

Lower Adrenocortical and Adrenomedullary Responses to Hypoglycemia in Premenopausal Women with Systemic Sclerosis

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ABSTRACT. *Objectives.* To evaluate function of the hypothalamic-pituitary-adrenal (HPA) axis, adrenomedullary hormonal system (AMHS), and sympathetic noradrenergic system (SNS) in premenopausal women with systemic sclerosis (SSc).

Methods. Insulin-induced hypoglycemia (0.1 IU/kg) was performed in 17 longterm, glucocorticoid-naive SSc patients with low disease activity and in 18 healthy women matched for age and body mass index (BMI). Concentrations of glucose, adrenocorticotrophic hormone (ACTH), cortisol, androstenedione (ASD), dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulfate (DHEAS), 17 α -hydroxyprogesterone (17OHP), epinephrine (EPI), norepinephrine (NE), interleukin 1 β (IL-1 β), IL-6, and tumor necrosis factor- α (TNF- α) were analyzed in plasma.

Results. Basal plasma levels of cortisol, ASD, 17OHP, DHEAS, IL-1 β , IL-6, and TNF- α were not significantly different in SSc compared to controls. Patients had higher basal ACTH (6.76 ± 1.0 pmol/l in SSc vs 4.14 ± 0.45 pmol/l in controls; $p < 0.05$), lower basal DHEA (9.02 ± 1.64 nmol/l in SSc vs 17.0 ± 2.8 nmol/l in controls; $p < 0.05$), and lower basal NE (1.61 ± 0.26 nmol/l in SSc vs 2.57 ± 0.38 nmol/l in controls; $p < 0.05$). Patients had comparable responses of glucose and ACTH to hypoglycemia. General linear model for repeated measurements, with BMI and age as covariates, revealed that the responses of 17OHP ($p < 0.05$), ASD ($p < 0.05$), DHEA ($p < 0.01$), EPI ($p < 0.001$), and NE ($p < 0.001$) to hypoglycemia were lower in SSc compared to controls. Cortisol response to hypoglycemia tended to be lower in SSc patients ($p = 0.06$) compared to controls.

Conclusion. Our data indicate decreased adrenocortical and adrenomedullary functions in premenopausal women with SSc. Whether the observed changes in the neuroendocrine system are secondary to chronic disease deserves further investigation. (J Rheumatol 2006;33:2235–41)

Key Indexing Terms:

SYSTEMIC SCLEROSIS

ADRENAL CORTEX HORMONES

HYPOGLYCEMIA

Systemic sclerosis (SSc) is a multisystem disorder characterized by functional and structural vasculopathy and inflammation, leading to fibrosis of internal organs and skin¹. It is generally thought that the neuroendocrine system exerts control over microvascular, immune, and connective tissues by means of immunomodulatory hormones^{2–4}. Thus, altered neuroendocrine function may represent an important factor in the complex pathogenesis of SSc.

Unlike other chronic inflammatory diseases with higher

prevalence in the population, such as rheumatoid arthritis (RA) or systemic lupus erythematosus, substantial data on neuroendocrine status of SSc patients are lacking. Nevertheless, lower levels of adrenal androgens hallmarking hypothalamic-pituitary-adrenal (HPA) findings in women with RA^{5–7} were also reported in women with SSc^{8,9}. In RA, a subclinical adrenal hypocompetence, as marked by lower adrenal androgen levels, is believed to permit enhanced immunoreactivity to escape from physiological control exerted by adrenal steroids over immune and microvascular tissues¹⁰. Subsequent failure of this important homeostatic mechanism may contribute to onset or progression of chronic inflammation not only in RA¹⁰ but also in other inflammatory diseases, including SSc.

Concerning reports of lower dehydroepiandrosterone sulfate (DHEAS) in women with SSc^{8,9}, it is clear that more complex evaluation of the HPA function is necessary to address the possible dysfunction of the HPA axis in women with SSc. In addition to a measurement of baseline concentration of adrenal steroids, HPA stimulation provides further important information on the axis function.

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We evaluated HPA function in patients with SSc using insulin-induced hypoglycemia, which was proved to be a well-controlled stimulus of the neuroendocrine system⁵.

Dysregulation of the autonomic nervous system (ANS) in SSc has been suspected since the initial description of SSc-associated paroxysmal vasoconstriction of the digits, a phenomenon first described by Raynaud¹¹. A recent review focusing on ANS perturbations in SSc described an ambiguous picture of cardiovascular, urogenital, skin, and eye autonomic function in SSc⁴. Surprisingly, data on function of the adrenomedullary hormonal system (AMHS), one of the principal components of the ANS, are lacking in SSc. Epinephrine (EPI), the main catecholamine of the adrenal medulla, and norepinephrine (NE), released from sympathetic nerve terminals and from the adrenal medulla, not only participate in regulation of vascular tonus but also modulate immune responses¹²⁻¹⁴. Therefore, an assessment of AMHS and sympathetic noradrenergic system (SNS) function during insulin-induced hypoglycemia may provide additional information on the complex neuroendocrine-immune-vascular network in SSc.

MATERIALS AND METHODS

Seventeen (n = 17) female patients fulfilling the criteria of LeRoy for SSc¹⁵ were studied. They were recruited from the National Institute of Rheumatic Diseases in Piestany, Slovakia. Eighteen (n = 18) healthy female subjects matched for age and body mass index (BMI), recruited from laboratory staff of the Institute of Experimental Endocrinology, Slovak Academy of Sciences, Bratislava, Slovakia, served as controls. Characteristics of all subjects are shown in Table 1. All patients and controls had negative history of diabetes or impaired glucose tolerance. The disease activity of SSc patients, assessed on the EULAR Systemic Sclerosis Activity Score¹⁶, was "inactive." All the SSc patients were positive for the following antibodies: antinuclear, anticentromere, and anti-Scl-70. No patient had been treated during the past 5 years with glucocorticoids or other drugs known to interfere with neuroendocrine function. Drugs used by SSc patients and the respective number of patients using each type of therapy were: metalcapase (D-penicillamine; 14 patients),

Table 1. Basic characteristics of patients with SSc and healthy controls. Data are mean (SEM) unless otherwise indicated. No patient had renal, muscle, or tendinous involvement at the time of the study.

	SSc	Controls
No. of subjects	17	18
Age, yrs	38.4 (2.7)	40.9 (1.6)
Body mass index, kg × m ²	21.8 (0.52)	22.6 (0.8)
Disease duration*, yrs	6.4 (2.6)	—
C-reactive protein, μg/ml**	2.15 (0.1–17.8)	1.47 (0.07–10.7)
TNF-α, pg/ml**	4.6 (0–178)	5.7 (0–48.3)
IL-6, pg/ml**	0 (0–121.9)	0 (0–6.8)
IL-1β, pg/ml**	0.66 (0–394)	0.39 (0–28.1)
Skin involvement, limited/diffuse	14/3	—
Joint involvement	7	—
Radiographic abnormalities	8	—
Esophageal involvement	7	—
Lung involvement	13	—
Heart involvement	3	—
Raynaud's phenomenon	17	—

* Time from occurrence of the first SSc symptoms. ** Median (range).

pentoxifylline, naftidrofuryl, xantinol, nitroglycerin or other vasodilatation drugs¹⁷, ginkgo biloba extract (5), nonsteroidal antiinflammatory drugs (7), calcium channel blockers (2), alendronate (2), azathioprine (1). The last dose of medication was administered 24 h prior to investigation. All subjects gave informed written consent, and the study was approved by the Ethics Committee of the National Institute of Rheumatic Diseases, Piestany, Slovakia.

Patients and controls were investigated under the same conditions. The investigations started at 8:00 AM, after overnight fast. An indwelling catheter was inserted into the cubital vein for blood sampling. To eliminate stress effect of a venipuncture, the blood sample for basal values was taken 30 min after inserting the catheter. Intravenous bolus of insulin (0.1 IU per kg, Actrapid HM, Novo Nordisk A/S, Bagsvaerd, Denmark) was administered afterwards. At intervals shown in Figure 1, blood samples were collected into polyethylene tubes containing EDTA as anticoagulant and immediately cooled. Heparin as anticoagulant was used for catecholamine measurements. After centrifugation, plasma aliquots were stored at –20°C until analyzed. The methods for hormone concentration measurements were the following: immunoradiometric assay [for adrenocorticotrophic hormone (ACTH), DHEAS, 17α-hydroxyprogesterone (17OHP), androstenedione (ASD)]; radioimmunoassay (for IL-6); radioimmunoassay after extraction in diethyl ether (for DHEA); enzyme linked immunosorbent assay [for C-reactive protein (CRP), IL-1β, and TNF-α]; intraassay and interassay coefficients were below 12.4% for all tests applied.

All kits were manufactured by Immuntotech AS (Beckman Coulter Company, Prague, Czech Republic). Concentrations of EPI and NE were measured by radioenzymatic method¹⁷. Plasma glucose concentrations were analyzed by glucose-oxidase method (Hitachi, Japan). Measurement of each hormone, cytokine, and glucose was performed in a single assay.

Area under response curve (AUC) of each hormone was calculated after subtraction of basal value (time 0 min) from the concentrations in 15–90 min hypoglycemia testing using the trapezoidal rule. Comparisons of basal hormone and cytokine concentrations were evaluated by unpaired t test or other appropriate nonparametric test depending on data normality. General linear model repeated measures (GLM-RM) procedure, with age and BMI as covariates, was used to determine the differences in endocrine responses to hypoglycemia between patients and controls. Spearman's nonparametric correlations adjusted for age and BMI as well as unadjusted nonparametric correlations were used to test relations between endocrine, inflammatory, and clinical data. Statistical evaluation was performed using SPSS 11.5 (SPSS Inc., Chicago, IL, USA) software. All data are expressed as mean (SEM). Significance was set at p < 0.05.

RESULTS

Glucose concentration immediately before and after insulin administration in SSc patients was not significantly different from that in controls. Insulin administration resulted in decreased (> 50% of basal values; p < 0.001) plasma glucose concentrations in all subjects (Figure 1).

Basal ACTH concentrations were significantly higher in SSc patients versus controls (6.76 ± 1.0 pmol/l in SSc vs 4.14 ± 0.45 pmol/l controls; p < 0.05). Hypoglycemia-induced increases in plasma ACTH concentration were comparable between patients and controls. Insulin administration resulted in a significant (p < 0.001) rise in plasma ACTH concentration in SSc patients and in controls (Figure 1).

The mean basal cortisol levels were comparable in SSc patients and in controls. During insulin-induced hypoglycemia, plasma cortisol concentration significantly (p < 0.001) increased in SSc and in controls. Cortisol response to hypoglycemia, as assessed by GLM-RM, tended to be lower

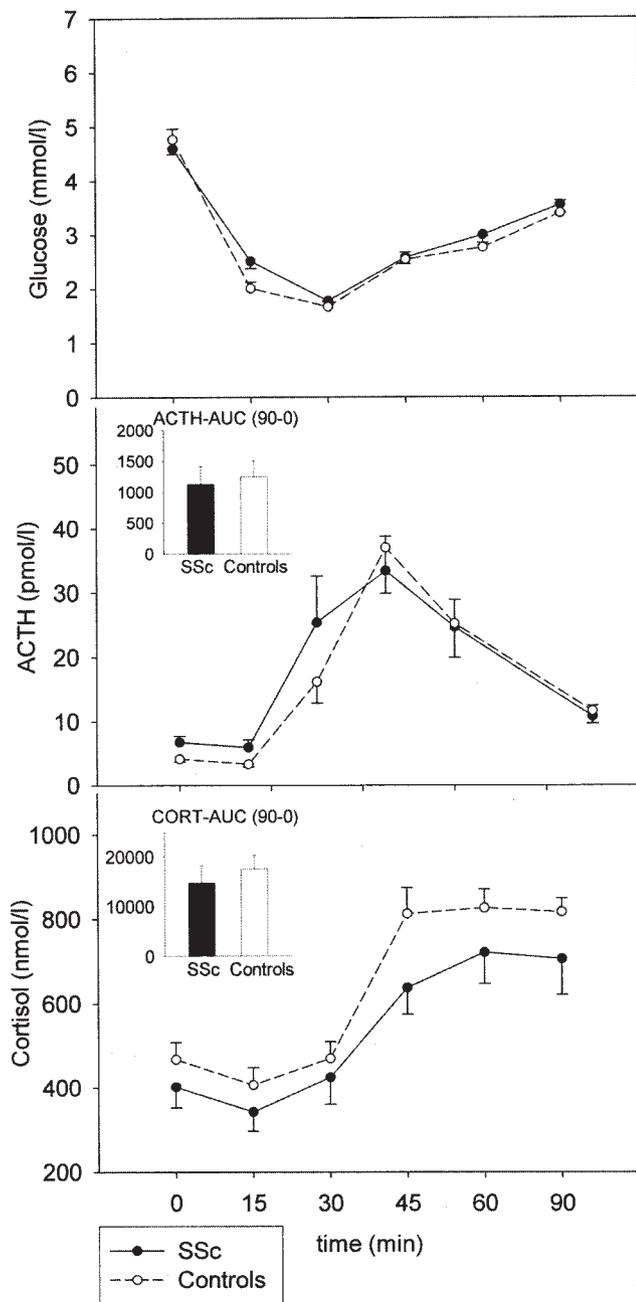


Figure 1. Concentrations of glucose (top panel), adrenocorticotropic hormone (ACTH) (middle), and cortisol (bottom), in plasma of 17 patients with SSc and 18 healthy controls during insulin-induced hypoglycemia. Data are means, error bars = SEM. Insets in panels indicate values of areas under response curve (AUC) in arbitrary units of ACTH from 0 min to 90 min and AUC of cortisol from 0 min to 90 min, in patients with SSc and controls.

in SSc patients compared to controls ($p = 0.06$, $F = 3.82$); however, the AUC of cortisol ($CORT_{AUC\ 0-90}$) did not differ significantly in the SSc group versus controls (Figure 1).

Basal 17OHP concentrations were comparable between patients and controls. Insulin administration resulted in a significant ($p < 0.001$) rise in plasma 17OHP concentration in SSc patients and in controls. A hypoglycemia-induced

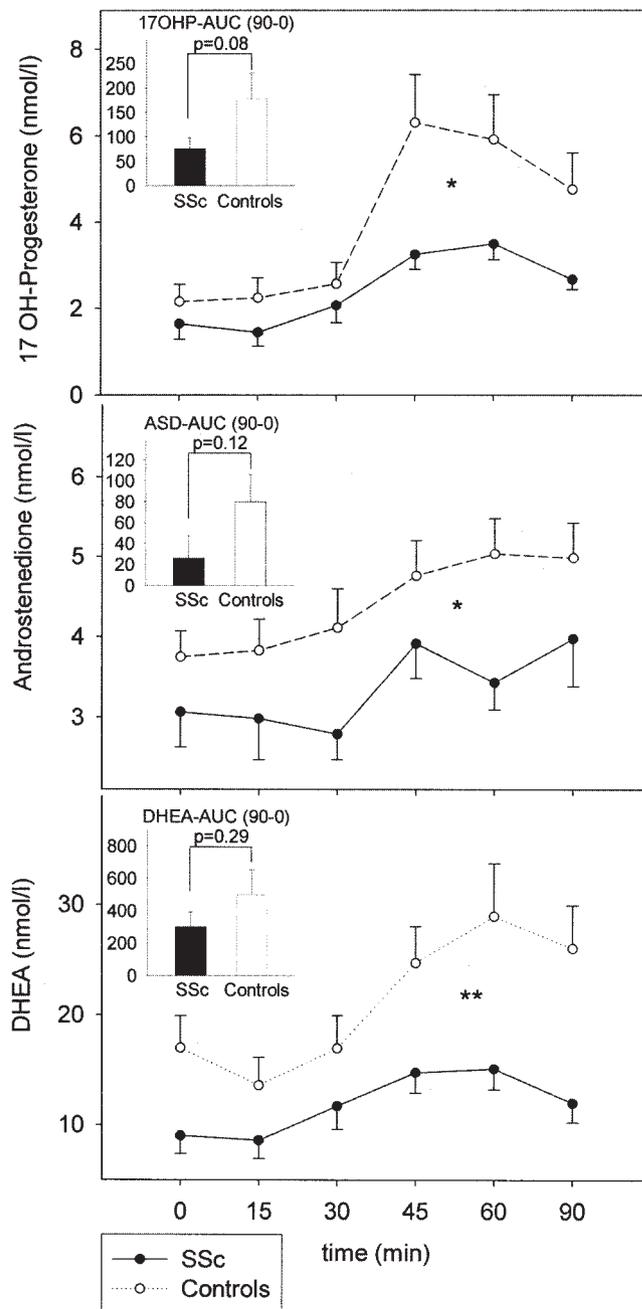


Figure 2. Concentrations of 17α -hydroxyprogesterone (top panel), δ -4-androstenedione (middle), and dehydroepiandrosterone (bottom) in plasma of 17 patients with SSc and 18 healthy controls during insulin-induced hypoglycemia. Data are means, error bars = SEM. * $p < 0.05$, ** $p < 0.01$ SSc vs controls, with age and BMI as covariants. Insets indicate values of areas under response curve (AUC) in arbitrary units of respective hormones from 0 min to 90 min, in patients with SSc and controls.

increase in plasma 17OHP concentration was significantly lower in SSc patients compared to controls ($p < 0.05$, $F = 6.2$, GLM-RM). The AUC of 17OHP ($17OHP_{AUC\ 0-90}$) tended to be lower in SSc patients versus controls ($p = 0.08$) (Figure 2).

Basal ASD concentrations were comparable between patients and controls. Insulin administration resulted in a sig-

nificant ($p < 0.001$) rise in plasma ASD concentration in SSc patients and in controls. Hypoglycemia-induced increases in plasma ASD concentration were significantly lower in SSc patients compared to controls ($p < 0.05$, $F = 5.2$, GLM-RM); however, AUC of ASD ($ASD_{AUC\ 0-90}$) did not differ between patients and controls ($p = 0.12$) (Figure 2).

Basal DHEA concentration was lower in SSc patients compared to controls: 9.02 ± 1.64 nmol/l in SSc vs 17.0 ± 2.8 nmol/l controls ($p < 0.05$). Insulin administration resulted in a significant ($p < 0.001$) rise in the mean plasma DHEA concentration in SSc and in controls. The responses of DHEA to hypoglycemia were significantly different between SSc and controls ($p < 0.01$, $F = 8.22$, GLM-RM); however, $DHEA_{AUC\ 0-90}$ values did not significantly differ between patients and controls (Figure 2).

Basal DHEAS levels were not significantly different in SSc patients compared to controls: 3.5 ± 2.7 nmol/l in SSc versus 4.7 ± 3.3 nmol/l controls ($p = 0.25$).

The mean basal EPI concentration did not differ between SSc patients and controls. Insulin administration resulted in a significant ($p < 0.001$) rise in mean plasma EPI concentration in SSc and in controls. SSc patients had lower EPI response to hypoglycemia ($F = 15.9$, $p < 0.001$) compared to controls. $EPI_{AUC\ 0-60}$ was also diminished ($p < 0.001$) in SSc patients compared to controls (Figure 3).

The mean basal NE concentrations were lower in SSc patients compared to controls: 1.61 ± 0.26 nmol/l in SSc versus 2.57 ± 0.38 nmol/l controls ($p < 0.05$). Insulin administration resulted in a significant ($p < 0.01$) rise in mean plasma NE concentration in controls but not in SSc patients. Response of NE in SSc patients was significantly reduced compared to controls ($F = 15.9$, $p < 0.001$). $NE_{AUC\ 0-60}$ was lower ($p < 0.001$) in SSc patients compared to controls (Figure 3).

Plasma levels of IL-1 β , IL-6, TNF- α , and CRP did not differ significantly between SSc patients and controls (Table 1).

As expected, several significant correlations were found among basal levels of adrenal steroids as well as AUC of respective hormones in both SSc and control groups (data not shown). Controlling for age and BMI as possible confounders, TNF- α was negatively correlated with $17OHP_{AUC\ 0-90}$ ($r = -0.7$, $p = 0.002$, $N = 17$) as well as with $DHEA_{AUC\ 0-90}$ ($r = -0.49$, $p = 0.048$, $N = 17$) in patients with SSc. TNF- α was not correlated with any endocrine characteristic measured in the study in healthy controls, controlling or not for age and BMI as covariates, including correlations between TNF- α and $17OHP$, TNF- α , and DHEA, TNF- α and ASD/ $17OHP$ ratio, as well as TNF- α and DHEAS/DHEA ratio (Figure 4).

DISCUSSION

Our results of decreased basal levels of DHEA and lower responses of this steroid to hypoglycemia suggest downregulated production of adrenal androgens in premenopausal women with SSc. In support of this, lower hypoglycemia-induced responses of another adrenal androgen intermediate,

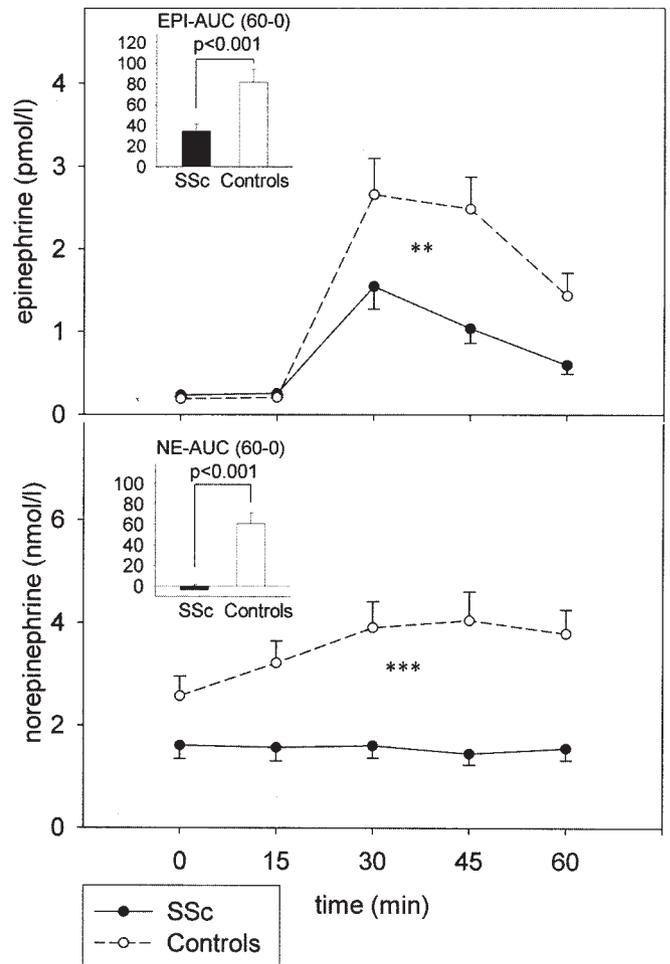


Figure 3. Concentrations of epinephrine (top panel) and norepinephrine (bottom) in plasma of 17 patients with SSc and 18 healthy controls during insulin-induced hypoglycemia. Data are means, error bars = SEM. ** $p < 0.01$, *** $p < 0.001$, SSc patients vs controls, with age and BMI as covariates. Insets indicate significantly lower values of areas under response curve (AUC) in arbitrary units of both epinephrine and norepinephrine from 0 min to 60 min, in patients with SSc and controls ($p < 0.001$).

ASD, were observed in the SSc group compared to healthy controls, suggestive of perturbed adrenal androgen production in those patients. While responses of ASD and DHEA to hypoglycemia were lower in SSc compared to controls, ACTH responses were similar in both groups, indicating normal hypothalamic-pituitary but decreased adrenal function in SSc. The results are in agreement with findings of other authors showing decreased levels of adrenal androgens in premenopausal women with SSc⁸, pre- and postmenopausal women with SSc⁹, and women with other chronic inflammatory diseases such as RA⁵⁻⁷.

Although basal levels of DHEA and DHEAS were positively correlated in both groups ($r = 0.69$, $p = 0.002$ for SSc and $r = 0.77$, $p < 0.001$ for controls), surprisingly, basal DHEAS levels were not found to be significantly different in SSc patients compared to controls. Plasma DHEAS concen-

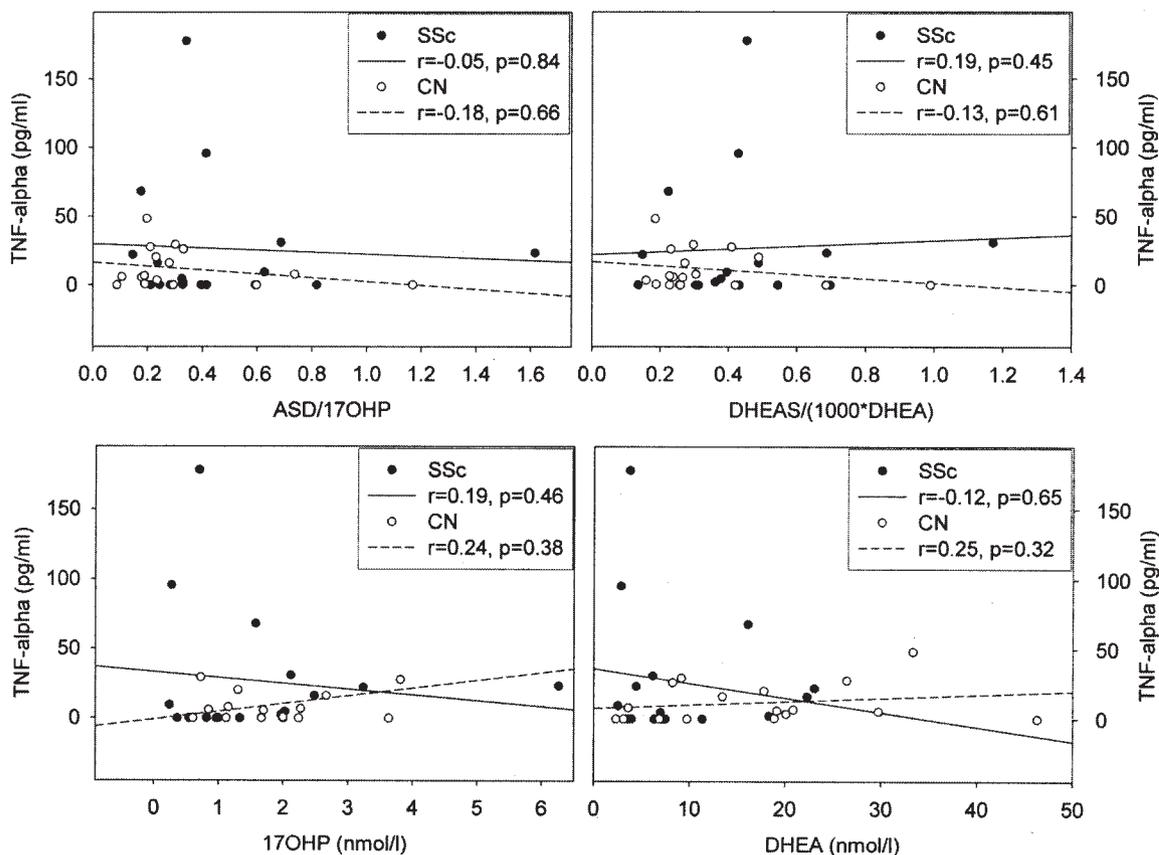


Figure 4. Spearman's rho correlations between TNF- α and 17OHP, TNF- α and DHEA, TNF- α and ASD/17OHP ratio, and TNF- α and DHEAS/DHEA ratio, in 17 patients with SSc and 18 healthy controls (CN). Correlation coefficients r and level of statistical significance p are shown in inset panels.

tration provides cumulative information on HPA activity over a longer period of time (hours to days) due to long plasma half-life compared to unsulfated DHEA. Therefore, lack of difference in DHEAS levels between SSc patients and controls but lower DHEA in SSc patients could be partially attributed to more frequent activation of the HPA axis in SSc patients compared with controls.

Since inflammatory cytokines such as IL-1 β , IL-6, or TNF- α are well known for their ability to immediately activate the HPA axis under normal conditions¹⁸, an increase in DHEAS would be expected in patients with a high degree of inflammatory activity. However, available data consistently indicate normal or even lower levels of adrenal androgens in various chronic inflammatory diseases⁵⁻⁹. Further, disease activity has been shown to be inversely related to DHEAS levels in chronic inflammatory diseases such as SSc⁹. This apparent paradox has been suggested to result from inherited hypocompetent adrenals and/or due to other secondary factors such as chronic modulatory effects of inflammatory mediators on steroidogenesis. Therefore, a low degree of inflammatory activity in our SSc patients could explain the lack of difference in DHEAS levels between SSc patients and controls.

Inflammatory cytokines have been shown to exert modulatory effects on steroidogenesis in steroid-producing tissues including the adrenal cortex, as well as on downstream conversion of steroid precursors to their active products in target tissues^{18,19}. An observed shift of production from adrenal androgens to adrenal glucocorticoids in chronic inflammatory diseases has been attributed in part to the effects of inflammatory cytokines on steroidogenesis²⁰. In particular, TNF- α has been shown to inhibit gene expression of the P450c17 enzyme, which catalyzes the 2-step conversion from pregnenolone and progesterone to DHEA and ASD, respectively, in human fetal adrenocortical cells²¹. Further, TNF- α appears to inhibit sulfatase-catalyzed conversion of DHEAS to DHEA in target tissues¹⁹, which might also contribute to relatively lower DHEA levels in chronic inflammatory diseases. Despite relatively low inflammatory activity in our SSc patients we attempted to explore such complex relations between adrenal androgens and TNF- α . However, we did not find any significant relationships between TNF- α and endocrine characteristics indicative of adrenal androgen production/peripheral conversion (Figure 4).

We observed lower responses of 17OHP to hypoglycemia.

Similarly, cortisol responses also tended to be lower in SSc patients compared to controls. Bearing in mind comparable ACTH responses to hypoglycemia in patients and controls, the results are indicative of decreased functional capacity or sensitivity of the adrenals to ACTH in SSc. Interestingly, higher basal ACTH found in SSc patients may suggest higher hypothalamic-pituitary "drive," compensating for decreased functional capacity of the adrenals by means of a negative feedback loop of cortisol.

Peripheral neuropathy characterized by structural and/or functional alterations in autonomic nerves, and possibly in sensory nerve fibers, represents a potential mechanism underlying ANS dysfunction in SSc^{4,22-24}. The AMHS is an integral part of the ANS, and central control of catecholamine secretion from the adrenal medulla is mediated through preganglionic sympathetic nerves. Further, adrenomedullary chromaffin cells of the adrenal medulla, which secrete catecholamines into the bloodstream, are regarded as specialized ganglionic neurons²⁵. Therefore, the factors involved in development of dysautonomia in SSc might also affect AMHS function, giving a rationale for AMHS evaluation in SSc.

To our knowledge, this is the first study investigating AMHS responses to hypoglycemia in patients with SSc. Our results showed lower EPI and NE responses to hypoglycemia in SSc patients compared to healthy controls. Recently, under a similar protocol, we demonstrated decreased EPI and NE responses in premenopausal women with RA⁵. Collectively, the findings are suggestive of downregulated or possibly defective AMHS in RA and SSc.

Insulin-induced hypoglycemia represents a potent stimulus eliciting release of EPI, and to a lesser extent that of NE, from the adrenal medulla. Thus, during hypoglycemia, increased plasma venous catecholamines provide reliable information on the function of the adrenomedullary hormonal component of the ANS²⁵.

Additionally, measurement of venous plasma NE also provides information on sympathetic noradrenergic activation during hypoglycemia. Activation of the sympathetic noradrenergic system during insulin-induced hypoglycemia occurs mainly due to hemodynamic effects of released EPI from the adrenal medulla, in addition to changes in plasma volume²⁶. In accord with the observed lower EPI response to hypoglycemia, we found significantly diminished NE response in SSc patients, suggesting low activation of the sympathetic noradrenergic system during the testing.

In one of the first studies of venous catecholamines in SSc, Sapira and coworkers did not report any significant differences in resting EPI or NE levels¹¹. In contrast, another group reported extremely elevated resting EPI and inappropriate fluctuations of plasma catecholamines during head-up tilt and hand-grip tests in SSc patients compared to healthy controls, which they interpreted as sympathetic overreactivity and instability²⁷. Our results failed to confirm such an extreme elevation of resting EPI or NE levels in SSc. In line with our

results, Kazzam and coworkers reported comparable levels of NE and sympathetic cotransmitter neuropeptide Y in SSc patients and in healthy controls²⁸.

Analyses of characteristics indicating the activity of sympathetic noradrenergic and parasympathetic cholinergic components in cardiovascular regulation in SSc suggest parasympathetic or mixed sympatho-parasympathetic impairment with sympathetic hyperactivity^{24,27,29-32}. It might be hypothesized that functional downregulation of the AMHS and compensating changes in sympathetic and parasympathetic systems might have occurred in patients with SSc, resulting in decreased responsiveness of the AMHS to stress stimulus. However, it seems more likely that primary pathology affecting sympathetic and parasympathetic nerves, e.g., by means of autoantibodies, also affects adrenomedullary function. Further research in mechanisms of autonomic dysfunction in SSc will be necessary.

There are also limitations in the findings of lower adrenomedullary hormonal responses to hypoglycemia in our patients with SSc. The majority (82%) of these patients have been receiving D-penicillamine (D-Pen, metalcaptase), a nitric oxide (NO)-donating compound. The drug has been shown to exert modulating effects on sympathetic neurotransmission³³. Also, neuropathy resulting from treatment with D-Pen has been reported in RA³⁴. Because effects of NO-donor drugs on sympathetic neurotransmission are very rapid and short-lasting, it seems unlikely that administration of D-Pen 24 hours prior to the investigation might have significantly affected AMHS responsiveness to hypoglycemia in our patients with SSc. However, longterm effects of chronic treatment cannot be excluded. On the other hand, xanthine-derived drugs, used by all our patients with SSc, may even enhance EPI secretion from the adrenal medulla in response to stimulation, as shown in rats³⁵. In humans, the xanthine-derived adenosine antagonist pentoxifylline had no effect on baseline EPI levels³⁶, diminishing the likelihood of interference of the drug with catecholamine levels in our study. Due to the relatively small number of subjects studied, Type I and II statistical error cannot be excluded.

In conclusion, decreased basal levels of DHEA and decreased responses of DHEA and ASD to hypoglycemia in premenopausal women with SSc indicate altered production of adrenal androgens in those patients. EPI and NE responses to hypoglycemia were also lower in SSc compared to healthy controls, suggesting downregulation or possible defect of the AMHS in SSc.

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