ORIGINAL RESEARCH

Nesfatin-1 and cortisol: potential novel diagnostic biomarkers in moderate and severe depressive disorder

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Background: This study aimed to determine whether plasma nesfatin-1, cortisol, and inflammatory cytokines could be used as novel noninvasive biomarkers for the diagnosis of moderate and severe depressive disorder (MSDD).

Materials and methods: A total of 70 patients with MSDD and 70 healthy subjects were assessed. Patients with MSDD were selected from Hefei Fourth People's Hospital, Anhui Mental Health Center, and subjects in the control group were selected from healthy volunteers. Hamilton Depression Rating Scale-17 (HAMD-17) was used to evaluate the two groups. ELISA was used for the measurement of plasma nesfatin-1, cortisol, IL-6, C-reactive protein (CRP), and tumor necrosis factor-α (TNF-α) levels. The diagnostic value of plasma nesfatin-1, cortisol, IL-6, CRP, and TNF-α for MSDD was assessed.

Results: Compared to healthy controls, the HAMD-17 scores and average nesfatin-1, cortisol, IL-6, and CRP levels in patients with MSDD were significantly increased. Moreover, multivariate linear regression analysis showed that HAMD-17 score was positively associated with plasma nesfatin-1 and cortisol. Furthermore, the results of the receiver operating characteristic (ROC) curve analysis revealed an area under curve (AUC) of 0.985 with 94.3% sensitivity and 97.1% specificity of nesfatin-1, and an AUC of 0.957 with 91.4% sensitivity and 85.7% specificity of cortisol in discriminating patients with MSDD from healthy volunteers. A combined ROC analysis using nesfatin-1 and cortisol revealed an AUC of 0.993 with a sensitivity of 97.1% and a specificity of 98.6% in separating patients with MSDD from healthy volunteers.

Conclusion: These results suggest that plasma nesfatin-1 and cortisol might be potential novel biomarkers for the diagnosis of MSDD.

Keywords: C-reactive protein, cortisol, IL-6, depression, nesfatin-1, tumor necrosis factor-α

Introduction

Depression, a serious debilitating mental disease, is among the leading causes of disability leading to a high rate of suicide and economic burden and affecting up to 120 million people worldwide.[1] The current diagnosis of depression is mainly based on patient interviews and symptoms, with uncertainties as high as 30% as a consequence.[2] Thus, it is important to find an objective examination method to improve the diagnostic accuracy of depression.

The stress response and the resulting hyperactivity of the hypothalamic pituitary adrenal (HPA) axis are considered to be one of the causes of depressive episodes.[3,4] Preclinical studies have shown that hyperfunctioning of the HPA axis and elevated serum corticosterone levels could be observed in animal models of depression.[5] Con-

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sistent with this finding, clinical studies have suggested that HPA axis dysfunction, such as excessive secretion of cortisol, occurs in patients with depression.[6] Therefore, the plasma concentration of cortisol was considered to be a reliable independent marker for diagnosing patients with moderate and severe depressive disorder (MSDD) in the present study.

Anorexigenic and orexigenic peptides have been considered to be associated with the pathogenesis of depression.[7,8] Nesfatin-1 is a new anorexigenic peptide that plays an important role in the integration of food intake, energy expenditure, and glucose homeostasis.[9] Recently, a link may have been discovered between nesfatin-1 and depression due to the findings that intracerebroventricular injection of nesfatin-1 could activate the HPA axis in rats and induce fear and anxiety-like behavioral changes.[10] Moreover, our recent studies have shown that peripheral injection of nesfatin-1 could also induce depressive behavioral changes and activate the HPA axis in rats.[11] Furthermore, higher plasma nesfatin-1 level was found in patients with major depressive disorder (MDD) than in healthy controls.[12] Taken together, the fact that plasma nesfatin-1 level is associated with the severity of depression,[13] it is rational to assume that nesfatin-1 might act as a potential diagnostic biomarker in depression.

Numerous studies support the notion that the occurrence of depression is often accompanied by activation of the inflammatory system, and overexpression of pro-inflammatory factors is thought to play a role in the pathogenesis of depression.[14,15] Meta-analysis shows that the most closely related inflammatory factors of depression are IL-6, C-reactive protein (CRP), and tumor necrosis factor-alpha (TNF-α).[16] In addition, more and more studies have shown that elevated levels of IL-6 and CRP in plasma are believed to increase the risk of depression and even predict the following symptoms of depression.[17] Therefore, whether these inflammatory factors could serve as objective and reliable biological indicators for the diagnosis of depression was evaluated in the present study.

Because of the dysfunction of the HPA axis, with the important role of nesfatin-1 and inflammatory markers in the pathophysiology of depression, the aim of the present study was to investigate whether plasma nesfatin-1, cortisol, and inflammatory cytokines could be used as novel noninvasive biomarkers for MSDD.

Materials and methods
Subjects
This study was conducted at Hefei Fourth People's Hospital, Anhui Mental Health Center, between July 2016 and April 2018. A total of 1,489 subjects with depression were screened via psychiatric interviews by an experienced researcher in accordance with the guidelines of the structured clinical interview according to the Diagnostic and Statistical Manual for Psychiatric Disorders-Fourth Version (DSM-IV), and 70 patients with MSDD were selected from the above patients. The criteria for inclusion in the group are as follows: 1) age 18–65 years; 2) Hamilton Depression Rating Scale-17 (HAMD-17) scores >17; and 3) currently not receiving any medication (Blood was collected when the patient with MSDD was just admitted to the hospital for treatment). Exclusion criteria were as follows: 1) suffering from other mental disorders; 2) diagnosed with alcohol or other substance dependent diseases; and 3) suffering from a major neurological or medical illness. Seventy healthy volunteers were included in the control group. The recruitment process is summarized in Figure 1. In accordance with the principles of the Declaration of Helsinki, all subjects provided informed written consent prior to participation. The Ethics Committee of Hefei Fourth People's Hospital, Anhui Mental Health Center, approved this study.

Measurements
The weight, height, and body mass index (BMI) of all subjects were measured. The blood sample was taken from the participants' vein between 8:00 A.M. and 9:00 A.M. All subjects were fasting while giving blood. Tubes containing EDTA were used for collecting the samples. The blood samples were immediately centrifuged at 3,000 rpm for 5 minutes at 4°C. Extract supernatant as plasma sample was collected. The extracted plasma was stored at −80°C until detection. Commercially available ELISA kits were used to measure the concentrations of nesfatin-1 (Cusabio Biotech. Co., LTD, Wuhan, Hubei, China), cortisol (Enzo Life Sciences, Inc., Farmingdale, NY, USA), IL-6, CRP, and TNF-α (Yuanye Biotech.Co., LTD, Shanghai, China) according to the manufacturer's instructions.

Statistical analysis
Data were analyzed using SPSS version 16.0 statistical analysis software (SPSS, Chicago, IL, USA). The differences between groups (age, BMI, HAMD-17 scores, and concentrations of nesfatin-1, cortisol, IL-6, CRP, and TNF-α) were evaluated by Student's *t*-test. The sex differences between the groups were analyzed by chi-squared test. Relationships between HAMD-17 scores and other variables were analyzed by Pearson correlation tests and independent relationships were determined by multivariate linear regression analysis. The receiver operating characteristic (ROC) curve analysis
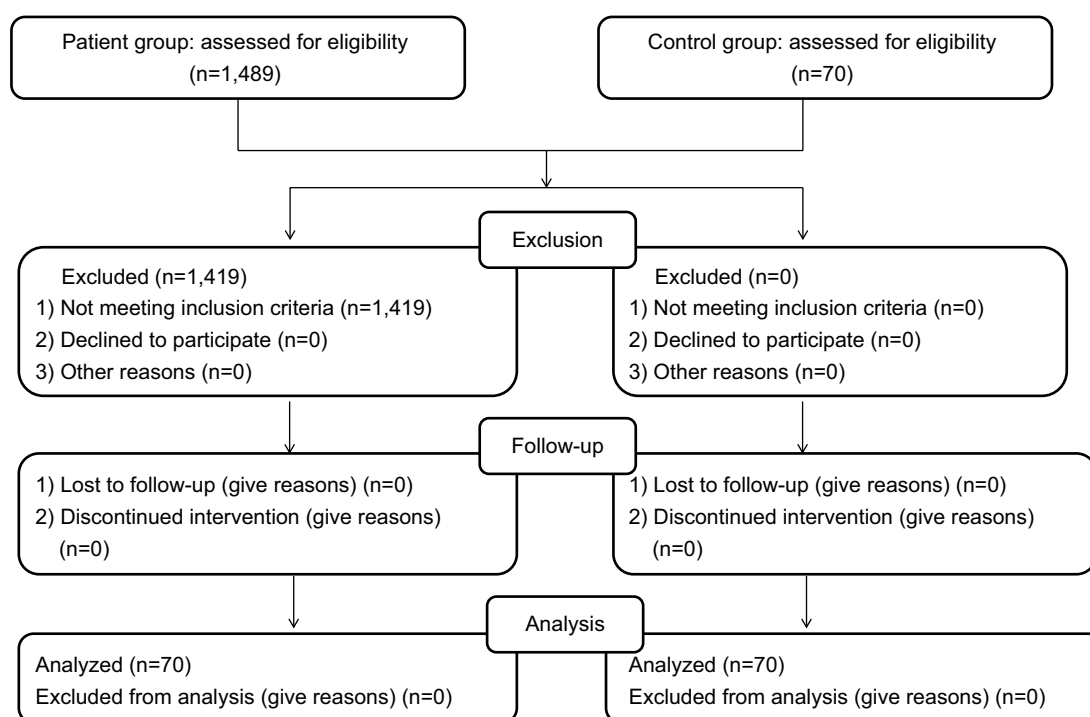
Figure 1 Flowchart showing the recruitment of participant.

was used to determine the area under curve (AUC) and cut-off values of plasma nesfatin-1 and cortisol. A *P*-value of <0.05 was considered statistically significant.

Results

Demographic values were not significantly different between the MSDD patients and healthy controls. As shown in Table 1, there were no significant differences in age, BMI, or sex between the two groups. The mean HAMD-17 scores were significantly higher in the MSDD group than in the control group ($t=-14.144$, $P<0.01$, Table 1). Similarly, the MSDD patients showed elevated nesfatin-1 ($t=-19.349$, $P<0.01$, Table 1) and cortisol ($t=-14.694$, $P<0.01$, Table 1) levels when compared with the healthy subjects. Additionally, the results of covariance analysis showed that the differences of plasma nesfatin-1 and cortisol between the MSDD groups and healthy control groups were not influenced by age and sex (Supplemental materials, Tables S1–S4). For inflammatory cytokines, the plasma concentrations of IL-6 ($t=-4.037$, $P<0.01$) and CRP ($t=-3.87$, $P<0.01$) were significantly higher than those in the healthy control group, whereas no statistical difference was observed in TNF-α levels ($t=-0.445$, $P=0.657$) between the two groups.

As shown in Table 2, the results of the Pearson correlation analysis showed that the HAMD-17 scores were positively correlated with plasma nesfatin-1 ($r=0.647$, $P<0.01$), cortisol ($r=0.566$, $P<0.01$), IL-6 ($r=0.236$, $P=0.005$), and CRP ($r=0.291$, $P<0.01$) concentrations. However, no correlation between HAMD-17 scores and TNF-α ($r=0.012$, $P=0.887$) was observed.

Multivariate linear regression analysis showed that the HAMD-17 score was positively associated with plasma nesfatin-1 and cortisol (Table 3).

Additionally, the ROC curve analysis indicated the potential diagnostic values of plasma nesfatin-1 and cortisol (Table 4): The AUCs for nesfatin-1 and cortisol were 0.985 (95% CI: 0.959–1.000) and 0.957 (95% CI: 0.927–0.986), respectively. At a cut-off point of 1.135 ng/mL for nesfatin-1, the sensitivity and specificity were 94.3% and 97.1% for separating patients with MSDD from healthy volunteers. At a cut-off point of 325.245 nmol/L for cortisol, the sensitivity and the specificity were 91.4% and 85.7%, respectively. When the test results for nesfatin-1 and cortisol were considered jointly, the ROC analysis revealed an AUC of 0.993 with a sensitivity of 97.1% and specificity 98.6% in discriminating patients with MSDD from healthy volunteers, indicating that the combination of nesfatin-1 and cortisol could improve the accuracy of diagnosis of MSDD (Figure 2).

Table 1 Comparison of age, gender, BMI, HAMD-17 scores, plasma nesfatin-1, cortisol, IL-6, CRP, and TNF-α of MSDD group and control group (mean ± SEM)

Variables	Control group	MSDD group	Statistics (t or χ^2)	P-value
Age	34.81±12.19	35.86±15.78	−0.438	0.662
Gender (female/male)	39/31	35/35	0.459[a]	0.498
BMI (kg/m²)	23.34±1.67	23.29±1.44	0.206	0.837
HAM-D	6.60±3.10	27.41±11.92	−14.144	<0.01[b]
Nesfatin-1 (ng/mL)	0.83±0.16	1.61±0.29	−19.349	<0.01[b]
Cortisol (nmol/L)	196.78±102.76	431.55±85.50	−14.694	<0.01[b]
IL-6 (pg/mL)	59.50±13.17	66.65±6.78	−4.037	<0.01[b]
CRP (mg/L)	2.48±0.29	2.66±0.25	−3.87	<0.01[b]
TNF-α (ng/L)	167.15±27.82	168.92±18.29	−0.445	0.657

Notes: [a]Shows χ^2 score. [b]$P<0.01$ was considered statistically significant.
Abbreviations: BMI, body mass index; CRP, C-reactive protein; HAM-D, Hamilton Depression Rating Scale; MSDD, moderate and severe depressive disorder; TNF-α, tumor necrosis factor-α.

Table 2 Correlation between plasma HAMD-17 scores and plasma nesfatin-1, cortisol, IL-6, CRP, and TNF-α (Pearson correlation analysis)

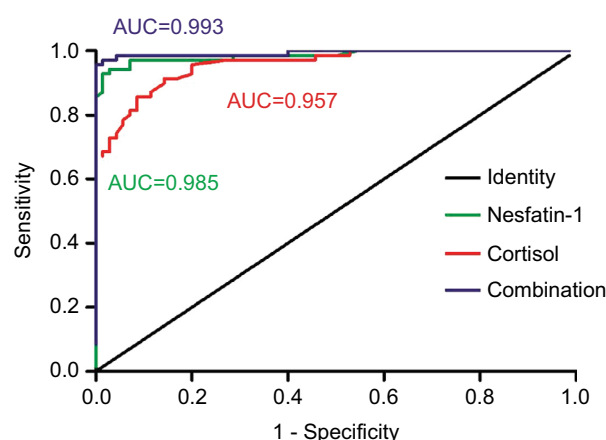| Variables	r	P-value
Nesfatin-1	0.647	<0.01[a]
Cortisol	0.566	<0.01[a]
IL-6	0.236	0.005[a]
CRP	0.291	<0.01[a]
TNF-α	0.012	0.887

Note: [a]$P<0.01$ was considered statistically significant.
Abbreviations: CRP, C-reactive protein; HAM-D, Hamilton Depression Rating Scale; TNF-α, tumor necrosis factor-α.

Table 4 ROC analysis of various indicators for diagnosis of MSDD

Variables	AUC	Cut-off	Sensitivity	Specificity
Nesfatin-1	0.985	1.135	0.943	0.971
Cortisol	0.957	325.245	0.914	0.857
Nesfatin-1+ Cortisol	0.993		0.971	0.986
IL-6	0.679			
CRP	0.672			
TNF-α	0.535			

Abbreviations: AUC, area under curve; CRP, C-reactive protein; MSDD, moderate and severe depressive disorder; ROC, receiver operating characteristic; TNF-α, tumor necrosis factor-α.

Table 3 Multiple linear regression analysis between HAMD-17 scores (dependent variable) and plasma nesfatin-1, cortisol, IL-6, CRP, and TNF-α (independent variables)

Variables	B	t	P-value	95% CI	
				Lower bound	Upper bound
Constant	−18.293	−2.043	0.043	−35.997	−0.589
Nesfatin-1	13.921	5.186	<0.01[a]	8.612	19.230
Cortisol	0.020	2.556	0.012[b]	0.005	0.036
IL-6	−0.004	−0.046	0.963	−0.169	0.161
CRP	4.755	1.511	0.133	−1.467	10.976

Note: [a]$P<0.01$, [b]$P<0.05$ was considered statistically significant.
Abbreviations: CRP, C-reactive protein; HAM-D, Hamilton Depression Rating Scale; TNF-α, tumor necrosis factor-α.

Figure 2 ROC curve of plasma nesfatin-1 and cortisol in identification of the patients with MSDD.
Abbreviations: AUC, area under curve; MSDD, moderate and severe depressive disorder; ROC, Receiver operating characteristic.

Discussion

In the present study, we demonstrated that the HAMD-17 score and the plasma nesfatin-1, cortisol, IL-6, and CRP concentrations were significantly higher in MSDD patients than in healthy controls. Moreover, multivariate linear regression analysis showed that HAMD-17 score was positively associated with plasma nesfatin-1 and cortisol.

Furthermore, the results of the ROC analysis revealed an AUC of 0.985 with 94.3% sensitivity and 97.1% specificity of nesfatin-1, and an AUC of 0.957 with 91.4% sensitivity and 85.7% specificity of cortisol in discriminating patients with MSDD from healthy controls, suggesting that plasma

nesfatin-1 and cortisol might be potential novel biomarkers for the diagnosis of MSDD.

Stress and the subsequent hyperactivity of the HPA axis have been involved in the development of depression.[18] Numerous studies have confirmed that disturbance in the HPA axis function, indicated by elevated plasma corticosterone concentrations, was observed in a rat model of depression (chronic unpredictable mild stress,[19] restraint stress,[20] or water avoidance stress).[21] Similar dysfunction in the HPA axis is also observed in depressed patients.[22] In line with these results, in the present study, patients with MSDD showed higher plasma cortisol levels than healthy control subjects. Recently, numerous studies have indicated that biomarker panels that monitor changes in cortisol, as well as other HPA axis factors, could provide important information for the diagnosis of depression.[23] Based on the ROC analysis, the plasma cortisol cut-off point of 325.245 nmol/L showed 91.4% sensitivity and 85.7% specificity, indicating that plasma cortisol has a superior diagnostic value (AUC=0.957) of MSDD.

Nesfatin-1, a novel satiety molecule, has been reported to be involved in a variety of affective disorders, including anxiety disorder,[24] depression,[13] and psychosis.[25] Clinical studies have shown that the average nesfatin-1 level in MDD patients is statistically higher than that in the control group.[12] Similarly, plasma nesfatin-1 levels were also increased in rat subjected to avoiding stress, an acute depression model.[26] Consistent with these findings, the MSDD patients showed elevated nesfatin-1 levels when compared with the healthy subjects in the present study. On the other hand, the intraperitoneal administration of nesfatin-1, in a single dose or multiple doses, led to despair behavior and HPA axis hyperactivity in rats.[11] These results provide more information on the link between nesfatin-1 and depression. Recently, another study has demonstrated that the plasma nesfatin-1 level is positively correlated with the severity of depression.[13] Consistently, a positive correlation between the nesfatin-1 levels and HAMD-17 scores was found in the present study. As mentioned above, it is rational to hypothesize that plasma nesfatin-1 might be a potential biomarker for the diagnosis of MSDD. The results of ROC curve analysis showed that the AUC for nesfatin-1 was 0.985 and the cut-off point of 1.135 ng/mL showed 94.3% sensitivity and 97.1% specificity in separating patients with MSDD from healthy controls. Thus, nesfatin-1 may be a useful biomarker for detection of MSDD due to its high sensitivity and specificity.

Combining the detection of different biomarkers has been reported to be a useful strategy in several studies, for the reason that it can increase the sensitivity and specificity of each biomarker. In the present study, both nesfatin-1 and cortisol exhibited high specificity and sensitivity. In a combined ROC analysis using the two markers, both sensitivity and specificity reached a relatively high value in discriminating patients with MSDD from healthy controls. To date, no reliable independent biomarkers have been established for MSDD. Our results suggest that the combination of nesfatin-1 and cortisol plasma assays may offer a useful tool for the detection of MSDD.

Accumulated data support a certain link between depression and inflammatory response. It has been indicated that depressed patients[27] and animals[28] showed increased concentrations of IL-6 and peripheral injection of IL-6 can induce depressive behavioral changes in mice.[29] In addition, elevated CRP levels were observed in patients with depression and high CRP levels were considered to be positively associated with the degree of depression.[30] Consistently, the present study showed that plasma levels of IL-6 and CRP were significantly increased in the patients with MSDD, which again suggests that IL-6 and CRP may play an important role in the development of depression. Although Pearson correlation analysis showed positive correlations were found between the HAMD-17 scores and the plasma IL-6 and CRP concentrations, these correlations were not observed by multivariate linear correlation analysis and the AUCs of IL-6 and CRP were less than 0.7. These results suggest that inflammatory factors (IL-6 and CRP) are closely related to depression, but may not be suitable as a biomarker for the diagnosis of MSDD.

There are several limitations in the present study. Firstly, this study is a single-center study and the sample size is relatively small. Secondly, we measured the plasma concentration of nesfatin-1 in only those patients who were not taking medication within 2 weeks; the long-term effects of previous drugs on plasma nesfatin-1 remain unknown. Thirdly, when collecting the general data of subjects, the patients' smoking status should be included, for the reason that smoking status may potentially affect their inflammatory factor levels.

Conclusion

In conclusion, the detection of plasma nesfatin-1 and cortisol may prove to be of clinical value for the diagnosis of MSDD. Multicentric and longitudinal studies are clearly required to validate the potential of nesfatin-1 and cortisol as novel biomarkers for MSDD.

Acknowledgment

Disclosure

The authors report no conflicts of interest in this work.

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Supplementary materials

Tables S1 and S2 show the results of analysis of covariance with age and sex as covariates (dependent variable: nesfatin-1), respectively. Tables S3 and S4 show the results of analysis of covariance with age and sex as covariates (dependent variable: cortisol), respectively.

The results of covariance analysis show that the differences of plasma nesfatin-1 and corticosterone between the MSDD groups and healthy control groups are not influenced by age and sex.

Table S1 Tests of between-subjects effects

Dependent variable: nesfatin-1

Source	Type III sum of squares	df	Mean square	F	P-value
Corrected model	21.132[a]	7	3.019	52.342	0.000
Intercept	133.913	1	133.913	2321.814	0.000
Age	0.013	3	0.004	0.074	0.974
Group	12.721	1	12.721	220.562	0.000
Age × group	0.113	3	0.038	0.653	0.582
Error	7.613	132	0.058		
Total	237.758	140			
Corrected total	28.746	139			

Notes: R^2=0.735 (Adjusted R^2=0.721). The differences in plasma nesfatin-1 between the MSDD groups and healthy control groups are not influenced by age (P>0.05).

Table S2 Tests of between-subjects effects

Dependent variable: nesfatin-1

Source	Type III sum of squares	df	Mean square	F	P-value
Corrected model	21.055[a]	3	7.018	124.110	0.000
Intercept	208.025	1	208.025	3678.658	0.000
Gender	0.044	1	0.044	0.784	0.377
Group	20.749	1	20.749	366.928	0.000
Gender × group	0.001	1	0.001	0.011	0.918
Error	7.691	136	0.057		
Total	237.758	140			
Corrected total	28.746	139			

Notes: R^2=0.732 (Adjusted R^2=0.727). The differences in plasma nesfatin-1 between the MSDD groups and healthy control groups are not influenced by gender (P>0.05).

Table S3 Tests of between-subjects effects

Dependent variable: cortisol

Source	Type III sum of squares	df	Mean square	F	P-value
Corrected model	1.950E6	7	278560.062	30.335	0.000
Intercept	9146742.680	1	9146742.680	996.090	0.000
Age	10032.684	3	3344.228	0.364	0.779
Group	1077392.415	1	1077392.415	117.329	0.000
Age × group	14107.698	3	4702.566	0.512	0.675
Error	1212109.577	132	9182.648		
Total	1.698E7	140			
Corrected total	3162030.007	139			

Notes: R^2=0.617 (Adjusted R^2=0.586). The differences in plasma cortisol between the MSDD groups and healthy control groups are not influenced by age (P>0.05).

Table S4 Tests of between-subjects effects

Dependent variable: cortisol

Source	Type III sum of squares	df	Mean square	F	P-value
Corrected model	1.941E6	3	647137.777	72.104	0.000
Intercept	1.377E7	1	1.377E7	1533.734	0.000
Gender	366.221	1	366.221	0.041	0.840
Group	1902079.346	1	1902079.346	211.928	0.000
Gender × group	11989.871	1	11989.871	1.336	0.250
Error	1220616.678	136	8975.123		
Total	1.698E7	140			
Corrected total	3162030.007	139			

Notes: R^2=0.614 (Adjusted R^2=0.605). The differences in plasma cortisol between the MSDD groups and healthy control groups are not influenced by gender (P>0.05).