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# Interleukin-6 Production and Deregulation of the Hypothalamic–Pituitary–Adrenal Axis in Patients with Major Depressive Disorders

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**The present study was designed to determine whether an association exists between HPA activity and cytokine production in major depression (MD). In 9 patients with MD and 11 control subjects of both sexes, all drug-free, activity of the HPA axis was evaluated by circadian rhythm of plasma cortisol, 24-h free urinary cortisol, an overnight 1 mg dexamethasone suppression test, and an oCRF stimulation test. Spontaneous and LPS-stimulated production of IL-1β, IL-6, and TNFα by peripheral blood mononuclear cells were also determined. We found a significantly elevated spontaneous production of IL-6 in patients with MD (3541.2 ± 726.8 vs 380.4 ± 77.5 pg/mL in controls, $p < 0.05$), while LPS-stimulated production was significantly lower in patients than in control subjects (19,867.7 ± 3649.2 vs 33,142.2 ± 15,47.2 pg/mL, $p < 0.05$). The adrenocorticotropic hormone response to oCRF, evaluated as the area under the curve ($AUC_{ACTH}$) was significantly lower in patients than in control subjects ($p = 0.02$). A positive correlation between $AUC_{ACTH}$ and LPS-stimulated IL-6 secretion was observed in patients with MD ($r = 0.75$, $p < 0.05$) but not in controls. These findings suggest that the activation of the inflammatory response described in depression might be associated with long-term hyperactivity of the HPA axis.**

**Key Words:** IL-6; major depressive disorders; HPA axis; LPS.

## Introduction

A clear association between major depressive disorders (MDD) and hyperactivity of the hypothalamus–pituitary–adrenal (HPA) axis has been described *(1–3)*. High plasma levels of adrenocorticotropic hormone (ACTH) and cortisol *(1,4,5)* as well as the early escape of cortisol to dexamethasone suppression described in depression *(6)* might be the result of an increased release of corticotropin releasing factor (CRF) in depression, as suggested by the elevated levels of CRF found in cerebrospinal fluid in patients with major depression *(7)*. The blunted ACTH response to exogenous CRF described in these patients *(8,9)* suggests that the excessive release of CRF found in patients with MDD could be of suprahypothalamic origin *(10)*. Based on these findings, it has been postulated that CRF, a neuropeptide with endocrine, neurochemical, immunological, and behavioral effects, would be inadequately downregulated in major depression, resulting in a persistent activation of the HPAaxis *(3,5,11,12)*.

Proinflammatory cytokines have endocrine, electrophysiological, and behavioral effects *(10,13)*. Interleukin-1 (IL-1) and interleukin-6 (IL-6) are able to activate the HPA axis *(14)* at the central level by directly stimulating the hypothalamic secretion of CRF *(15,16)*. Based on this evidence, and considering that the acute administration of cytokines may reproduce many of the physical symptoms of depression**,** it has been suggested that cytokines play an important role in the pathohysiology of major depression *(13,17–19)*.

Although high levels of IL-6, IL-1, and acute-phase proteins have been reported in depressive disorders *(19,20)*, neither a typical cytokine profile nor a correlation between cytokine production and HPA axis function have been determined. Therefore, the exploration of the actual relevance of proinflammatory cytokine overproduction on the HPA axis hyperactivity found in patients with MDD seems crucial to the comprehension of this disorder and toward the development of new therapeutical strategies. For this reason, in the formulation of the present study, we considered an integrated view including endocrine, immune, and psychiatric aspects of depression to determine if proinflammatory cytokine production is related to HPA axis dysfunction in MDD.

## Results

There were no significant differences in age, gender, body mass index (BMI), 24-h FUC, and cortisol circadian rhythm (8 AM, 3 PM, and 11 PM) between patients with MDD and

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**Table 1**
Demographic, Clinical, and Baseline Laboratory Profile of Major Depressive Patients and Control Subjects

| | Depressive patients ($n$ = 9) | Control subjects ($n$ = 11) | Statistical difference |
|---|---|---|---|
| Gender (M/F) | 2/7 | 3/8 | NS |
| Age (yr) | 31 ± 9.4 | 33 ± 8.7 | NS |
| BMI (kg/m$^2$) | 26.5 ± 0.97 | 26.8 ± 1.2 | NS |
| HDRS-21 | 35 ± 3.8 | 5.3 ± 1.8 | <0.05 |
| MADRS | 35 ± 4.6 | 5.3 ± 2.1 | <0.05 |
| FUC (µg/24 h) | 54.9 ± 21.9 | 55.6 ± 18.3 | NS |
| Plasma cortisol (µg/dL) | | | |
| 8 AM | 20.1 ± 3.7 | 19.5 ± 7.7 | NS |
| 3 PM | 9.9 ± 2.3 | 9.6 ± 4.0 | NS |
| 11 PM | 4.8 ± 2.1 | 3.6 ± 1.9 | NS |
| Patients Suppressors to Dexa | | | |
| <4 mg/dL | 8/9 | 11/11 | <0.05 |
| <1.8 mg/dL | 7/9 | 10/11 | <0.05 |

HDRS: Hamilton Depression Rating Scale; MADRS: Montgomery-Asberg Rating Scale; BMI: body mass index; FUC: free urinary cortisol; NS: not significant.

control subjects (Table 1). Although there was no significant difference between patients with MDD and control subjects in FUC, the proportion of subjects with a FUC higher than 70 µg/24 h was higher among patients with MDD ($p$ = 0.01).

In most cases, the current episode of depression was the first. In only one patient a previous episode of depression had been diagnosed and a 10-mo antidepressant treatment had successfully been accomplished more than 12 mo before the current episode. Interestingly, this case presented the lowest 11 PM cortisol value of the group and the strongest ACTH response to oCRH. The cortisol response of this case remained similar to the mean value for the MDD group.

Considering a 4 µg/dL cortisol level cut-point *(6)*, eight patients with MDD and all control subjects were suppressors (S) in response to dexamethasone. Only one patient with MDD, a woman for which this was a first episode of depression and who had never taken antidepressants, was a non-suppressor (NS). Using the 1.8 µg/dL cut-point *(27)*, two patients with MDD and only one control subject were NS ($p < 0.05$).

### *HPA Assessment*

The pituitary and adrenal responses to oCRF are shown in Fig 1. Cortisol and ACTH responses were lower and delayed in patients with MDD as compared with control subjects. This difference was significant at the 5th minute after stimulation for ACTH (Fig. 1A) and at min 45 for cortisol (Fig. 1B) ($p < 0.05$). The $AUC_{ACTH}$ was significantly decreased in patients with MDD when compared to control subjects: 8341 ± 3389.9 pg/mL/min vs 18,610 ± 4104.8 pg/mL/min ($p$ = 0.02). The $AUC_{cortisol}$ was similar in both groups: 1146.8 ± 113.6 µg/dL/min in patients with MDD and 1391.8 ± 1123 µg/dL/min in control subjects.

In patients with MDD, a negative correlation between BMI and basal cortisol was observed ($r$ = –0.66, $p$ = 0.04). On the other hand, BMI showed a positive correlation to the rate of increment in ACTH and cortisol after oCRF stimulation ($r$ = 0.7, $p$ = 0.02 and $r$ = 0.8, $p$ = 0.01, respectively).

### *Cytokine Measurements*

The ex vivo spontaneous IL-6 production by PBMC from patients with MDD was significantly higher than control subjects: 3541.2 ± 726.8 pg/mL vs 380.4 ± 77.5 pg/mL ($p <$ 0.001). LPS-induced IL-6 production by PBMC from patients with MDD was significantly lower than in control subjects: 19,867.7 ± 3649.2 pg/mL vs 33,142.2 ± 1547.2 pg/mL ($p$ < 0.005). The delta between spontaneous and LPS-induced IL-6 production was significantly lower for patients with MDD (Fig. 2).

A positive correlation between the $AUC_{ACTH}$ response to oCRF and LPS-activated IL-6 secretion was observed in patients with MDD, with a Spearman $R$ of 0.75 ($p < 0.005$) (Fig. 3A). We found no correlation between these variables in control subjects (Fig. 3B). No correlation between BMI and spontaneous or induced IL-6 could be determined in either group.

No difference in LPS-stimulated IL-1β and TNFα production by PBMC between patients with MDD and control subjects was found. Spontaneous production was undetectable for both cytokines in PBMC from patients and control subjects.

## Discussion

The most consistent finding of our study was a higher spontaneous IL-6 secretion by PBMC from patients with MDD as compared to control subjects. Conversely, LPS-stimulated IL-6 secretion was lower in patients with MDD.

In our study, a negative correlation between BMI and basal cortisol was observed, as well as a direct correlation between BMI and the response of ACTH and cortisol to oCRF. This finding supports the hypothesis that associates depression as well as obesity with a chronic activation of the HPA axis *(28)*. Nevertheless, no correlation between BMI and spontaneous or induced IL-6 production could be determined. This finding differs with previous reports *(29,30)*. The composition of our study group could explain this difference, since most patients and control subjects had a BMI under 30 kg/m$^2$ and women were mainly premenopausal. On the other hand, the ex vivo determination of cytokines used in our study cannot be directly compared with the serum concentration of cytokines obtained in these reports.

Our study found two patients with a dexamethasone suppression test over 1.8 µg/dL *(27)*. Cortisol levels were under

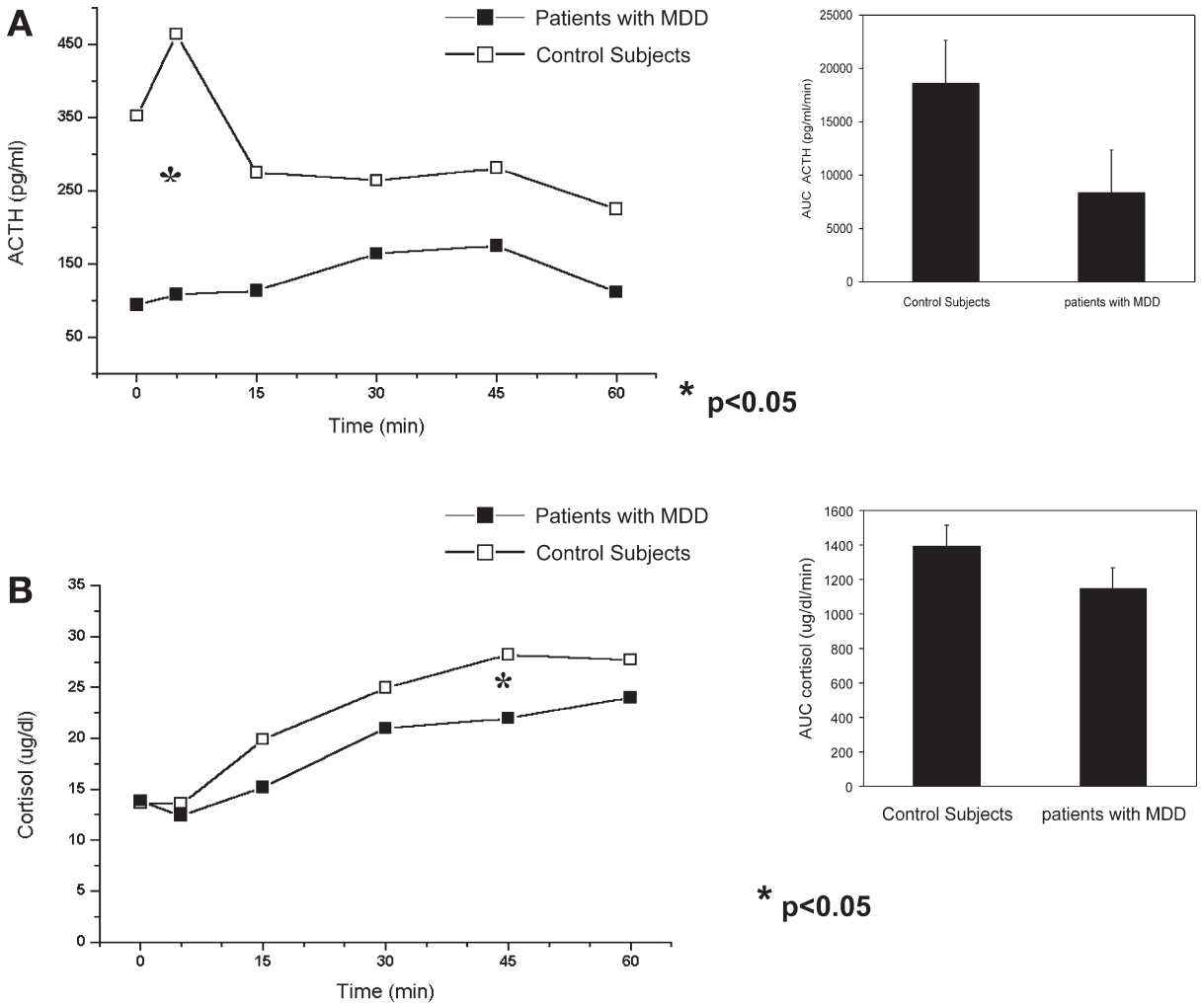


**Fig. 1.** (**A**) ACTH response to oCRF in patients with MDD and control subjects. (**B**) Cortisol response to oCRF in patients with MDD and control subjects.

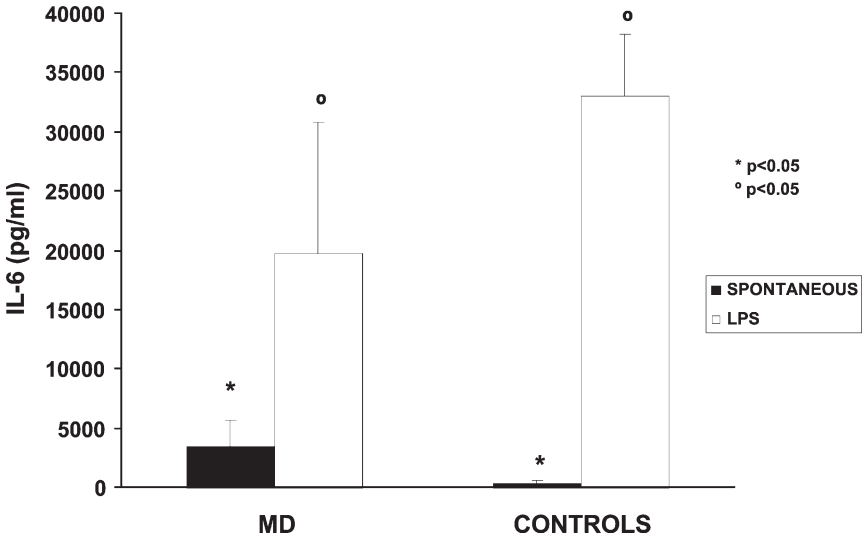


**Fig. 2.** Spontaneous and LPS-stimulated IL-6 production in patients with MDD and control subjects.

1 µg/dL in the majority of control subjects, while only 50% of patients with MDD had cortisol levels under this level. Previous studies have already reported higher rates of cortisol hypersecretion after dexamethasone administration in depressive inpatients compared with outpatients *(31,32)*. The study of subtypes of depression should thus be considered

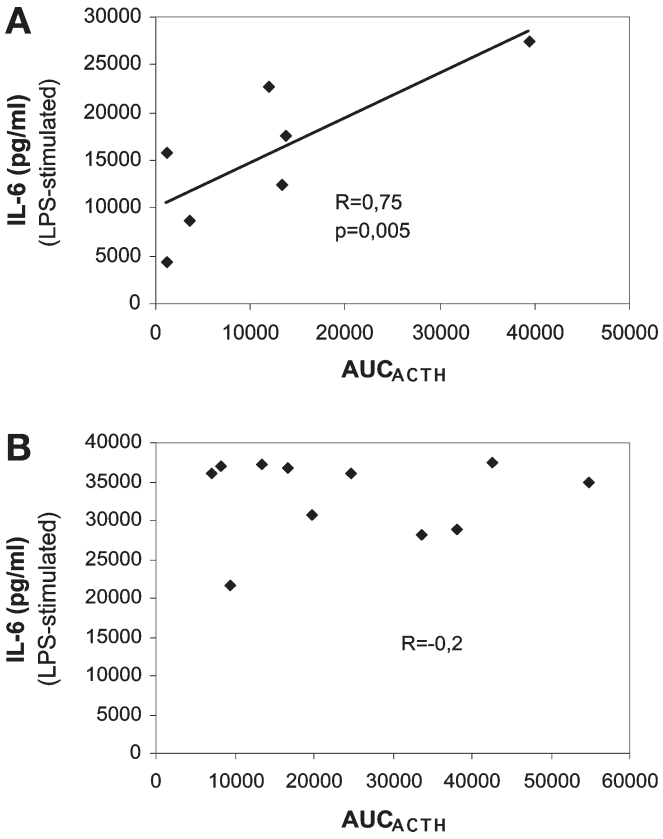


**Fig. 3.** (**A**) $AUC_{ACTH}$ and LPS-stimulated IL-6 secretion in patients with MDD. (**B**) Correlation between $AUC_{ACTH}$ and LPS-stimulated IL-6 secretion in control subjects.

in the interpretation of this test, as well as ethnical differences in pharmacokinetics of dexamethasone or in glucocorticoid-receptor polymorphism *(33,34)*.

The alteration of immune parameters associated with depressive disorders has widely been discussed elsewhere *(13, 18–20,35)*. Although studies on whole blood have reported increased serum levels of IL-6 in patients with depression *(19,35–37)*, serum determinations cannot be directly compared to the production obtained from blood cells in culture. Nevertheless, the high spontaneous in vitro cytokine production found in our study could be associated with high plasma levels of IL-6.

Studying blood cells in culture, Maes et al. described high IL-6 levels in PHA-stimulated PBMC from inpatients with melancholic depression under medication *(38)*. Similarly, Seidel et al. *(39)* reported slightly elevated IL-1 and IL-6 levels in activated PBMC in a study that included only depressive inpatients. Nevertheless, different inclusion criteria and methodological differences do not permit a direct comparison with our results. Many clinical factors, including age, gender, individual stress associated with assessment conditions or with hospitalization, as well as the subtype or severity of depression may account for some of these discrepancies. Inpatients represent a particular group of patients with depression, including chronic, treatment-resistant depressive disorders, patients with suicidal ideation, major sleep disorders, and significant weight loss *(31)*. On the other hand, some studies have shown that depressive outpatients have a higher proportion of lymphocyte type B cells and a better response to LPS stimulation as compared with inpatients *(40)*. Our study only included healthy subjects and patients with major depression with no associated personality disorders, and all free of antidepressants, which affect the CRF receptor balance as well as cytokine production *(2,12,17)*. Moreover, considering that the severity of depression has been associated with the degree of imbalance in the HPA axis, two depression scales were assessed to avoid severity-related differences.

We assume that the chronic stress associated with MDD might induce a continuous IL-6 secretion, probably stimulated by central and autonomic nervous system catecholamines *(41)*. Consequently, this would produce a blunted response to further stimulation (LPS challenge). Considering the process of cytokine production and release, specifically the fact that no intracellular storage system exists for cytokines, we suggest that the low IL-6 secretion under challenging conditions (LPS) in patients with MDD could be a consequence of the high basal release in these patients associated with the state of chronic stress underlying the depressive disorder. This continuous maximal IL-6 secretion could impair a normal response to LPS. Some studies have, on the other hand, demonstrated a reduced function of the lymphocyte glucocorticoid receptor in depressive patients *(42)*, another argument that could explain the inability of lymphocytes to respond to the LPS challenge

Our study establishes an association between HPA axis dysfunction and altered cytokine secretion, as manifested by the positive correlation between blunted $AUC_{ACTH}$ and secretion of IL-6 by activated PBMC from patients with MDD. In animal models, IL-6 is able to produce an increase of ACTH and corticosterone in the acute response, but might decrease the ACTH response in the chronic state, suggesting that high levels of this cytokine could modulate the activity of the HPA axis *(2,11)*. Thus, IL-6 affects CRF, ACTH, and cortisol secretion permanently, leading to a global imbalance of the regulatory mechanisms, as evidenced by the attenuated ACTH response to oCRH seen in our patients.

These findings suggest the importance of the IL-6 imbalance associated with the dysfunction of the HPA axis, main stress regulator of the organism. An alteration in the contraregulatory mechanism of cytokines should be further investigated because glucocorticoids normally inhibit IL-6 production by PBMC, while in patients with MDD there is a coexistence of increased spontaneous cytokine production and HPA hyperactivity.

### *Conclusions*

The blunted response of adrenocorticotropic hormone to ovine corticotropine-releasing factor associated with in-

creased spontaneous production of interleukin-6 and decreased interleukin-6 secretion after stimulation with an endotoxin (LPS) observed in patients with MDD could be considered as a manifestation of interleukin-6-mediated chronic hyperactivity of the hypothalamus–pituitary–adrenal axis. Considering that cortisol levels show a wide variability in depressive disorders, and the fact that ovine corticotropine-releasing factor response test is difficult to assess in ordinary practice, we think that interleukin-6 determination could become a marker of severity of hypothalamus–pituitary–adrenal axis deregulation.

Our aim to obtain a group of untreated cases, and to avoid any situation that could interfere with the interpretation of cytokine determinations, resulted in a rigorous inclusion criteria and, hence, a reduced study group. Although the small size of our population could represent a statistical limitation, the results reported here constitute a preliminary approach toward a better understanding of the association between HPA dysfunction and proinflammatory cytokine disorder in major depression.

## Patients and Methods

### *Patients*

The present study was approved by the Institute of Nutrition and Food Technology of the University of Chile Ethics in Human Research Committee. Informed written consent was obtained from each patient and control subject.

Nine patients with MDD, two men and seven women, aged between 19 and 46 yr, were selected for this study. A trained researcher assessed the potential subjects with the structured clinical interview for axis I disorders according to DSM-IV criteria *(21)*. The 21-item Hamilton Depression Rating Scale (HDRS) *(22)* and the Montgomery-Asberg Rating Scale for Depression (MADRS) *(23)* were also assessed. Subjects with any other DSM-IV axis I disorder, suicidal ideation, a medical condition, or any endocrine disorder were excluded from the study. Use of antidepressant, lithium, anticonvulsant, or neuroleptic treatment during the last 12 mo was also an exclusion criterion. All patients were free of drugs known to interfere with immune or endocrine function, they had never taken major psychotropic drugs (except benzodiazepines), and were not regular drinkers.

HDRS and MARDS were assessed during the first interview, before basal cortisol determinations, and repeated a week later. Score variations greater than 10% between the first and second assessment represented an exclusion criterion. Minimal doses of benzodiazepines were used as a symptomatic treatment during this period of time.

Eleven healthy controls, three men and eight women in the same range of age were selected from volunteers with no lifetime history of axis II or I disorders and no history of axis I disorder in first-degree relatives. They were all healthy and had not received any psychotropic medication during the last year.

None of the participants received non-steroidal antiinflamatory or glucocorticoid drugs during at least 3 wk prior to the study. None of them presented a current infectious disease. Because oral contraceptives increase proinflammatory cytokine production and interfere with plasma cortisol determination by increasing cortisol binding globulin levels *(24)*, subjects affected by the beginning or decline of gonadal function or undergoing any kind of hormonal therapy were also excluded.

### *HPA Assessment*

Twenty-four hour urine collections were obtained during the first day of the study for free urinary cortisol (FUC) assessment. Subjects were then invited to the Endocrinology Unit of the University of Chile Clinical Hospital at 8 AM, 3 PM, and 11 PM for three separate plasma cortisol determinations. After the last baseline cortisol sampling, 1 mg of oral dexamethasone (Oradexon®) was administered to all subjects at 11 PM and plasma cortisol levels were determined 9 h later, according to Carroll's protocol for the dexamethasone suppression test (DST) *(25)*.

The ovine-corticotropine-releasing factor (oCRF, Bachem, Torrance, CA) stimulation test was assessed 7 d later *(26)*.

An intravenous heparinized cannula was inserted into each arm at 8 AM. After 30 min of supine rest, baseline samples were obtained (−15 and 0) for basal cortisol and ACTH determinations. oCRF was administered as an intravenous bolus injection of 1 μg per kilogram of body weight using the first cannula. Blood samples were obtained from the other cannula at 5, 15, 30, 45, and 60 min for ACTH and cortisol response assessment. An additional blood sample was drawn before the injection of oCRF for cytokine assessment. Plasma was stored at −20°C and processed within the first 30 d after sampling.

### *Cortisol and ACTH Determinations*

Cortisol and ACTH were determined by commercial chemoluminescent enzyme immunometric assays (Immulite, Diagnostic Products Corporation, Los Angeles, CA). The intraassay variation coefficient was 7%, with an interassay coefficient of 10% for both hormones.

### *Cytokine Induction and Determination*

Peripheral blood mononuclear cells (PBMC) were isolated by Hypaque gradient (Sigma Diagnostics, St Louis MO), washed twice in PBS, and $2.5 \times 10^6$ cells/mL were resuspended in RPMI 1640 culture medium supplemented with gentamicin, glutamine 0.5%, heat-inactivated AB plasma, and 1 μg/mL indomethacin. For each sample, six 200 μL per well of cell suspension were dispensed, two in each of three 96-well flat-bottom microtiter plates, in the absence (spontaneous cytokine release) and presence of endotoxin: lipopolysaccharide (LPS, Sigma) for 24 h at 37°C, in 5% $CO_2$/95% air. Cell viability after incubation was

over 90%. Supernatants were collected and stored at –20ºC until assay for proinflammatory cytokines.

All cytokine determinations were performed using specific, commercial enzyme-linked immuno-absorbent assays (ELISA): (RD Systems, Minneapolis, MN), the assay sensibility being 3.6 pg/mL.

### *Statistical Analysis*

Results are expressed as mean ± SE. Two-way analysis of variance (ANOVA) was used to compare parametric values. The total and integrated ACTH and cortisol responses to oCRF were calculated by the trapezoid method and expressed as the area under the concentration-time curve (AUC) from 0–60 min. The Mann–Whitney test was used to assess the difference of means and chi-square between groups. The association of variables was assessed using the Spearman's rank order correlation coefficient. A $p$-value ≤ 0.05 was considered statistically significant.

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