PLASMA CORTICOSTEROID, PLASMA INSULIN
AND BLOOD SUGAR OF NORMAL FASTING AND
DIABETIC SUBJECTS WITH OR WITHOUT
HYPERCORTICISM

By
Vivian Harding Asfeldt and Kai R. Jørgensen

ABSTRACT

Transient, maximum stimulation with \( \beta^{1-24} \) corticotrophin has been carried out in nine normal fasting subjects, in two fasting diabetics without hypercorticism and in three fasting diabetics with hypercorticism. Fluorimetric determinations of corticosteroids and determinations of immunological detectable insulin in plasma and blood sugar were made during stimulation. No significant variation in the blood sugar or the plasma insulin during transient, maximum ACTH stimulation was found either in normal fasting subjects or in fasting diabetics with or without hypercorticism. Moreover, in two diabetics with hypercorticism the plasma insulin response was measured during an oral glucose tolerance test. After treatment for approximately seven months with glucocorticosteroids, a reduced glucose tolerance and an increased plasma insulin response were found in one of these two patients. Four and a half months after the termination of steroid treatment, normal glucose tolerance and normal insulin responses were observed. In one patient, after several years of hypercorticism, a reduced glucose tolerance and a markedly reduced plasma insulin response were found.

The diabetogenic effect of adrenal cortex steroids, particularly the glucocorticosteroids, is well known. This relationship was first described by Long & Lukens (1936), who observed that pancreatic diabetes mellitus in cats was reduced after adrenalectomy. Ingle (1941) was the first to produce steroid
diabetes in force-fed normal rats, and Ingle et al. (1951) showed that corticotrophi
n (ACTH) injections under the same experimental conditions, also caused steroid diabetes.

These observations were later used for the diagnosis of latent diabetes mellitus. Berger (1952) used ACTH to increase the sensitivity of the oral glucose tolerance test (GTT), while on the other hand, Fajans et al. (1954) found that cortisone was more suitable.

As a result of its organotrophic effect, ACTH must be regarded as a diabetogenic factor. Certain observations indicate that in addition, ACTH has certain extra-adrenal effects on carbohydrate metabolism.

Westermeyer & Raben (1954) and Engel & Engel (1954), independently of each other, observed a fall in the blood sugar in intact and adrenalectomized mice and rats, 30 minutes to three and a half hours after injection of oxycel-purified ACTH.

These observations have been confirmed by others (Engel et al. 1958; Marshall et al. 1962; Genuth & Lebovitz 1965). The extra-adrenal hypoglycaemic effect seems to be due to an increased insulin production brought about by a direct stimulating influence on the islets of Langerhans by ACTH. Thus Engel et al. (1958) showed that oxycel-purified ACTH in intact and adrenalectomized rats produced a significantly increased glucose tolerance as well as an increase in dorsal brown adipose tissue glycogen.

Miller & Krake (1963) did not find any hypoglycaemic effect in alloxan-diabetic mice after AGTH injection. Genuth & Lebovitz (1965) confirmed this observation. On the other hand, in intact mice a hypoglycaemic effect is found simultaneously with a 5–10 times increase in immunological detectable plasma insulin, 15 minutes after the injection of ACTH. D-mannoheptulose, which is known to inhibit insulin secretion, partly masks the ACTH-induced hyperinsulinaemia. In vitro experiments with pancreas of mice indicate that ACTH has a direct stimulating effect on the release of insulin.

Similarly, Love et al. (1965) demonstrated an increase in immunological detectable plasma insulin in adrenalectomized rats 15 minutes after the injection of ACTH.

However, an extra-adrenal peripheral antagonism between ACTH and insulin has also been described. Westermeyer & Raben (1954) and Genuth & Lebovitz (1965) have found that ACTH inhibits the hypoglycaemic effect of insulin in adrenalectomized mice.

In intact mice and adrenalectomized rats where insulin secretion reaches a maximum by force-feeding and cortisone treatment Marshall et al. (1962) observed a hyperglycaemic response up to four hours after ACTH injection.

The purpose of the present work was to investigate the effect of a transient maximum ACTH stimulation on the blood sugar and plasma insulin, in normal fasting subjects as well as in fasting diabetics with or without hypercorticism.
In diabetics with hypercorticism, moreover, the plasma insulin response was measured during oral GTT.

**MATERIALS AND METHODS**

The material consisted of 9 normal subjects, 2 diabetics without hypercorticism and 3 diabetics with hypercorticism.

**I. Normal subjects**

This group consisted of 7 men and 2 women, aged 19–20. They were all of normal weight, without any endocrine disorders or familial history of diabetes mellitus.

**II. Case reports (see also Table 1)**

**Diabetics without hypercorticism**

A. H. Diabetes mellitus diagnosed 1½ years previously. No diabetic complications. Treatment: 1300 calories/24 h.


**Diabetics with hypercorticism**

S. Y. In 1960 suspicion of Cushing’s syndrome. In October 1964 diabetes mellitus was diagnosed. Diabetes was well regulated by diet only.

13. XII. 1965 admitted to Steno Memorial Hospital (S. M. H.) with typical signs of Cushing’s syndrome. Diabetes remained well regulated by diet only. X-rays showed sella turcica to be normal. Ophthalmic examination showed normal conditions. Treatment with dexamethasone for four days (according to Liddle (1960)) suppressed urinary 17-ketogenic steroids (17-KGS) to 6.0 mg/24 h.

E. G. November 1961 admitted to S. M. H. with Cushing’s syndrome. By radiological methods the presence of a left-sided adrenal tumour was established. The sella turcica was found to be normal. Ophthalmic examination showed normal conditions. 1. XII. 1961 (K. A. S. Gentofte) the adrenal cortex tumour was removed. Histological examination of the tumour: Carcinoma solidum corticale suprarenale (Prosector Dr. A. Søeborg Ohlsen). For the next two years the patient was in relatively good health. After 1963 increasing Cushing-like signs appeared again. 14. II. 1967 admitted to S. M. H. with marked symptoms of Cushing’s syndrome. Diabetes well regulated by diet only. The patient deteriorated rapidly and died on 5. IV. 1967. Post-mortem examination showed no tumour recurrence on the left adrenal gland site. Right adrenal gland was atrophic. In the right lung, tumour tissue, on the pleura and the diaphragm was found of precisely the same histological character as in the adrenal tumour found in 1961.

G. V. November 1965 admitted to a dermatological ward because of hirsutism. As a test she was treated from 5. I. 1966 to 24. V. 1966 with 15 mg cortisone daily; from 16. VI. 1966 to 12. VII. 1966 with 2 mg dexamethasone daily, and from 12. VII. 1966 to 8. IX. 1966 with 4 mg dexamethasone daily. During this treatment clinical symptoms
Table 1.
Some data on patients with diabetes mellitus.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age in years</th>
<th>Height in cm</th>
<th>Weight in kg</th>
<th>Mean value and range in mg/24 h of 17-ketogenic steroids (17-KGS)*</th>
<th>Blood pressure in mm Hg</th>
<th>Glucose tolerance test</th>
</tr>
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<tbody>
<tr>
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<td></td>
<td>17-ketosteroids (17-KS)**</td>
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<tr>
<td>without hypercorticism</td>
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</tr>
<tr>
<td>A. H.</td>
<td>♂</td>
<td>45</td>
<td>172</td>
<td>83.1</td>
<td>9.3 (7.5–11.1) (n = 2)</td>
<td>125/80</td>
<td>diabetic</td>
</tr>
<tr>
<td>R. W.</td>
<td>♂</td>
<td>64</td>
<td>158</td>
<td>77.6</td>
<td>5.2 (3.3–7.0) (n = 2)</td>
<td>145/70</td>
<td>diabetic</td>
</tr>
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<td></td>
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</tr>
<tr>
<td>Diabetics</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>S. Y.</td>
<td>♂</td>
<td>51</td>
<td>158</td>
<td>68.0</td>
<td>20.0 (25.2–14.8) (n = 2)</td>
<td>180/110</td>
<td>diabetic</td>
</tr>
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<tr>
<td>E. C.</td>
<td>♂</td>
<td>50</td>
<td>158</td>
<td>62.7</td>
<td>36.9 (33.0–40.8) (n = 2)</td>
<td>165/90</td>
<td>diabetic</td>
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<tr>
<td>with hypercorticism</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>sept. 1966</td>
<td>♂</td>
<td>23</td>
<td>174</td>
<td>59.8</td>
<td>18.1 (n = 1)</td>
<td>130/70</td>
<td>diabetic</td>
</tr>
<tr>
<td>G. V.</td>
<td>♂</td>
<td>23</td>
<td>174</td>
<td>59.0</td>
<td>24.2 (n = 1)</td>
<td>130/70</td>
<td>normal</td>
</tr>
<tr>
<td>jan. 1967</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
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</tr>
</tbody>
</table>

characteristic of Cushing's syndrome developed. 7. IX. 1966 admitted to S. M. H. Corticosteroid treatment was discontinued. X-rays showed sella turcica to be normal. Ophthalmic examination showed normal conditions. X-rays showed the thorax to be normal. Treatment with dexamethasone for four days (according to Liddle (1960)) suppressed urinary 17-KGS to 4.8 mg/24 h. 27. I. 1967 admitted to S. M. H. Cushing characteristics and signs were rapidly diminishing.

**Procedure**

In nine normal subjects, two diabetics (A. H. and R. W.), two patients with Cushing's syndrome (S. Y. and E. C.) and in one glucocorticoid-treated patient (G. V.) ACTH stimulation was carried out. All the patients were fasting, 4 patients (cases no. 8, 9, R. W. and G. V.) had, however, eaten lunch between 1 p. m. and 2 p. m. 

0.25 mg $\beta^{1-24}$ corticotrophin $30.920 \text{Ba}^*$ dissolved in 500 ml 0.9 % NaCl was given as an intravenous drip from 9 a. m. until 1 p. m. Heparinized blood for the determination of plasma insulin and for the fluorimetric determination of corticosteroids was taken by venepuncture at hour zero (9 a. m.) and at each successive complete hour. the final occasion being at 2 p. m. At the same time capillary blood was taken from the ear for blood sugar determinations.

**Methods**

The blood sugar was determined in duplicate according to the method of Hagedorn et al. (1946). Plasma corticosteroid determination was carried out by a fluorimetric method according to De Moor & Steeno (1963), slightly modified by Nielsen & Asfeldt (1967).

Plasma insulin concentration was measured as immunologically detectable insulin by the method of Hales & Randle (1963) using $^{125}$I-insulin or $^{131}$I-insulin as tracer (see Jørgensen (1966) and Brunfeldt & Jørgensen (1967)).

Reproducibility of the insulin assay calculated from duplicate determinations, expressed as S. E. M. for double determinations on plasma samples were 1.3, 1.3, 1.5, 2.6 and 6.7 $\mu$units/ml corresponding to concentration ranges of 7.8–15.6, 15.6–31.3, 31.3–62.5, 62.5–125 and 125–250 $\mu$units/ml. The recovery of crystalline pig insulin added to the plasma was found to be 98–105 % (Jørgensen, to be published).

**RESULTS**

I. Normal subjects

Plasma insulin, blood sugar and fluorimetrically determined corticosteroids during stimulation with $\beta^{1-24}$ corticotrophin are shown in Table 2. Fig. 1 shows the mean values ± S. E. M.

Stimulation of the adrenal cortex with $\beta^{1-24}$ corticotrophin gave a significant response, as expressed by the rise in the fluorimetrically determined plasma corticosteroids.

Fasting blood sugar values before and during the transient maximum adrenal cortex stimulation were all normal. No significant change in blood sugar

* made available by courtesy of A/S Ciba.
Table 2.
Plasma insulin, fluorimetric corticosteroids and blood sugar under ACTH-stimulation.

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Sex</th>
<th>Plasma insulin (μunits/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>a. m.</td>
</tr>
<tr>
<td>1.</td>
<td>♂</td>
<td>14</td>
</tr>
<tr>
<td>2.</td>
<td></td>
<td>21</td>
</tr>
<tr>
<td>3.</td>
<td></td>
<td>16</td>
</tr>
<tr>
<td>4.</td>
<td></td>
<td>15</td>
</tr>
<tr>
<td>5.</td>
<td></td>
<td>24</td>
</tr>
<tr>
<td>6.</td>
<td></td>
<td>12</td>
</tr>
<tr>
<td>7.</td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>8.</td>
<td>♀</td>
<td>22</td>
</tr>
<tr>
<td>9.</td>
<td></td>
<td>12</td>
</tr>
</tbody>
</table>

Mean values
S. E. M.
15.56  16.50  15.22  15.11  14.67  14.00
2.06   2.95   1.91   2.11   2.74   1.57

Diabetics without hypercorticisim

A. H.  ♀  23  24  26  27  34  23
R. W.  ♀  38  52  30  37  37  73*

Diabetics with hypercorticisim

S. Y.  ♀  12 12 17*
E. C.  -  20 20 21 21 20 19
G. V.  -  11 14 10 9 14 114*

* Non-fasting values. Plasma insulin values at 2 p.m. in cases no. 8 and 9 are not included in calculation of mean value.

during adrenal stimulation is seen (Fig. 1). By Student’s t-test on mean values it is found that \( P > 0.1 \); the exception being the mean values for 10 a.m. and 2 p.m. where \( 0.1 > P > 0.05 \).

Plasma insulin concentrations in the 9 normal fasting subjects were all within the normal range (6–26 μunits/ml, found by Jørgensen, to be published). Plasma insulin at 2 p.m. in cases no. 8 and 9 were, however, raised as expected as these patients had eaten shortly after 1 p.m. There is no significant variation in plasma insulin during transient maximum adrenal cortex stimulation \( (P > 0.1) \).

II. Diabetics without hypercorticisim

A. H. and R. W. (Table 1) were both overweight and had maturity onset diabetes. The response in the fluorimetrically determined corticosteroids during
Table 2.
Plasma insulin, fluorimetric corticosteroids and blood sugar under ACTH-stimulation.

<table>
<thead>
<tr>
<th>Fluorimetric plasma corticosteroids (μg/100 ml)</th>
<th>Blood sugar (mg/100 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>09 a.m. 10 a.m. 11 a.m. 12 a.m. 01 p.m. 02 p.m.</td>
<td>09 a.m. 10 a.m. 11 a.m. 12 a.m. 01 p.m. 02 p.m.</td>
</tr>
<tr>
<td>16 27 31 32 39 37</td>
<td>96 88 80 84 93 93</td>
</tr>
<tr>
<td>13 29 36 43 44 49</td>
<td>90 82 80 84 90 98</td>
</tr>
<tr>
<td>21 34 41 42 44 49</td>
<td>82 76 84 80 80 93</td>
</tr>
<tr>
<td>15 32 37 39 44 46</td>
<td>93 94 93 96 94 94</td>
</tr>
<tr>
<td>19 29 33 38 43 41</td>
<td>86 88 98 88 100 88</td>
</tr>
<tr>
<td>25 40 45 46 52 57</td>
<td>17.50 31.22 37.22 40.67 45.89 46.11</td>
</tr>
<tr>
<td>19 35 38 43 46 46</td>
<td>1.55 1.73 2.03 2.20 2.85 2.49</td>
</tr>
<tr>
<td>20 33 46 52 67 58</td>
<td>89.40 85.60 87.00 86.40 91.40 93.20</td>
</tr>
<tr>
<td>9.5 22 28 31 34 32</td>
<td>1.00 1.00 1.00 1.00 1.00 1.00</td>
</tr>
</tbody>
</table>

stimulation with β1-24 corticotrophin is in agreement with the response in the 9 normal subjects.

Fasting plasma insulin was normal in A. H. and increased in R. W. Fasting blood sugar was elevated in both diabetics. No definite variation in plasma insulin and blood sugar during stimulation with β1-24 corticotrophin is seen.

**Diabetics with hypercorticism**

For results see Table 1, 2, and 3. A graphic reproduction of the variations in blood sugar and plasma insulin during oral GTT in G. V. and E. C. is seen in Fig. 2.

S. Y. and E. C. had typical steroid diabetes secondary to Cushing’s syndrome with adrenal cortex hyperplasia (S. Y.) and adrenal cortex carcinoma (E. C.). In S. Y. Cushing’s syndrome had been present for 1–2 years. Fasting plasma

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Mean values ± S. E. M. of plasma insulin, blood sugar and fluorimetrically determined corticosteroids in normal subjects during ACTH stimulation.

**Fig. 1.**

Table 3.
Plasma insulin and blood sugar during oral glucose tolerance test.

<table>
<thead>
<tr>
<th></th>
<th>Plasma insulin (μunits/ml)</th>
<th>Blood sugar (mg/100 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>min</td>
<td>min</td>
</tr>
<tr>
<td>0</td>
<td>15  30  60  90  120  160</td>
<td>0  15  30  60  90  120  150</td>
</tr>
<tr>
<td>G. V. 6. X. 1966</td>
<td>21  39  90  197  139  190  146</td>
<td>103  125  161  187  154  148  118</td>
</tr>
<tr>
<td>31. I. 1967</td>
<td>12  46  107  111  92  46  53</td>
<td>77  105  121  146  99  95  86</td>
</tr>
<tr>
<td>E. C. 1961</td>
<td>16  34  32  35  40  42  39</td>
<td>83  140  167  198  175  167  147</td>
</tr>
<tr>
<td>1967</td>
<td>16  34  32  35  40  42  39</td>
<td>88  132  167  212  252  246  209</td>
</tr>
</tbody>
</table>

Insulin was normal while, on the other hand, fasting blood sugar was elevated. The increase in the fluorimetrically determined corticosteroids during stimulation with β1-24 corticotrophin was abnormally high. However, no variation in plasma insulin and blood sugar was found.

The Cushing's syndrome in E. C. had been present for at least six years.

On stimulation with β1-24 corticotrophin a minimal rise in plasma cortico-
Plasma insulin and blood sugar during oral glucose tolerance test before and after corticosteroid treatment (G. V.) and in a case of Cushing's syndrome (E. C.).

steroid was observed, which is characteristic for patients with carcinoma of the adrenal cortex. No variation in plasma insulin and blood sugar during stimulation was found.

From 1961 to 1967 (Fig. 2) GTT had clearly changed in a more pathological direction. The response in plasma insulin was in agreement with that seen in the low insulin output types of diabetes (Berson & Yalow 1965).

Under treatment with corticosteroids in increasing doses (biologically equivalent to up to 120 mg cortisone daily) clinical symptoms of Cushing's syndrome were aggravated in G. V. GTT on 6. X. 1966 (28 days after the termination of the corticosteroid treatment) was diabetic in type.

During GTT an abnormally high rise and a delayed fall in plasma insulin was found, i.e. a condition similar to the response to GTT in high insulin output types of diabetes (Berson & Yalow 1965). About 4½ months after the termination of corticosteroid treatment (31. I. 1967) the blood sugar and plasma insulin response during GTT was normal.

**COMMENTS AND DISCUSSION**

While in the abovementioned animal experiments genuine corticotrophin (39 amino-acids) was used, in the present experiments use was made of synthetic corticotrophin consisting of N-terminal amino-acids 1–24. Comparative ex-
periments in humans show, however, that there is no significant difference between the effect of naturally occurring corticotrophin and that of $\beta^{1-24}$ corticotrophin on urinary 17-ketogenic steroids, 17-ketosteroids and plasma corticosteroids (Karl 1963; Landon et al. 1964; Lamberg et al. 1966). The synthetic $\beta^{1-24}$ corticotrophin has a biological activity equivalent to 106 IU/mg (Schuler et al. 1963) when given subcutaneously.

The abovementioned effect of the biologically produced ACTH was observed in animal experiments with non-physiological large doses. In the present investigation a more physiological dosage of the synthetic $\beta^{1-24}$ corticotrophin was used. With $\beta^{1-24}$ corticotrophin in doses larger than 0.25 mg/4 hours a greater response in plasma corticosteroids was not obtained. Half the dose is probably sufficient to obtain the maximum response (Landon et al. 1964; Asfeldt & Nielsen 1965).

A possible effect on blood sugar and plasma insulin of the transient maximum ACTH stimulation may be the sum of the extra-adrenal and of the adrenal effect of $\beta^{1-24}$ corticotrophin.

In normal subjects, diabetics without hypercorticism and diabetics with hypercorticism there was no variation during transient maximum ACTH stimulation. The organotrophic effect (transient increased corticosteroid production) or the above described extra-adrenal effect of ACTH within the short period in which ACTH stimulation occurred had, in consequence, scarcely any physiological significance with regard to the regulation of blood sugar. It appears therefore hardly probable that transient alterations in the activity of the pituitary-adrenal axis during fasting plays any part in the regulation of blood sugar.

The long-term effects of corticosteroids, however, manifests themselves in reduced glucose tolerance (Fajans et al. 1954). It is known that long-continued treatment with glucocorticosteroid produces islet hyperplasia in a number of experimental animals (Franckson et al. 1953; Hausberger & Ramsay 1953; Volk & Lazarus 1959; Vranic 1965). Corresponding conditions have been described after the injection of anterior pituitary extracts in dogs (Richardson & Young 1938), and of ACTH in rats (Kinash & Haist 1954).

An increased proportion of $\beta$ to $\alpha$ cells indicates that the proliferation of the islet $\beta$ cells is greater than that of the other islet cell types. The $\beta$ cells are found enlarged and degranulated, which indicates the marked hyperfunction of these cells (Hausberger & Ramsay 1953; Vranic 1965).

This explains the diabetic GTT with increased plasma insulin response in G. V. (Table 3; Fig. 2) after about 7 months' treatment with glucocorticosteroids. The condition at this point is, however, reversible in that the GTT and the insulin response is normalized about 4 1/2 months after termination of the glucocorticosteroid treatment.

In experiments in which adrenal cortex steroids produce marked hyper-
glycaemia – diabetes mellitus – degeneration of the β cells has been demonstrated (Richardson & Young 1938; Hausberger & Ramsay 1953). In dogs with diabetes produced by anterior pituitary extracts, Best et al. (1939) found a reduced content of extractable insulin in the pancreas. The abovementioned histological changes in the islet tissue related to the reduced content of the extractable insulin in the pancreas indicates an exhaustion of the β cells caused by over-work.

E.C. (Table 3; Fig. 2) is typical of this development. Even in 1961 the GTT was diabetic. After 6 years of massive hypercorticism the GTT showed deterioration and at that time a hypofunction of the β cells clearly occurred, in so far as a poor rise could only be registered in plasma insulin during oral GTT.

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