Papers and Originals

Oral Glucose Tolerance and Related Factors in a Normal Population Sample

I. Blood Sugar, Plasma Insulin, Glyceride, and Cholesterol Measurements and the Effects of Age and Sex

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Summary: Oral glucose tolerance tests were performed on 220 people, a representative sample of the employees of a large pharmaceutical company. Blood sugar and plasma immunoreactive insulin levels were measured on each sample of venous blood obtained before and at half-hourly intervals for two hours after 50 g. of glucose by mouth; plasma cholesterol and glycerides were measured on the fasting sample only.

Women had higher mean insulin levels throughout the test, though their mean blood sugar levels were higher only at 90 and 120 minutes. In both sexes there were positive correlations between age and the levels of blood sugar, plasma cholesterol, and plasma glycerides. Though the levels of glucose rose with age, those of insulin did not.

Introduction

During a diabetes screening survey in a large British pharmaceutical company the opportunity was taken to perform oral glucose tolerance tests on a representative sample of the employees in order to study the interrelationships of the circulating glucose and insulin responses to a glucose load with a number of other factors. In this paper the methodology of the study is described and the results in relation to age and sex are presented. In the accompanying paper an analysis of the interrelationships is presented.

Methods

Population.—The diabetes survey was carried out on the premises of Beecham Laboratories Ltd., where the population was mixed, containing people engaged in managerial, clerical, and both skilled and unskilled manufacturing occupations. Altogether 961 employees (84% of the total) took part in the screening survey, which also had a research aspect—namely, the study of various methods of preparation before tolerance testing. A total of 919 people were tested on two occasions, and this co-operating population was further asked to volunteer for the glucose tolerance tests of the present study. From the volunteers, 220 people were selected by a stratification procedure in such a way that there were about equal numbers in each age and sex group (Table I) and an even distribution of body build in the groups. The latter was achieved by calculating the “ponderal index” ($\text{height} \div \sqrt{\text{weight}}$) for all the

individuals in each age/sex group, then randomly selecting about equal numbers from the upper, middle, and lower tertiles of the distributions. Known diabetics and pregnant women were excluded before test, but no exclusions were made on the basis of the test results themselves.

TABLE I.—Age and Sex Composition of the Population Sample

Age	Men	Women
24 and below	24	22
25-34	22	22
35-44	20	20
45-54	22	23
55+	21	24
Mean	39.43	38.95
Range	16-74	15-63
Total No.	109	111

Testing Procedure.—After a clear explanation of the research nature of the examinations proposed, members of the subsample were requested to take extra carbohydrate in addition to their normal diet for three days, until the evening before the test, and to present themselves, fasting, at about 9 a.m. on the morning of the test. Venous blood samples were collected before and at 30, 60, 90, and 120 minutes after an oral load of liquid glucose (235 ml. of Lucozade, equivalent to 50 g. of anhydrous glucose). Blood was taken by repeated venepuncture through a tiny skin bleb of lignocaine (without adrenaline), put into chilled tubes containing heparin and Versenate, then carefully mixed and centrifuged at 2,000 r.p.m. for 15 minutes. The supernatant plasma was stored at -20°C . and not thawed until required for estimation.

Laboratory Methods

Blood Sugar.—This was measured on 0.1-ml. aliquots of the freshly drawn venous blood taken before centrifugation and diluted in 3 ml. of 1% aqueous solution of potassium fluoride. Estimations were carried out on the AutoAnalyzer (Technicon) by the microferricyanide reduction method (Whichelow *et al.*, 1967).

Plasma Immunoreactive Insulin.—Radioimmunoassay of the plasma samples against human insulin standard curves (human insulin was kindly supplied by Novo Industri A.S.) was carried out by a modification of the two-antibody method of Morgan and Lazarow (1963). The modification was minor, consisting chiefly of miniaturization, so that plasma samples of 0.1 ml. only were required. In addition, edetic acid was added to the buffer, as recommended by Sheldon and Taylor (1965). Estimates were carried out at least in duplicate. When plasma samples required dilution owing to high values, several dilution

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levels were assayed to ensure parallel slope conditions. Assays were arranged so that complete "sets" of samples were measured in the same experimental run and so that, so far as was possible, all the stratification groups were equally represented in each run. The standard error of the assay was about 5% of the value right across the operative range of the assay.

Glycerides.—Plasma glycerides were estimated by a semi-automated procedure based on the method of Lofland (1964). Aliquots of the isopropanol extract of plasma were treated with 0.1 N ethanolic KOH at 70° C. for 20 minutes in order to hydrolyse the glycerides and the free glycerol was estimated on the AutoAnalyzer by the chromotropic acid method of

Lambert and Neish (1950). A measure of the reproducibility of the method was obtained from 10 estimations of the glyceride concentration on a pooled serum sample. These gave a mean serum glyceride of 73 mg./100 ml. (S.D. ± 4.8 mg./100 ml.), which did not differ significantly from the mean of 10 gravimetric serum glyceride estimations on the same sample (71 ± 4.4 mg./100 ml.).

Cholesterol.—The total plasma cholesterol was estimated by the automated method of Block *et al.* (1966). When applied to the pooled serum sample, 10 estimations gave a mean value 185 mg./100 ml. (S.D. ± 7.6 mg./100 ml.), which compared well with 10 gravimetric estimations on the same sample (182 ± 6.3 mg./100 ml.) (data of MacDonald and Jordan, (personal communication)).

Data analysis was performed by means of an IBM 360/65 computer at University College, London. The necessary programmes were written in Fortran IV and use was also made of the BMD (Biomedical) computer programmes (Dixon, 1965).

Results

Blood Sugar and Plasma Insulin

The frequency distribution of blood sugar and plasma insulin values for the sexes separately are shown as histograms in Figs. 1 and 2, which also give the mean values, standard deviations, and range. The distribution of the plasma insulin values is positively skewed, but logarithmic transformation (Fig. 3) makes the distribution more nearly normal, a phenomenon previously reported by Welborn *et al.* (1966) and Chlouverakis *et al.* (1967). The mean blood sugar and plasma insulin values, by sex, are shown graphically in Figs. 4 and 5. The blood sugar values in men are greater at 0, 30, and 60 minutes but less at 90 and 120 minutes. These differences are statistically significant at 30 ($0.02 > P > 0.01$), 60 ($0.05 > P > 0.02$),

FIG. 1.—Histograms showing the frequency distribution by sex of blood sugar values at the five time points of the glucose tolerance test. FIG. 2.—Histograms showing the frequency distribution by sex of plasma insulin values at the five time points of the glucose tolerance test. FIG. 3.—Frequency distribution of plasma insulin values after logarithmic transformation.

FIG. 4.—The mean ($\pm$ S.E.M.) levels of blood sugar by sex. FIG. 5.—The mean ($\pm$ S.E.M.) levels of plasma insulin by sex.

TABLE II.—Mean Blood Sugar Values (with Standard Deviation) at the Five Time Points of the Glucose Tolerance Test by Age and Sex

Age	Sex	n	Blood Sugar at									
			0 min.		30 min.		60 min.		90 min.		120 min.	
			Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
24	M	24	78.8	5.4	108.5	16.5	86.5	19.1	68.0	17.2	67.1	14.1
	F	22	80.1	7.3	113.5	21.4	91.5	22.2	86.0	17.6	81.7	13.8
25-34	M	22	81.3	10.4	120.5	25.9	97.5	23.8	73.2	20.7	68.3	13.4
	F	22	82.6	8.4	109.4	20.2	88.1	17.8	79.9	16.9	80.7	10.9
35-44	M	20	84.8	8.7	124.5	15.7	113.5	30.5	84.8	16.9	76.0	13.3
	F	20	78.7	7.7	112.9	22.8	101.2	39.1	89.4	28.9	77.0	18.0
45-54	M	22	85.4	11.0	133.9	27.5	122.5	31.0	86.8	28.0	71.9	20.2
	F	23	80.8	8.1	120.6	29.4	112.4	34.0	90.8	29.2	76.4	17.1
55+	M	21	85.8	11.8	139.7	23.4	135.9	32.5	95.6	35.1	74.6	26.1
	F	24	83.5	8.9	126.5	21.0	114.0	29.5	90.8	24.2	82.1	21.2

TABLE III.—Mean Plasma Insulin Levels (and Standard Deviation) at the Five Time Points of the Glucose Tolerance Test by Sex and Age Group

Age	Sex	n	Plasma Insulin at									
			0 min.		30 min.		60 min.		90 min.		120 min.	
			Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
24	M	24	15.2	4.4	72.5	35.9	47.3	17.1	25.2	10.3	17.9	7.0
	F	22	19.0	12.3	101.3	89.4	74.0	48.3	55.7	47.9	33.3	27.5
25-34	M	22	14.7	4.4	75.6	35.8	59.4	27.1	27.7	22.7	16.7	6.0
	F	22	17.6	5.7	75.7	44.1	60.9	43.7	35.4	16.1	31.2	33.2
35-44	M	20	15.7	4.7	78.8	54.2	65.4	30.2	36.4	19.1	20.8	11.2
	F	20	14.9	6.9	86.0	62.1	72.9	52.6	46.0	27.5	24.8	14.3
45-54	M	22	16.6	6.8	72.6	45.4	70.8	34.5	36.8	23.2	24.6	16.9
	F	23	14.6	5.2	76.1	36.7	74.1	38.6	43.3	24.5	23.7	12.5
55+	M	21	12.5	5.8	64.9	36.8	29.9	34.7	34.7	22.3	22.2	15.6
	F	24	16.5	6.1	79.6	52.6	81.0	47.5	42.3	24.7	29.2	21.9

TABLE IV.—Correlation of Age with Blood Sugar, Plasma Insulin, and Log Insulin

Time (min.)	Men (n=109)						Women (n=111)					
	Blood Sugar		Insulin		Log Insulin		Blood Sugar		Insulin		Log Insulin	
	R	P	R	P	R	P	R	P	R	P	R	P
0	0.241	<0.02	-0.1	—	-0.183	—	0.073	—	-0.182	—	-0.133	—
30	0.440	<0.001	-0.056	—	-0.091	—	0.213	<0.05	-0.134	—	-0.096	—
60	0.535	<0.001	0.202	<0.05	0.191	—	0.309	<0.01	0.064	—	0.091	—
90	0.356	<0.001	0.161	—	0.144	—	0.118	—	-0.105	—	-0.051	—
120	0.124	—	0.166	—	0.066	—	-0.043	—	-0.110	—	-0.091	—

and 120 ($P<0.001$) minutes. By contrast, women have higher insulin values throughout the test, the differences reaching statistical significance at 60 ($P=0.02$), 90 ($P<0.001$), and 120 ($P<0.001$) minutes.

In Tables II and III the blood sugar and plasma insulin values, respectively, are given for each time point of the tolerance test and also in relation to age. In both men and women the mean blood sugar level (Table II) tends to rise with age at each time point except at 120 minutes. When linear correlation coefficients of age against blood sugar were calculated, statistically significant associations were found at 0, 30, 60, and 90 minutes for the men and at 30 and 60 minutes for the women. By contrast, plasma insulin levels, both arithmetic and logarithmic (Table III), did not show any significant trends with age. The correlation coefficients are given in Table IV.

Glycerides

The mean levels of fasting plasma glyceride by age and sex are given in Table V. The mean level in each age group is higher for the men. In both sexes there was a trend upwards with age, which was highly significant for the men ($r=0.414$; $P<0.001$) but only just significant for the woman ($r=0.204$; $P<0.05$). This rise in glycerides with age is similar to that found by Schaefer (1964) and Fredrickson *et al.* (1967), who also noted higher levels in men.

Cholesterol

The mean levels of total plasma cholesterol by age and sex group are shown in Table VI. The mean values, which are strikingly similar to those of Fredrickson *et al.* (1967), rise with age in both sexes.

TABLE V.—Mean Fasting Plasma Glyceride Levels (With Standard Deviation) by Age and Sex

Age	Plasma Glyceride (mg./100 ml.)					
	Men			Women		
	Glyceride	S.D.	n	Glyceride	S.D.	n
<24	77.7	16.9	23	75.8	30.6	21
25-34	83.3	26.4	17	73.0	22.9	21
35-44	96.6	21.2	18	88.6	27.0	17
45-54	101.0	36.0	22	84.7	26.0	18
55+	109.0	27.7	20	89.9	27.3	19
Overall mean	93.4	28.5	100	81.9	27.2	96
Range	47-210			36-185		

TABLE VI.—Mean Fasting Plasma Cholesterol Levels (With Standard Deviation) by Age and Sex

Age	Total Plasma Cholesterol (mg./100 ml.)					
	Men			Women		
	Cholesterol	S.D.	n	Cholesterol	S.D.	n
≤24	190.3	41.3	22	192.3	30.7	22
25-34	212.4	37.7	17	191.8	27.0	20
35-44	235.8	31.7	17	219.9	32.4	17
45-54	249.1	35.7	21	238.8	48.7	18
55+	258.4	43.2	20	249.1	40.1	19
Mean	228.9	45.6	97	216.9	42.9	96
Range	133-328			152-363		

Discussion

Analysis of the blood sugar data confirms the different "shape" of the mean glucose tolerance curve in men and women previously described by Butterfield (1964), Keen (1964), and Sharp (1964) and the effect of age on oral glucose tolerance

(Butterfield, 1964 ; Gordon, 1964 ; Keen, 1964 ; Sharp, 1964). In both men and women the mean blood sugar two hours after the glucose load was unrelated to age, at least up to the seventh decade. This contrasts with the peak blood sugars, but confirms the results of the glucose tolerance tests performed on the Bedford random population sample, in which a mean two-hour blood sugar rise was observed in only the over-70 age group (Butterfield, 1964 ; Keen, 1964 ; and Sharp, 1964). This fact affects the choice of the one-hour or two-hour blood sugar sample for population screening for diabetes, a topic which is discussed in detail elsewhere (Keen and Jarrett, 1969).

There is a considerable literature concerning the levels of blood sugar and plasma insulin, both fasting and after a glucose load, but the insulin values reported in this paper are the first, to our knowledge, to be based on a normal population sample. Most reports are of small series (see Buchanan and McKiddie, 1967, for references), and direct comparison of reported levels is difficult because of differences in technique of measurement and the lack of universal insulin standards. In general, however, it has been found that the shape of the insulin curve parallels that of the blood sugar curve and that the distribution of the insulin levels is log normal.

The present study shows a sex difference in plasma insulin levels after a glucose load, not obviously attributable to differences in blood sugar levels. Thus women had higher mean insulin levels throughout the tolerance test, though their mean blood sugar levels were higher only at 90 and 120 minutes. The difference also could not be attributed to the effects of oral contraceptives, for only four women were using them at the time. In addition, one postmenopausal woman was receiving oestrogens.

In this study there was little evidence of a positive relationship between age and insulin levels, with the single exception of the peak levels in men. This is apparently at variance with an earlier study (Chlouverakis *et al.*, 1967) in which serum insulin was found to rise with age in a control group, but not in a group of borderline diabetics. This rise with age was statistically significant only when the fasting levels were considered, but a similar trend was apparent at one hour.

There are several differences between the two studies, however. Firstly, and probably most important, the control population in the first study was preselected by virtue of having a blood sugar less than 120 mg./100 ml. two hours after 50 g. of glucose on two occasions. Perhaps because of this selection, in this control population, blood sugar levels did *not* rise with age, a phenomenon which could be related, or even attributed, to the rise with age of circulating levels of insulin. This hypothesis is reinforced by the study of Hales *et al.* (1968), in which a positive correlation was found between age and insulin levels at 60 and 90 minutes after oral glucose. This was again

in a selected population, with normal glucose tolerance, in which blood glucose levels did not rise with age. The second major difference lies in the timing of the glucose tolerance tests. In the earlier study they were performed in the afternoon, and it has since become apparent that afternoon glucose tolerance and insulin responses may differ considerably from morning ones (Bowen and Reeves, 1967 ; Jarrett and Keen, 1968 ; Malherbe *et al.*, 1968).

At present little is known about the diurnal variation in insulin response to glucose. It is likely that it will be quantitatively rather than qualitatively different, and if that is true then the results of the present study and those of Chlouverakis *et al.* (1967) and of Hales *et al.* (1968) may be combined. The conclusion with respect to age is that, in the whole population, insulin levels do not rise with age, though glucose levels do. However, in selected subgroups, in which there is no positive correlation between age and blood glucose levels, there is discernible a positive correlation, albeit of low degree, between age and insulin levels. As we suggested earlier (Chlouverakis *et al.*, 1967), "older people, maintaining the same blood sugar levels as young ones, only do so under the influence of elevated circulating levels of insulin."

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