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CLINICAL STUDY

Decreased steroidogenic enzyme 17,20-lyase and increased 17-hydroxylase activities in type 2 diabetes mellitus

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Abstract

Objective: To analyze activities of adrenal steroidogenic enzymes in type 2 diabetes mellitus, serum levels of 11 steroid hormones were measured simultaneously.
Subjects: We studied 130 patients with type 2 diabetes mellitus (74 men and 56 women between the ages of 40 and 69 years), whose blood glucose control had been poor (more than 10% in HbA_{1c}). Age-matched normal subjects served as the control group.
Methods: Serum levels of steroid hormones (pregnenolone (Preg), progesterone (Prog), deoxycorticosterone (DOC), corticosterone (B), 17-hydroxypregnenolone (17-OH-Preg), 17-hydroxyprogesterone (17-OHP), 11-deoxycortisol (S), cortisol (F), dehydroepiandrosterone (DHEA) and Δ4-androstenedione (Δ4A)) were measured by HPLC/RIA methods. Fasting plasma glucose (FPG), HbA_{1c}, ACTH, serum immunoreactive insulin (IRI) and DHEA sulfate (DHEA-S) were also measured. We analyzed product/precursor ratios to assess relative activities of adrenal steroidogenic enzymes.
Results: Serum levels of ACTH and F were high and DHEA and DHEA-S were low in both male and female patients under poor blood glucose control. Following 6-months treatment with diet only or with sulfonylurea, FPG and HbA_{1c} improved, and blood concentrations of ACTH and F decreased while DHEA and DHEA-S levels increased to within the normal range. DHEA/17-OH-Preg and Δ4A/17-OHP ratios, reflecting 17,20-lyase activity, were low before treatment and recovered to the normal range after treatment, and 17-OH-Preg/Preg and 17-OHP/Prog ratios, reflecting 17-hydroxylase activity, were high before treatment, and fell within the normal range after treatment. 3β-Hydroxysteroid dehydrogenase, 21-hydroxylase and 11β-hydroxylase activities remained within the normal range both before and after treatment.
Conclusions: These data suggest that the decrease in DHEA and DHEA-S concentrations together with the high F levels that occur in patients with type 2 diabetes mellitus is associated with low 17,20-lyase and high 17-hydroxylase activity in the adrenal steroidogenic enzymes. High insulin concentrations may further lower DHEA and DHEA-S levels.

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Introduction

Abnormalities of secretion and metabolism of many steroid hormones occur in diabetes mellitus. In poorly controlled insulin-dependent diabetes mellitus (IDDM), serum concentrations of dehydroepiandrosterone (DHEA) and its sulfate (DHEA-S) decrease (1) while plasma adrenocorticotropin (ACTH) and cortisol levels increase in non-insulin-dependent diabetes mellitus (NIDDM) (2). Low levels of DHEA and DHEA-S in type 2 diabetes mellitus are associated with hyperinsulinemia (3–7). Most studies have analyzed any one of glucocorticoid, mineralocorticoid or sex steroids. In the present study, to investigate activities of adrenal steroidogenic enzymes in type 2 diabetes mellitus we measured the serum levels of 11 steroid hormones simultaneously (8), and the analysis of product/precursor ratios was used to measure indirectly the relative activities of adrenal steroidogenic enzymes in type 2 diabetic patients with respect to the degree of blood glucose control.

Materials and methods

Subjects

The subjects were type 2 diabetic patients seen regularly at the outpatient clinic of Toho University Hospital. We chose 130 patients, whose blood glucose control had been poor (more than 10% in HbA_{1c}).

Their medication was managed by diet only or with sulfonylurea and patients under insulin therapy were excluded. The patient group consisted of 74 men and 56 women between the ages of 40 and 69 years. Age-matched normal subjects served as the control group. Informed consent was obtained from each subject before the study. Clinical characteristics of the patients and the normal controls are described in Table 1.

Methods

Blood samples were obtained from patients with type 2 diabetes mellitus and normal subjects between 0900 and 1000 h after an overnight fast. From patients with type 2 diabetes mellitus, blood samples were obtained before and 6 months after the treatment. Serum levels of 11 steroid hormones (pregnenolone (Preg), progesterone (Prog), deoxycorticosterone (DOC), corticosterone (B), 17-hydroxypregnenolone (17-OH-Preg), 17-hydroxyprogesterone (17-OHP), 11-deoxycortisol (S), cortisol (F), DHEA, DHEA-S, Δ4-androstenedione(Δ4A)), plasma ACTH, fasting plasma glucose (FPG), HbA_{1c}, and serum immunoreactive insulin (IRI) were measured. Steroid hormones were determined by the previously reported HPLC/RIA methods (8) except DHEA-S, which was measured using an RIA kit (Mitsubisi Chemcal Co., Tokyo, Japan), FPG which was measured by the glucose oxidase method, HbA_{1c} which was measured by HPLC, and IRI and plasma ACTH which were measured by commercial kits (Daiichi, Tokyo, Japan).

Data are shown as means±S.D. Variables were compared by Bonferroni's analysis and *P*-values less than 0.05 were considered to indicate statistical significance.

Results

Compared with age-matched normal subjects, serum levels of DHEA and DHEA-S were low and those of F were high in both male and female patients with type 2 diabetes mellitus across the entire age range studied (Figs 1 and 2). Plasma levels of ACTH and IRI were high in all groups before the treatment (Table 1). Following 6-months treatment, FPG and HbA_{1c} improved and IRI decreased in most patients (Table 1). In parallel with the improvement in FPG and HbA_{1c}, blood concentrations of ACTH and F decreased, and DHEA and DHEA-S levels increased to within the normal range in all the groups (Figs 1 and 2). Product/precursor ratios in serum steroids as an index of the relative activities of steroidogenic enzymes are shown in Fig. 3 and Tables 2–4. In male and female patients with type 2 diabetes mellitus, both DHEA/17-OH-Preg and Δ4A/17-OHP ratios, reflecting 17,20-lyase activity, were low before treatment and recovered to the normal range after treatment (Fig. 3). Both 17-OH-Preg/Preg and 17-OHP/Prog ratios, reflecting 17-hydroxylase activity, were high in diabetic patients before treatment, and fell within the normal range

Table 1 Clinical characteristics of type 2 diabetic patients before and after treatment and in age-matched normal subjects.

	Number	**BMI** (kg/m^2)	**FPG** (mmol/l)	**HbA_{1c}** (%)	**IRI** (pmol/l)	**ACTH** (pmol/l)
Male 40 years						
Before treatment	22	25.1±1.5*	10.2±0.9*	11.6±1.2*	68.9±23.7*	7.9±2.0*
After treatment	22	24.2±1.2**	6.2±0.8**	7.2±0.6**	53.8±11.5**	5.5±1.8**
Normal	20	22.3±0.9	5.2±0.3	5.2±0.3	40.9±12.2	4.8±1.1
Male 50 years						
Before treatment	29	24.4±1.2*	9.6±1.0*	11.7±1.2*	72.5±23.0*	8.1±2.0*
After treatment	29	23.6±1.1	5.9±0.8**	6.8±0.6**	49.5±9.3**	6.9±2.1**
Normal	25	22.7±0.9	5.2±0.3	5.1±0.3	42.3±10.0	5.7±0.9
Male 60 years						
Before treatment	23	25.0±1.5*	9.8±1.1*	11.4±1.1*	75.3±30.9*	7.3±1.8*
After treatment	23	24.1±1.2	5.6±0.8**	6.7±0.6**	52.4±7.9**	6.2±1.7**
Normal	20	22.4±0.7	5.0±0.4	5.2±0.2	40.2±7.9	5.3±1.1
Female 40 years						
Before treatment	17	24.4±2.3*	9.6±0.9*	12.0±1.1*	54.5±17.2*	7.7±2.0*
After treatment	17	23.8±1.4	5.5±0.6**	7.0±0.6**	56.0±6.5**	5.5±1.4**
Normal	15	21.7±1.5	5.2±0.4	5.1±0.2	44.5±9.3	5.3±1.3
Female 50 years						
Before treatment	23	23.8±1.0*	9.2±0.9*	11.6±0.8*	65.3±26.5*	7.9±2.2*
After treatment	23	23.3±0.6	5.2±0.3**	6.7±0.4**	46.6±8.6**	6.0±1.3**
Normal	20	22.6±0.7	5.1±0.4	5.1±0.3	38.7±7.2	5.7±1.5
Female 60 years						
Before treatment	16	24.2±1.1*	9.7±1.1*	11.9±1.2*	59.6±21.5*	8.4±2.2*
After treatment	16	23.3±0.9**	5.4±0.5**	6.8±0.5**	48.8±10.8**	7.2±1.4
Normal	15	22.7±0.6	5.2±0.3	5.3±0.3	38.0±6.5	5.5±1.3

*$P < 0.05$ vs normal, **$P < 0.05$ vs before treatment.

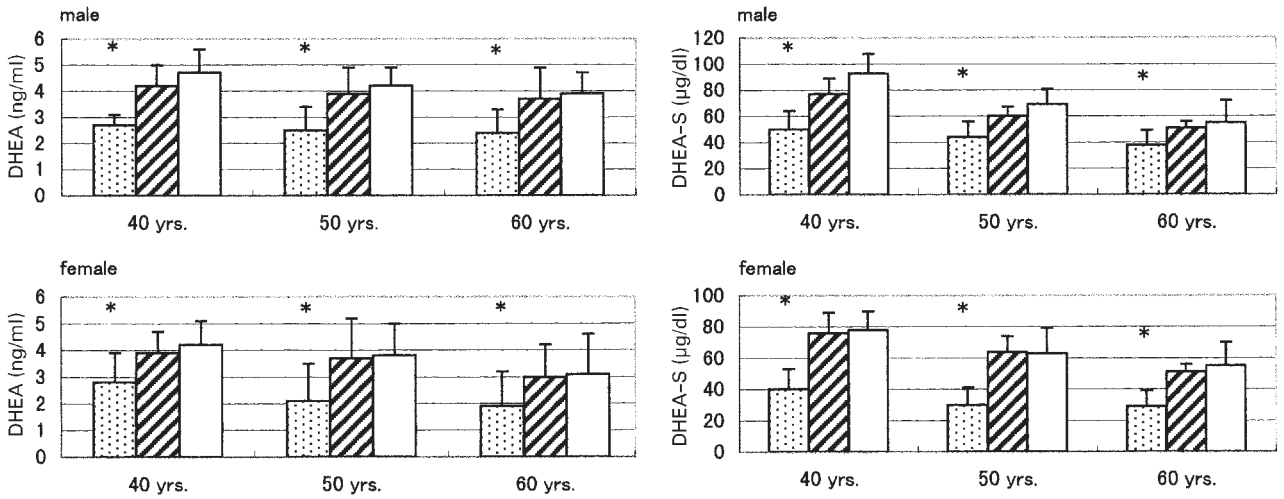

Figure 1 Serum DHEA and DHEA-S levels in male and female type 2 diabetic patients before (stippled bars) and after (hatched bars) treatment and in age-matched normal subjects (open bars). *$P < 0.05$ compared with values after treatment and with normal values.

after treatment (Fig. 3). 3β-Hydroxysteroid dehydrogenase activity assessed by Prog/Preg, 17-OHP/17-OH-Preg, and Δ4A/DHEA ratios (Table 2), 21-hydroxylase activity assessed by DOC/Prog and S/17-OHP ratios (Table 3) and 11β-hydroxylase activity assessed by B/DOC and F/S ratios (Table 4) remained within the normal ranges both before and after treatment.

Discussion

We have examined serum steroid hormone profiles in patients with type 2 diabetes mellitus under poor therapeutic control stratified by three decades in age. We have also assessed the enzymatic activities by analysis of product/precursor ratios of C-21 and C-19 steroid hormones in the steroidogenic pathway to determine how their ratios may change with the control of type 2 diabetes mellitus. As most previous studies were mainly concerned with steroid hormones in one of three steroidogenic pathways – to either cortisol,

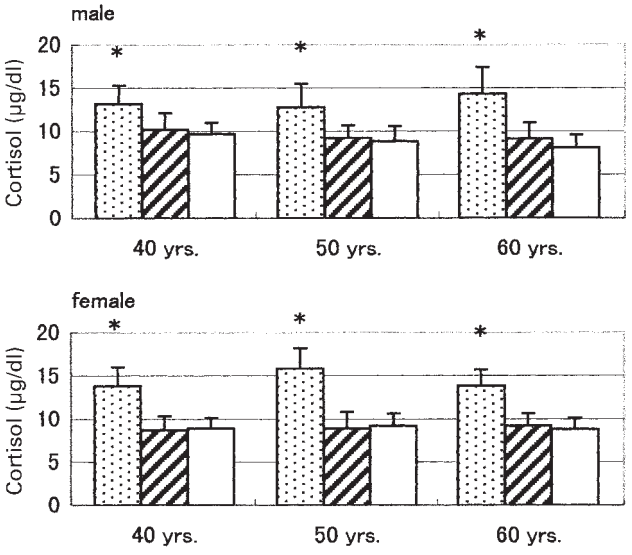

Figure 2 Serum cortisol levels in male and female type 2 diabetic patients before (stippled bars) and after (hatched bars) treatment and in age-matched normal subjects (open bars). *$P < 0.05$ compared with values after treatment and with normal values.

Table 2 3β-HSD activities assessed by Prog/Preg, 17-OHP/17-OH-Preg and Δ4A/DHEA ratios in male and female type 2 diabetic patients before and after treatment and in age-matched normal subjects.

	Prog/Preg	17-OHP/17-OH-Preg	Δ4A/DHEA
Male 40 years			
Before	0.22±0.09	1.08±0.25	0.40±0.18*
After	0.25±0.10	0.99±0.31	0.26±0.19
Normal	0.23±0.08	0.95±0.28	0.24±0.06
Male 50 years			
Before	0.27±0.11	1.21±0.19	0.26±0.13
After	0.26±0.12	1.03±0.28	0.25±0.18
Normal	0.15±0.10	0.98±0.31	0.26±0.10
Male 60 years			
Before	0.24±0.13	1.24±0.21	0.46±0.14*
After	0.25±0.19	0.98±0.29	0.29±0.17
Normal	0.21±0.12	0.96±0.32	0.26±0.13
Female 40 years			
Before	0.55±0.18	1.30±0.14	0.35±0.12
After	0.59±0.13	1.13±0.19	0.27±0.13
Normal	0.51±0.14	1.14±0.21	0.26±0.15
Female 50 years			
Before	0.56±0.21	1.42±0.18	0.44±0.14*
After	0.57±0.18	1.18±0.15	0.25±0.18
Normal	0.51±0.15	1.36±0.19	0.26±0.13
Female 60 years			
Before	0.48±0.18	1.22±0.14	0.45±0.19*
After	0.39±0.14	1.01±0.13	0.29±0.11
Normal	0.42±0.13	1.12±0.19	0.27±0.12

*$P < 0.05$ vs normal value and after treatment.
3β-HSD, 3β-hydroxysteroid dehydrogenase.

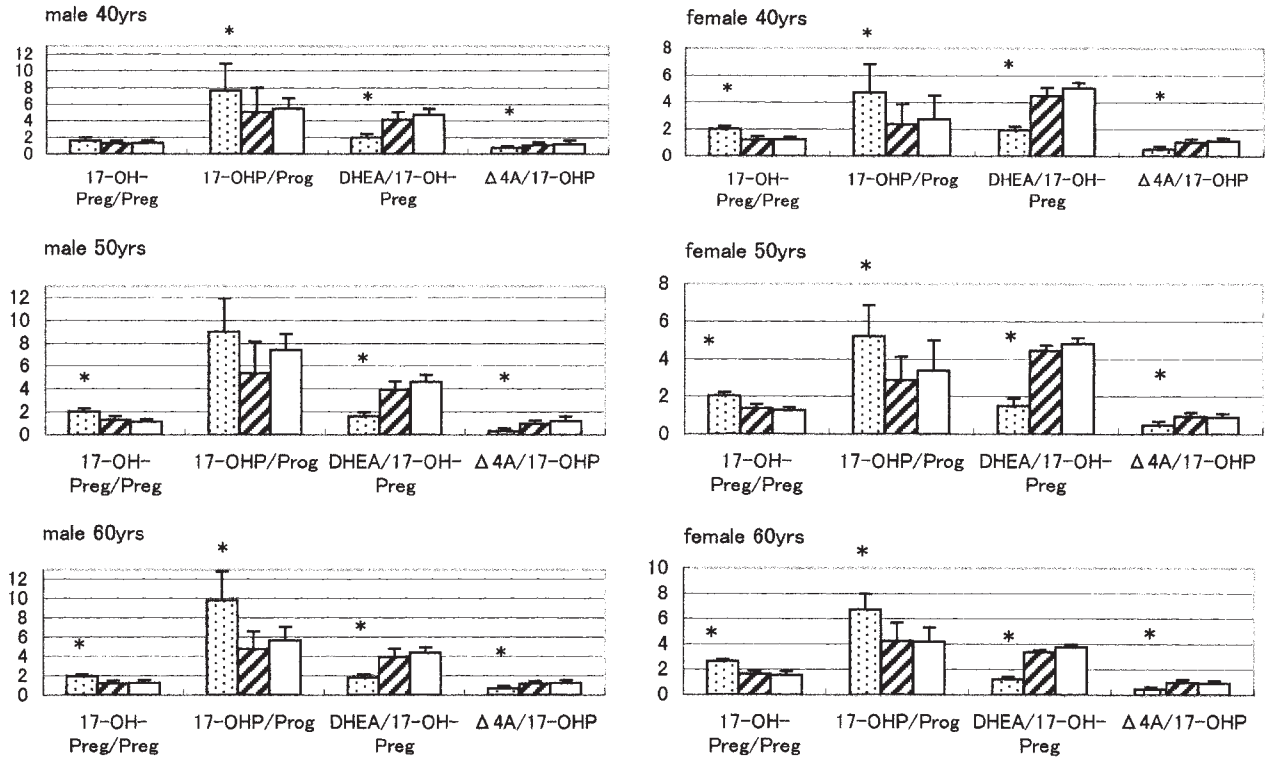

Fig. 3 17-Hydroxylase activities assessed by 17-OH-Preg/Preg and 17-OHP/Prog ratios and 17–20-lyase activities assessed by DHEA/17-OH-Preg and Δ4A/17-OHP ratios in male and female type 2 diabetic patients before (stippled bars) and after (hatched bars) treatment and in age-matched normal subjects (open bars). *$P < 0.05$ compared with values after treatment and with normal values.

Table 3 21-Hydroxylase activities assessed by DOC/Prog and S/17-OHP ratios in male and female type 2 diabetic patients before and after treatment and in age-matched normal subjects.

	DOC/Prog	S/17-OHP
Male 40 years		
Before	0.29±0.18	0.88±0.24
After	0.32±0.13	0.85±0.31
Normal	0.35±0.14	0.81±0.23
Male 50 years		
Before	0.32±0.21*	0.71±0.23
After	0.38±0.18	0.90±0.28
Normal	0.56±0.19	0.97±0.27
Male 60 years		
Before	0.28±0.12	0.85±0.21
After	0.26±0.14	0.96±0.24
Normal	0.37±0.18	0.92±0.18
Female 40 years		
Before	0.13±0.09	0.83±0.19
After	0.14±0.11	0.91±0.18
Normal	0.17±0.12	0.86±0.15
Female 50 years		
Before	0.15±0.10	0.75±0.21
After	0.19±0.13	0.95±0.14
Normal	0.19±0.14	0.81±0.19
Female 60 years		
Before	0.18±0.09	0.73±0.19
After	0.28±0.12	1.02±0.16
Normal	0.23±0.14	0.91±0.21

*$P < 0.05$ vs normal.

Table 4 11β-Hydroxylase activities assessed by B/DOC and F/S ratios in male and female type 2 diabetic patients before and after treatment and in age-matched normal subjects.

	B/DOC	F/S
Male 40 years		
Before	25.9±10.3	10.2±2.9
After	22.2±10.9	11.7±2.4
Normal	25.3±9.7	12.7±2.1
Male 50 years		
Before	21.9±9.4	9.5±3.1
After	18.8±8.8	10.0±2.1
Normal	20.8±9.2	10.1±2.4
Male 60 years		
Before	27.9±8.8	10.1±2.7
After	25.4±9.4	10.3±1.9
Normal	23.0±7.9	10.2±2.1
Female 40 years		
Before	26.7±7.4	8.8±1.9
After	25.1±9.8	9.7±2.4
Normal	24.6±8.3	10.8±2.3
Female 50 years		
Before	23.1±8.2	10.6±2.1
After	21.8±7.8	9.5±1.4
Normal	22.2±8.8	10.4±1.6
Female 60 years		
Before	24.4±6.9	10.0±1.8
After	21.0±8.2	10.1±2.4
Normal	22.6±7.9	10.5±2.1

aldosterone or DHEA, this is the first report describing simultaneous analyses of steroid hormones of three different steroidogenic pathways in type 2 diabetes mellitus. In this study we demonstrated that serum DHEA and DHEA-S levels decreased markedly under poor control of type 2 diabetes mellitus and increased to age-matched normal values with the improvement of FPG and HbA_{1c} after 6 months treatment with diet and/or sulfonylurea. Barrett-Connor showed that DHEA and DHEA-S levels were also low in patients with non-insulin dependent diabetes mellitus (9), but she did not measure changes in these steroid hormones after treatment. Markedly reduced levels of DHEA and DHEA-S in type 2 diabetes mellitus under poor therapeutic control, with slightly increased plasma IRI are consistent with an association between DHEA synthesis and/or metabolism and insulin. Nestler and colleagues showed that insulin reduces serum DHEA and DHEA-S in men either by inhibiting their production or by increasing the metabolic clearance rate of DHEA (10, 11). The metabolic clearance rate of DHEA is reported to be increased two- to fivefold in obesity and in the insulin-resistant, hyperinsulinemic state (11). The infusion of high doses of insulin reduces serum DHEA levels, suggesting the involvement of the inhibition of adrenal 17,20-lyase activity. Administration of metformin, which inhibits hepatic glucose production and enhances peripheral tissue sensitivity to insulin, to healthy normal weight men and to obese men with hypertension but without diabetes mellitus decreased serum insulin levels and increased serum DHEA-S levels in obese men with hypertension and in healthy controls (12). Our data showing that both the DHEA/17-OH-Preg and the Δ4A/17-OHP ratios, reflecting 17,20-lyase activity, were low in patients with type 2 diabetes mellitus before treatment and recovered to the normal range after treatment are consistent with these reports. However, Yamauchi *et al.* reported that serum DHEA and DHEA-S levels are low even in patients with impaired glucose tolerance and low insulin response (13), and therefore the decrease in serum DHEA levels may not arise exclusively from the hyperinsulinemic state. Hyperglycemia may reduce 17,20-lyase activity and consequently serum DHEA may decrease. The improvement in plasma glucose control parallels the recovery of 17,20-lyase activity. When micronized DHEA (50 mg) was administered to healthy postmenopausal women, DHEA significantly enhanced insulin sensitivity over the placebo, attenuating the age-related decline in glucose tolerance (14). We found that both F and ACTH levels were high in patients with type 2 diabetes mellitus and they returned to normal with improvement in glycemic control. As both the 17-OH-Preg/Preg and 17-OHP/Prog ratios, reflecting 17-hydroxylase activity, were high in most patients with type 2 diabetes mellitus, the increase in this enzyme may produce the increased F secretion. The increase in ACTH and F was reported to correlate positively with the duration of diabetes mellitus (15) and with the degree of complications such as neuropathy (16) and retinopathy (17). It seems likely that hyperglycemia in patients with type 2 diabetes mellitus is also responsible for the relative hypersecretion of ACTH and cortisol due to a hyperactive hypothalamic–pituitary–adrenal (HPA) axis. It has been suggested that patients with type 2 diabetes mellitus have abnormalities in the HPA axis (2, 15), especially abnormalities in negative feedback regulation by cortisol at the pituitary level due to some metabolic disorders (3). Recent studies show the relationship between the HPA axis and insulin resistance syndrome (18, 19). Obese patients with visceral adiposity have a hyperactive HPA axis. In this study some patients were obese; however the body mass index in most patients did not change significantly before and after treatment; therefore the relationship between the HPA axis and visceral adiposity is unclear.

In conclusion, the data collectively suggest that the decrease in DHEA and DHEA-S concentrations together with the high IRI and F levels that occur in patients with type 2 diabetes mellitus is associated with low 17,20-lyase and high 17-hydroxylase activities in the steroidogenic enzymes. High insulin concentrations may further lower DHEA and DHEA-S concentrations.

References

1 Couch RM. Dissociation of cortisol and adrenal androgen secretion in poorly controlled insulin-dependent diabetes mellitus. *Acta Endocrinologica* 1992 **127** 115–117.

2 Hashimoto K, Nishioka T, Takao T & Numata Y. Low plasma corticotropin-releasing hormone (CRH) levels in patients with non-insulin dependent diabetes mellitus (NIDDM). *Endocrine Journal* 1993 **40** 705–709.

3 Nesler JE, Usiskin KS, Barlascini CO, Welty DF, Clore JN & Blackard WG. Supression of serum dehydroepiandrosterone sulfate levels by insulin: an evaluation of possible mechanisms. *Journal of Clinical Endocrinology and Metabolism* 1989 **69** 1040–1046.

4 Diamond MP, Grainger DA, Laudano AJ, Starick-Zych K & DeFronzo RA. Effect of acute physiological elevations of insulin on circulating androgen levels in nonobese women. *Journal of Clinical Endocrinology and Metabolism* 1991 **72** 883–887.

5 Hubert GD, Schriock ED, Givens JR & Buster JE. Suppression of circulating Δ4-androstenedione and dehydroepiandrosterone sulfate during oral glucose tolerance in normal females. *Journal of Clinical Endocrinology and Metabolism* 1991 **73** 781–784.

6 Smith S, Ravnikar VA & Barbieri RL. Androgen and insulin response to an oral glucose challenge in hyperandrogenic women. *Fertility and Sterility* 1987 **48** 72–77.

7 Schriock ED, Buffington CK, Hubert GD, Kurtz BR, Kitabchi AE, Buster JE *et al.* Divergent correlation of circulating dehydroepiandrosterone sulfate and testosterone with insulin levels and insulin receptor binding. *Journal of Clinical Endocrinology and Metabolism* 1988 **66** 1329–1331.

8 Ueshiba H, Segawa M, Hayashi T, Miyachi Y & Irie M. Serum steroid hormones in patients with Cushing's syndrome determined by a new HPLC/RIA method. *Clinical Chemistry* 1991 **37** 1329–1333.

9 Barrett-Connor E. Lower endogenous androgen levels and dyslipidemia in men with non-insulin dependent diabetes mellitus. *Annals of Internal Medicine* 1992 **117** 807–811.

10 Nestler JE, McClanahan MA, Clore JN & Blackard WG. Insulin inhibits adrenal 17,20-lyase activity in men. *Journal of Clinical Endocrinology and Metabolism* 1992 **74** 362–367.
11 Nestler JE. Regulation of human dehydroepiandrosterone metabolism by insulin. *Annals of the New York Academy of Sciences* 1995 **774** 73–81.
12 Nestler JE, Beer NA, Jakubowicz DJ & Beer RM. Effects of a reduction in circulating insulin by metformin on serum dehydroepiandrosterone sulfate in nondiabetic men. *Journal of Clinical Endocrinology and Metabolism* 1994 **78** 549–554.
13 Yamauchi A, Takei I, Nakamoto S, Ohashi N, Kitamura Y, Tokui M *et al.* Hyperglycemia decreased dehydroepiandrosterone in Japanese male with impaired glucose tolerance and low insulin response. *Endocrine Journal* 1996 **43** 285–290.
14 Bates GW Jr, Egerman RS, Unstot ES, Buster JE & Casson PR. Dehydroepiandrosterone attenuated study-induced declines in insulin sensitivity in postmenopausal women. *Annals of the New York Academy of Sciences* 1995 **774** 291–293.
15 Roy M, Collier B & Roy A. Hypothalamic–pituitary–adrenal dysregulation among diabetic outpatients. *Psychiatry* 1990 **31** 31–37.
16 Tsigos C, Young RJ & White A. Diabetic neuropathy is associated with increased activity of the hypothalamic–pituitary–adrenal axis. *Journal of Clinical Endocrinology and Metabolism* 1993 **76** 554–558.
17 Lu J, Li J, Pan C, Lu S, Ma S & Gao A. Changes of pituitary–adrenal functions in diabetics and their response to aminogluthetimide. *Chinese Medical Journal* 1988 **101** 587–590.
18 Vicennati V & Pasquali R. Abnormalities of the hypothalamic–pituitary–adrenal axis in nondepressed women with abdominal obesity and relations with insulin resistance: evidence for a central and a peripheral alteration. *Journal of Clinical Endocrinology and Metabolism* 2000 **85** 4093–4098.
19 Ljung T, Holm G, Friberg P, Andersson B, Bengtsson BA, Svensson J, Dallman M, McEwen B & Bjorntorp P. The activity of the hypothalamic–pituitary–adrenal axis and the sympathetic nervous system in relation to waist/hip circumference ratio in men. *Obesity Research* 2000 **8** 487–495.

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