonstrated that the addition of human serum albumin to the incubating medium is not associated with acceleration of glucose uptake by the epididymal fat pad.[6]

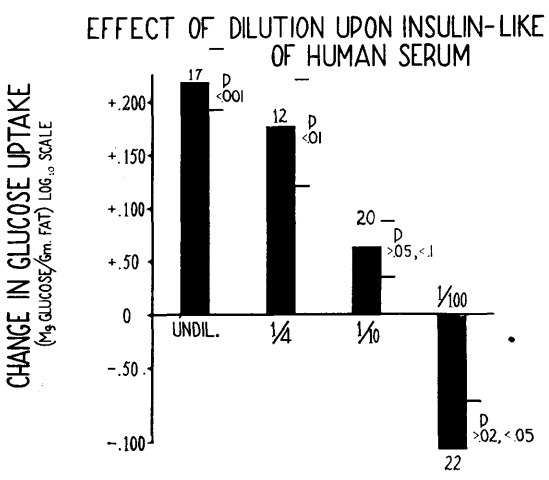

FIG. 1. Effect of dilution of serum upon glucose uptake by rat epididymal adipose tissue. Serum samples tested included no dilution, 1/4, 1/10 and 1/100 dilutions. Glucose uptake figures (log 10) are presented as differences between glucose uptakes obtained with serum in medium and without serum in medium, incubated with adipose tissue from same rat. Heights of bars represent mean change in glucose uptake. Brackets indicate S.E.M. Numbers above bars are numbers of determinations. P values, from Fisher's Tables, refer to significance of difference from buffer control.

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Serial determinations of serum insulin-like activity were made in the normal fasting state and at various intervals following oral ingestion of glucose or a meal. No consistent variations in serum ILA appeared which could be readily ascribed to the type of feeding the subject received. The sera were diluted 1/4 and 1/10. At both dilutions, it appears that there was little or no ILA present in the fasting state or up to 30 minutes postprandially, while significant serum ILA was evident thereafter (table 1, figures 2 and 3).

Considerable variation was evident even for serum samples from any one individual. In part, this could be attributed to the variability of the method, as indicated by the wide range of glucose uptake determinations

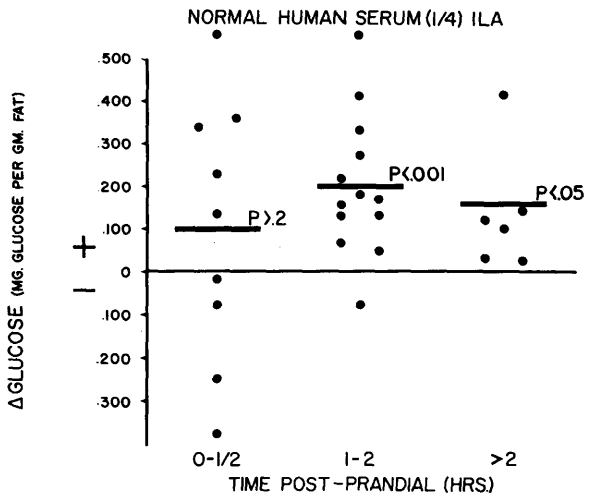

FIG. 2. Relationship of serum (diluted 1/4) insulin-like activity to time (hours) after eating meal or ingesting glucose. Each point represents insulin-like activity of a single serum. This is calculated as the mean difference of glucose uptake between medium with serum and medium without serum, incubated with adipose tissue from the same rat.

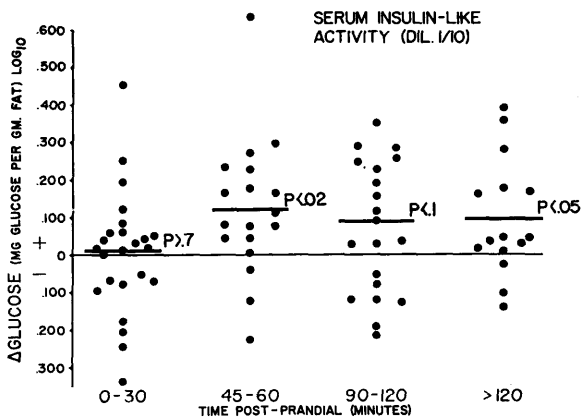

FIG. 3. Relationship of serum (diluted 1/10) insulin-like activity to time (minutes) after eating meal or ingesting glucose. Each point represents insulin-like activity of a single serum. This is calculated as the mean difference of glucose uptake between medium with serum and without serum, incubated with adipose tissue from the same rat.

obtained with each serum. However, the most important single factor influencing the serum insulin-like activity appears to be the length of time intervening between ingestion of glucose or food and venesection. There is also some association between serum glucose levels and insulin-like activity. Serum glucose levels greater than 140, occurring in eight instances, were never associated with measurable serum (diluted 1/10) ILA. These were generally obtained fifteen to thirty minutes postprandially. Conversely, serum glucose levels below 70 mg. per 100 ml. were associated with the presence of measurable activity in six of eight samples tested at a 1/10 dilution. These were specimens obtained one to three hours after meals.

One subject (BEI) was tested for serum insulin-like activity (dilution 1/10) seven times over about eight-

TABLE 1

Effect of time interval after eating upon serum insulin-like activity

Postprandial time interval	Serum dilution	Number of sera tested	Mean change log 10	Significance "P" of difference from Buffer control	Fasting serum	15-30 min. Postprandial serum
Fasting	1/4	6	+.129	>.4	—	—
15-30 min.	1/4	3	+.047	>.6	>.6	—
60 min.	1/4	4	+.177	>.02,<.05	>.7	>.2,<.3
120 min.	1/4	7	+.248	>.01,<.02	>.4	>.1,<.2
150-180 min.	1/4	5	+.189	>.02,<.05	>.7	>.2,<.3
Fasting	1/10	10	+.003	>.9	—	—
5-30 min.	1/10	15	—.017	>.6	>.8	—
45-60 min.	1/10	19	+.113	>.01,<.02	>.2,<.3	>.01,<.02
90-120 min.	1/10	20	+.071	>.05,<.1	>.3	>.05,<.1
150 min. or greater	1/10	15	+.106	>.02,<.05	>.2,<.05	>.02,<.05

TABLE 2

Variations in serum (dil. 1/10) insulin-like activity of a single individual (BEI)

Date	Interval of time postprandial	Number of determinations	Blood glucose (mg. per 100 ml.)	Mean change*	Significance "p"‡
5/19/58	½ hr.	7	96	+.226	<.01
7/18/58	Fasting	8	104	+.251	<.01
	½ hr.	6	147	+.002	>.9
	1 hr.	15	76	+.180	>.05,<.1
	2 hr.	8	53	(—.122)†	(>.4)†
9/16/59	1½ hr.	6	102	+.250	<.01
	2 hr.	4	81	+.206	>.02,<.05
3/23/59	2 hr.	5	—	+.010	>.8
4/27/59	1½ hr.	8	—	(—.093)†	(>.1,<.2)†
10/31/59	Fasting	9	105	+.133	>.1,<.2
	15 min.	8	135	+.033	>.7
	30 min.	9	125	+.094	>.1,<.2
	1 hr.	9	83	+.164	>.02,<.05
	2 hr.	8	86	+.190	<.01
	3 hr.	19	68	+.179	<.001

*Mean change of glucose uptake as compared with equivalent nonserum control values—mg. glucose/gm. adipose tissue (log 10).
†Parentheses enclose figures indicating decrease of glucose uptake.
‡Refers to significance of difference from buffer control.

een months (table 2). Multiple sera for two of these assays were acquired in the course of oral glucose tolerance tests. Five of the seven tests demonstrated significant serum insulin-like activity. Each glucose tolerance test was characterized by significant insulin-like activity in one or more serum specimens.

Sera appear to have greater insulin-like activity at 1/4 than at 1/10 dilution. This is consistent with the results of testing varying dilutions of pooled sera.

DISCUSSION

In this study serum dilution appears to be associated with a corresponding, fairly proportional, diminution in glucose uptake. These results do not parallel those obtained with the rat diaphragm assay technic, which were characterized by comparatively little change in glucose uptake consequent to dilution, particularly in the range 1/2 to 1/4.[6,7] Also, it is of possible importance that the present report indicated diminution of glucose uptake in the presence of serum diluted 1/100 significantly less than that of corresponding media containing 0.2 per cent albumin. This may reflect the need for further effort to define more fully the activity of protein "control" material, including albumin and gelatin. Alternately there may be important shifting of the balance between complex insulin-like and anti-insulin effects of whole serum associated with degree of serum dilution.

Vallance-Owen[8] and Seltzer and Smith,[9] employing the rat diaphragm method, and Baird and Bornstein,[10] utilizing blood glucose changes in the anesthetized adrenalectomized mouse, noted marked increase of circulating human insulin-like activity following feeding. This study appears to confirm these observations.

The marked individual variations noted in this study while, in part, a reflection of the inherent variability of the method, also suggest true differences between human subjects. It has been emphasized that this bio-assay measures the total "insulin-like" effect, which is the total balance between circulating insulin, insulin-like factors, and anti-insulin substances.[8] A preponderance of anti-insulin factors at certain times may explain, in part, the occurrence of the occasional significant negative glucose uptake figures obtained in this study.

No attempt is being made in this investigation definitively to relate glucose uptake figures to quantitative insulin levels. Rough estimations, based upon the results of testing serum diluted 1/4, suggest an effective mean normal level of about 100 μU/ml. in the fasting state and 400 μU/ml. one to two hours postprandially.

SUMMARY

Normal human sera were assayed by a method utilizing glucose uptake by adipose tissue. Maximum insulin-like activity appeared to occur one to two hours postprandially. Considerable individual variation was a notable feature. Decrease of insulin-like activity was demonstrated as a consequence of diluting pooled whole serum.

SUMMARIO IN INTERLINGUA

Activitate Insulinoide de Sero Human, Determinate per un Methodo de Bio-Essayage con le Uso de Tissu Adipose de Ratto

Normal seros human esseva essayate per un methodo utilisante le acceptation de glucosa per tissu adipose. Le maximo del activitate insulinoide pareva occurrer inter un e duo horas post prandios. Considerabile grados de variabilitate individual esseva un notabile characteristica. Un reduction del activitate insulinoide esseva demonstrate como consequentia de un dilution de collationate sero total.

ACKNOWLEDGMENT

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Mortality of Overweights with Impairments

Some impairments generally considered to be of minor significance produce an unduly large increase in the mortality of overweights. This is one of the major findings of the Build and Blood Pressure Study, 1959, by the Society of Actuaries, which investigated separately the records of insured persons with minor impairments and of those without known minor impairments. The facts will be reviewed for males only, since their examinations for life insurance were more extensive and more thorough in most cases than those for females. For purposes of this study, a minor impairment is one which would not bar the applicant from obtaining standard insurance.

Overweight men with moderate elevation of blood pressure experienced a mortality nearly 60 per cent above that for overweights with no known minor impairments. For overweights with small or moderate amounts of albuminuria, the mortality was increased about 30 per cent; for those with other genitourinary disorders (such as kidney stone) and for those with a history of nervous disorders (chiefly psychoneurosis) the increase was about 15 per cent. Among overweight men with a family history of early cardiovascular-renal disease—considered to be a minor impairment for purposes of this study—the mortality was 35 per cent higher than for overweights of corresponding degree without known minor impairments.

The mortality among the overweight men with moderate elevation of blood pressure according to age at issue and degree of overweight is clearly shown. Not only is the mortality of these men consistently higher than that for all standard risks, but the excess is also quite large when compared with the mortality for men in the corresponding weight group without known minor impairments. The relatively small rise in the mortality ratios with increase in degree of overweight reflects, in part at least, the careful selection of the more obese applicants with moderately elevated blood pressure.

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