PAPERS AND ORIGINALS

Diurnal Variation in Oral Glucose Tolerance: Blood Sugar and Plasma Insulin Levels Morning, Afternoon, and Evening

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Summary

Twenty-four subjects received three oral glucose tolerance tests, in the morning, afternoon, and evening of separate days. The mean blood sugar levels in the afternoon and evening tests were similar, and they were both significantly higher than those in the morning test. Plasma immunoreactive insulin levels, however, were highest in the morning test. The pattern of insulin levels during the afternoon and evening tests resembled that described as typical of maturity-onset diabetes.

Introduction

In previous communications (Jarrett and Keen, 1969, 1970) we have confirmed and extended the earlier observations of Roberts (1964) and of Bowen and Reeves (1967) on the diurnal variation in oral glucose tolerance. The earlier investigations compared, in the same individuals, blood sugar levels in oral glucose tolerance tests performed in the morning and late afternoon. In the present study we have extended the comparison to include a test performed in the late evening and, in addition to the blood sugar, we have also measured the simultaneous levels of plasma insulin.

Subjects and Methods

Subjects for this study were volunteers selected from the patients and staff at New Cross Hospital in the Guy's Hospital Group. Most of the patients were under investigation or receiving physical treatment for musculoskeletal or joint disorders and none were known to be suffering from conditions, or taking medications, likely to affect glucose tolerance. Details of the participants are given in Table I.

Before the tests all subjects were consuming a normal diet containing adequate amounts of carbohydrate. The period of fasting before each test was nine hours and the last meal before the fast was made similar in composition, consisting of about 50 g of carbohydrate as a jam or marmalade sandwich. Oral glucose tolerance tests were performed at intervals of two to seven days, and in random order, at 09.00, 15.00, and 20.00 hours. A standard load of 50 g of liquid glucose (as 235 ml of Lucozade) was used and capillary (ear-lobe) blood samples were taken fasting and at half-hourly intervals after the glucose drink for two hours. For subsequent blood sugar determination by the ferricyanide reduction micromethod (Technicon method N — 9a) on the AutoAnalyzer, 0·1 ml of blood was added to 0·9 ml of 1% sodium fluoride. Approximately 0·5 ml of capillary blood was taken into dry, previously heparinized, glass capillary tubes. The plasma was separated in these by centrifugation and the immunoreactive insulin subsequently determined by the method of Albano and Ekins (1970), using duplicate 10-µl aliquots of plasma, each made up to 50 µl with insulin-free (charcoaled) human plasma. Human insulin (Eli Lilly, batch 516-7343-33) was used as a standard.

TABLE I—*Characteristics of the 24 Subjects Studied*

Subject No.	Sex	Age	Diagnosis
1	M	49	Disseminated sclerosis
2	M	62	Fractured femur
4	F	54	Rheumatoid arthritis
6	M	86	Dermatitis herpetiformis
7	M	91	Social problems
8	M	63	Cerebellar infarction
9	M	58	Previous stroke
10	M	23	Prolapsed disc
11	M	43	Lumbosacral strain
12	M	73	Arthritic hips
13	M	44	Backache
14	M	38	Prolapsed disc
15	M	25	Backache
16	M	66	Arthritic hips
19	M	50	Prolapsed disc
20	M	19	Normal subject
21	M	21	Normal subject
22	M	51	Prolapsed disc
23	M	23	Prolapsed disc
24	M	26	Prolapsed disc
25	M	56	Chronic back strain
26	M	51	Arthritic hips
27	M	59	Ankylosing spondylitis
28	M	55	Arthritic spine

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TABLE II—*Mean Blood Sugar Levels (mg/100 ml) ± S.E. of Mean at the Five Time Points of the Three Glucose Tolerance Tests*

Time of Test	Time in Minutes				
	0	30	60	90	120
09·00	75·29 ± 1·52	125·71 ± 3·05	129·13 ± 6·73	108·5 ± 6·08	84·67 ± 5·35
15·00	76·86 ± 1·77	127·0 ± 5·17	159·62 ± 7·15	155·81 ± 9·55	126·10 ± 8·99
20·00	82·17 ± 2·40	131·22 ± 4·86	159·39 ± 6·29	151·09 ± 7·73	121·52 ± 8·12

TABLE III—*Mean Plasma Insulin Levels (μU/ml) ± S.E. of Mean at the Five Time Points of the Three Glucose Tolerance Tests*

Time of Test	Time in Minutes				
	0	30	60	90	120
09·00	8·18 ± 1·15 (n = 22)	30·0 ± 5·45 (n = 20)	33·05 ± 7·0 (n = 22)	33·47 ± 7·52 (n = 19)	16·77 ± 3·74 (n = 22)
15·00	9·00 ± 1·09 (n = 20)	17·5 ± 2·34 (n = 18)	23·63 ± 3·05 (n = 19)	28·26 ± 6·62 (n = 19)	25·68 ± 7·72 (n = 19)
20·00	8·14 ± 1·48 (n = 21)	17·29 ± 2·33 (n = 21)	24·09 ± 4·14 (n = 23)	18·4 ± 2·66 (n = 20)	17·64 ± 5·68 (n = 22)

Results

Blood Sugar.—The mean blood sugar levels in the three tests are presented in Table II and Fig. 1. The mean result of the evening test is almost identical with that of the afternoon test. In both tests the levels at 60, 90, and 120 minutes significantly exceed those in the morning test. Of the participants, two (Nos. 7 and 8) had morning test results which met the British Diabetic Association criteria for the diagnosis of diabetes (Fitzgerald and Keen, 1964).

Plasma Insulin.—The mean results of the plasma insulin levels in the three tests are presented in Table III and Fig. 2.

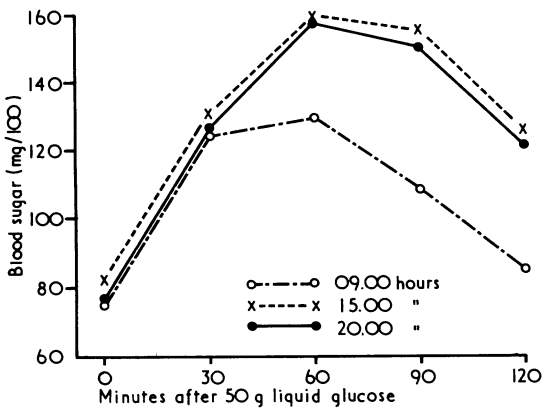

FIG. 1—Mean blood sugar curves in the three glucose tolerance tests.

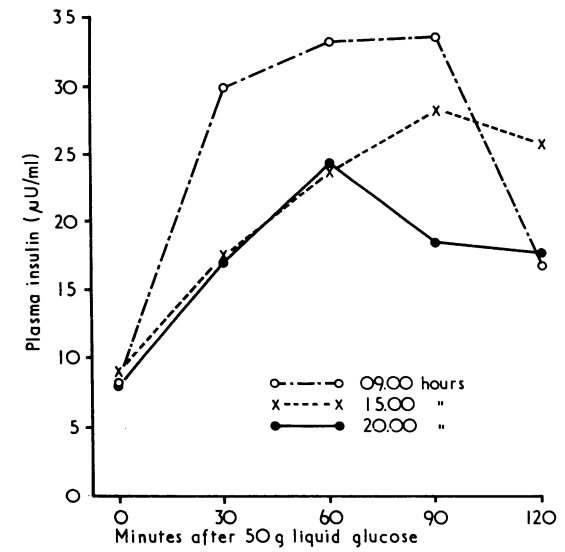

FIG. 2—Mean plasma immunoreactive insulin levels during the three glucose tolerance tests.

Not all the insulin values were available for analysis, usually owing to insufficient sample size. The trends, however, are of sufficient dimension to compensate for this. It is clear that the insulin levels reached in the afternoon and evening tests are lower than those in the morning test, despite the concomitant higher levels of blood sugar. While the only statistically significant ($P < 0.05$) difference is at the 30-minute time point, this analysis is relevant only to a consideration of the insulin values in isolation. It will be noted that the *form* of the insulin response to the glucose load in the afternoon and evening is like that said to be characteristic of diabetes, with a delayed rise and late peak response.

Effects of Age.—These were examined by dividing the population into those aged 50 and below (n = 11) and those aged more than 50 (n = 13). The mean blood sugar and insulin results

TABLE IV—*Mean Blood Sugar Levels (mg/100 ml) by Age Group*

Age Group	Time of Test	Time in Minutes				
		0	30	60	90	120
≤50	09.00	80·0	120·4	110·4	92·1	76·5
	15.00	74·8	121·4	143·3	137·2	110·6
	20.00	83·7	133·1	150·4	128·9	99·8
>50	09.00	71·3	130·2	145·0	122·4	91·6
	15.00	78·4	131·2	170·3	169·8	137·8
	20.00	81·0	129·8	166·3	168·2	138·2

TABLE V—*Mean Plasma Insulin Levels (μU/ml) by Age Group*

Age Group	Time of Test	Time in Minutes				
		0	30	60	90	120
≤50	09.00	7·0	38·4	31·1	21·9	9·3
	15.00	8·4	19·5	19·5	26·3	23·6
	20.00	7·1	14·5	25·7	17·8	10·8
>50	09.00	9·2	23·1	34·7	41·9	21·9
	15.00	9·4	16·5	26·6	29·4	27·2
	20.00	9·5	19·0	22·8	18·8	22·4

are presented in Tables IV and V. It can be seen that the results are qualitatively similar for both age categories. The older group, as expected, had higher postglucose blood sugar levels in the standard morning test, with a later peak, and these are reflected in the corresponding plasma insulin levels. The diurnal variation in blood sugar levels is proportionately less in the older group: +18·8% contrasted with 26·4% in the younger group when the areas under the 09.00 and 20.00 hour glucose tolerance curves are compared. This lesser variation may be attributable to the poorer glucose homoeostasis of the older group rather than to age per se (Jarrett and Keen, 1970).

Discussion

Previous studies of diurnal variation of oral glucose tolerance have compared tests performed in the morning with similar tests in the afternoon. The present study extends the observations to later in the day and shows that, for mean behaviour at least, the afternoon and evening results are virtually identical. It would seem that the reversion to the morning state of "best glucose tolerance" must occur during the night, though the possibility remains that the same reversion might result from a similar period of rest or sleep during the day.

These changes in blood sugar response to the glucose load are, not surprisingly, accompanied by changes in the pattern of plasma insulin response, and it is the direction of these changes which claims attention. In both the afternoon and evening tests the mean plasma insulin response is smaller, while the accompanying blood sugar rise is greater, than in the morning. The major difference lies in the early part of the test (Fig. 2). It seems reasonable to attribute the loss of glucose tolerance to a diminished pancreatic response to the glycaemic stimulus, so that the higher blood sugar levels observed in afternoon and evening tests are the result of insulinopenia. Support for this view can be adduced from the findings of Freinkel *et al.* (1968), who observed a diurnal variation in plasma insulin levels during periods of total fasting, with mean levels at 07.00-08.00 hours exceeding those at 15.00-16.00 hours. Lambert and Hoet (1965) also noted a diurnal pattern in insulin levels. They found high levels during the night, even though daytime meals were presumably stimulating insulin release from the pancreas. However, the same group in a later study (Malherbe *et al.*, 1969) investigated the insulin response to identical meals given at 07.30, 12.00, and 16.30 hours and found that both the mean and maximal insulin levels were greatest in response to the morning meal. Sensi *et al.* (1970) studied 11 healthy subjects and measured circulating levels of glucose and insulin before and one hour after 50 g glucose given by mouth at 08.00, 16.00, and 24.00 hours. The net increase above the basal value of insulin was higher in the morning than the other two times, a result similar to our own. At a different level Hellman and Hellerström (1959) observed diurnal variation in the nuclear size of the beta cells of the islets of Langerhans in the rat, an animal which also shows diurnal variation in glucose tolerance (Ben-Dyke, 1971). Further support for the islet cell as the basis of the diurnal variation in oral glucose tolerance comes from the observations of a diurnal variation in intravenous glucose tolerance (Abrams *et al.*, 1968; Nemeth *et al.*, 1970), which clearly cannot be attributed to changes in glucose absorption or intestinal hormone release, factors which might be implicated in any changes in oral glucose tolerance.

A strong circumstantial case can thus be made out in favour of the hypothesis that the diurnal variation in glucose tolerance is secondary to a rhythm in the islet cells which determines the amount of insulin released in response to a given stimulus. However, this may be an oversimplification. Several individual results from the present study are detailed in Table VI. Subject 8 has blood sugar levels which are almost identical in the morning and evening tests, yet the corresponding insulin levels are much lower in the evening. It might be argued that here the lower levels of insulin have been compensated by an increased effectiveness of the hormone. A similar argument could be applied to the findings of Freinkel *et al.* (1968) for, despite the distinct

TABLE VI—*Blood Sugar and Plasma Insulin Levels Observed in Three Subjects Showing Individual Variation in Type of Response*

Subject No.	Time of Test	Blood Sugar (mg/100 ml) at: 0	30	60	90	120 Minutes	Plasma Insulin (μU/ml) at: 0	30	60	90	120 Minutes
8	09.00	74	121	177	180	154	9	13	35	46	41
	20.00	67	103	168	203	147	6	12	12	22	20
19	09.00	71	125	134	103	80	7	62	58	36	20
	15.00	68	114	140	119	87	13	15	19	35	25
22	09.00	72	118	115	92	60	10	12	35	20	14
	15.00	77	162	175	157	123	11	15	29	17	11

afternoon fall in insulin levels, there was no corresponding change in the blood sugar. Subject 19 shows a similar phenomenon, though here, unlike Subject 8, both sets of blood sugar results are well within the normal range. Subject 22 exemplifies the mean behaviour of the whole group, with a considerable diurnal swing upwards in blood sugar levels, and a corresponding fall in insulin levels. The relative importance of circulating insulin levels and of insulin "sensitivity" or "effectiveness" clearly requires further study.

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