Table 2. Characteristics of Studies Included in Meta-Analysis on Comparison With Plasma L-Tryptophan Concentration Between Patients With MDD and Healthy Controls

Study	Subjects' Country (race)	Case Group Name (criteria)	Case N (n female/ n male)	Mean (SD) Plasma L-Tryptophan Concentration (μmol/L)	Control N (n female/ n male)	Mean (SD) Plasma L-Tryptophan Concentration (μmol/L)	Evaluation of Depressive State (mean [SD] score)	Drug Free in Patients[a] (period)
DeMyer et al, 1981[41]	USA	MDD (RDC)	18 (13/5)	42 (11)	10 (7/3)	56.9 (12)	HDRS-17 (22.4 [8.9])	Yes (3 wk)
Menna-Perper et al, 1983[40]	USA	MDD with melancholia (*DSM-III*)	9 (3/6)	42.8 (8.2)	6 (3/3)	46.3 (4.7)	HDRS, BDI (NA)	No
Joseph et al, 1984[39]	USA	MDD (*DSM-III*)	16 (10/6)	31.3 (7.2)	8 (5/3)	43.8 (16)	HDRS, BDI (NA)	Yes (1 wk)
Anderson et al, 1990[38]	UK	MDD (*DSM-III*)	31 (15/16)	38.2 (8.9)	31 (15/16)	45.4 (11.1)	HDRS-17 (22.8 [NA])	No
Chiaroni et al, 1990[37]	France, Switzerland	MRD (*DSM-III*)	25 (19/6)	48.8 (13.3)	33 (19/14)	59.5 (12.7)	AMDP (NA)	No
Russ et al, 1990[36]	USA	MDD (*DSM-III-R*)	16 (10/6)	59 (11)	9 (7/2)	52 (14)	HDRS-21 (31 [7])	No
Maes et al, 1990[24]	Belgium	MDD – melancholia (*DSM-III*)	22 (12/10)	56.6 (10.5)	16 (8/8)	60.6 (4.8)	HDRS (NA)	No
		MDD + melancholia (*DSM-III*)	13 (7/6)	50 (12.1)	16 (8/8)	60.6 (4.8)	HDRS (NA)	
Price et al, 1991[35]	USA	MDD (*DSM-III-R*)	109 (78/31)	35.3 (8.3)	58 (41/17)	36.7 (8.3)	HDRS-25 (34 [11])	No
Quintana, 1992[33]	Spain	MDD (RDC)	25 (15/10)	42.5 (8.3)	25 (NA)	47.6 (11.3)	HDRS (NA)	No
Lucca et al, 1992[34]	Italy	MDD (*DSM-III-R*)	19 (12/7)	52 (20)	29 (14/15)	74 (12)	HDRS-21 (24.7 [4.1])	No
Maes et al, 1993[25]	Belgium	MDD – melancholia (*DSM-III-R*)	7 (NA)	56 (14)	8 (NA)	79 (12)	HDRS (NA)	No
		MDD + melancholia (*DSM-III-R*)	10 (NA)	55 (15)	8 (NA)	79 (12)	HDRS (NA)	
Ortiz et al, 1993[26]	Spain	MDD adults (*DSM-III-R*)	10 (8/2)	69.0 (9.8)	10 (NA)	70.0 (14.7)	MADRS (26.8 [2.0])	No
		MDD elderly (*DSM-III-R*)	7 (5/2)	64.1 (8.8)	10 (NA)	70.0 (14.7)	MADRS (28.3 [1.4])	
Moller, 1993[32]	Denmark	All depressives (*DSM-III*)	26 (18/8)	36 (6)	55 (39/16)	39 (8)	HDRS-17 (24 [5])	No
Maes et al, 1995[27]	Belgium	MDD – melancholia (*DSM-III*)	47 (35/12)	61 (12)	50 (24/26)	66 (12)	HDRS-17 (21.3 [2.9])	No
		MDD + melancholia (*DSM-III*)	35 (21/14)	57 (12)	50 (24/26)	66 (12)	HDRS-17 (26.7 [3.2])	
Mauri et al, 1998[31]	Italy	MDD (*DSM-IV*)	29 (14/15)	33.3 (27.3)	28 (12/16)	56.7 (79.9)	HDRS (NA)	Yes (4 wk)
Song et al, 1998[30]	Belgium	MDD (*DSM-IV*)	6 (4/2)	69 (11)	14 (6/8)	73 (19)	(NA)	Yes (10 d)
Hoekstra et al, 2001[29]	Netherlands	MDD (*DSM-IV*)	20 (13/7)	35.5 (9)	29 (13/16)	45.6 (6.1)	HDRS-17 (31 [NA])	No
Mauri et al, 2001[28]	Italy	MDD (*DSM-IV*)	16 (11/5)	28.6 (34.1)	11 (2/9)	45.6 (13.0)	HDRS-17 (22.4 [5.6])	No
Myint et al, 2007[10]	Korea	MDD (*DSM-IV*)	58 (32/26)	65.8 (15.6)	189 (86/103)	69.7 (13.7)	HDRS-17 (27.2 [7.3])	No
Manjarrez-Gutierrez et al, 2009[8]	Mexico	MDD (*DSM-IV*)	8 (4/4)	48.1 (1.2)	9 (5/4)	57.7 (3.3)	(NA)	Yes (NA)
Sublette et al, 2011[12]	USA (white/ nonwhite)	MDD (*DSM-IV*)	30 (14/16)	59.2 (10.4)	31 (21/10)	60.2 (7.7)	HDRS-17 (20.1 [3.4]) BDI (25.9 [8.2])	No
Maes and Rief, 2012[7]	Germany	MDD (*DSM-IV*)	35 (22/13)	69.8 (14.4)	22 (8/14)	82.9 (15.9)	BDI (27.1 [8.3])	Yes (NA)
Pinto et al, 2012[11]	Brazil	MDD (*DSM-IV*)	5 (NA)	35 (6)	5 (NA)	36 (2)	HDRS (22 [2])	Yes (NA)
Xu et al, 2012[9]	China (Han Chinese)	MDD (*DSM-IV*)	26 (19/7)	42.9 (6.4)	25 (16/9)	49.8 (7.2)	HDRS-17 (24.2 [4.5])	Yes (NA)

[a]*Drug free* means being completely excepted from the administration of psychotropic drugs, not only antidepressants but also benzodiazepines and antipsychotics.

Abbreviations: AMDP = The Association for Methodology and Documentation in Psychiatry, BDI = Beck Depression Inventory, *DSM* = *Diagnostic and Statistical Manual of Mental Disorders*, HDRS = Hamilton Depression Rating Scale, MADRS = Montgomery-Asberg Depression Rating Scale, MDD = major depressive disorder, MRD = major recurrent depression, NA = not mentioned in the article, RDC = Research Diagnostic Criteria, TRP = plasma L-tryptophan.

and 203, respectively (Figure 2C). There was a significant heterogeneity ($P = .002$), and the random effects model was applied. As a result, there was a highly significant difference in standardized mean tryptophan concentration between the 2 groups (Hedges *g*, −0.84; 95% CI, −1.27 to −0.40; $P = .00015$; fail-safe number, 93). Funnel plot and Egger regression analysis did not indicate publication bias (intercept, −2.73; 95% CI, −6.92 to 1.47; $df = 7$; $P = .17$) (Figure 2D).

To elucidate the relationship between plasma tryptophan concentration and depression severity, we performed meta-regression analysis in 11 comparisons (Figure 3A). When we set the HDRS-17 score as an outcome variable and Hedges *g* value as an explanatory variable, we found that Hedges *g* value had a significant, albeit weak, effect on HDRS-17 score by adopting the fixed effects model as the estimation method ($\tau^2 = 0.068$, slope, −0.029; 95% CI, −0.057 to −0.00012; $P = .049$) (Figure 3B).

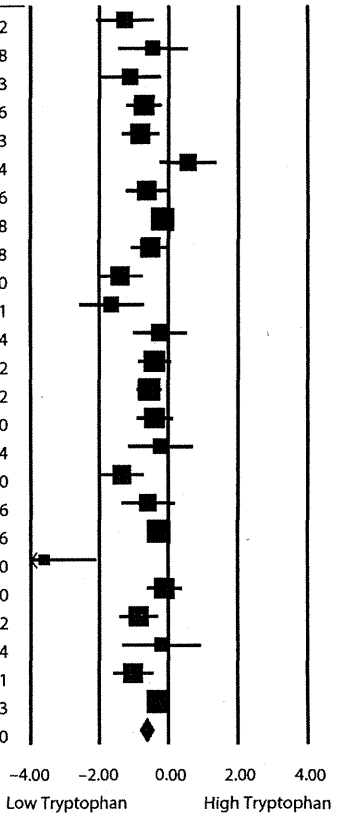
DISCUSSION

In our case-control study, the ANCOVA analysis controlling for age, sex, and BMI showed that the patients with MDD had significantly lower plasma tryptophan

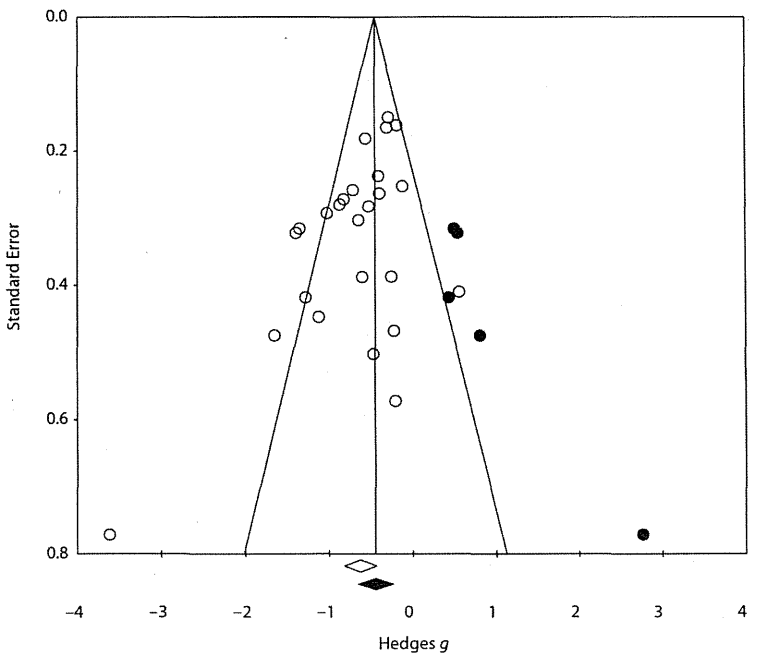
Figure 2. Forest Plots and Funnel Plots of Meta-Analysis[a]

A. Forest Plot of Meta-Analysis on 25 Comparisons

Study	Hedges *g*	Standard Error	Lower Limit	Upper Limit	*Z* Value	*P* Value
DeMyer et al, 1981[41]	−1.274	0.419	−2.095	−0.453	−3.040	.002
Menna-Perper et al, 1981[40]	−0.462	0.503	−1.449	0.524	−0.919	.358
Joseph et al, 1984[39]	−1.117	0.448	−1.995	−0.239	−2.492	.013
Anderson et al, 1990[38]	−0.705	0.259	−1.212	−0.198	−2.726	.006
Chiaroni et al, 1990[37]	−0.815	0.272	−1.348	−0.281	−2.992	.003
Russ et al, 1990[36]	0.558	0.411	−0.247	1.363	1.359	.174
Maes et al, 1990[24]	−0.635	0.304	−1.231	−0.040	−2.092	.036
Price et al, 1991[35]	−0.176	0.162	−0.493	0.142	−1.084	.278
Quintana, 1992[33]	−0.516	0.283	−1.071	0.039	−1.822	.068
Lucca et al, 1992[34]	−1.385	0.323	−2.018	−0.752	−4.289	.000
Maes et al, 1993[25]	−1.645	0.475	−2.577	−0.713	−3.461	.001
Ortiz et al, 1993[26]	−0.247	0.388	−1.008	0.513	−0.637	.524
Moller, 1993[32]	−0.400	0.238	−0.866	0.066	−1.683	.092
Maes et al, 1995[27]	−0.551	0.182	−0.907	−0.195	−3.035	.002
Mauri et al, 1998[31]	−0.389	0.264	−0.906	0.128	−1.475	.140
Song et al, 1998[30]	−0.223	0.469	−1.142	0.695	−0.476	.634
Hoekstra et al, 2001[29]	−1.341	0.316	−1.961	−0.721	−4.238	.000
Mauri et al, 2001[28]	−0.595	0.388	−1.356	0.166	−1.532	.126
Myint et al, 2007[10]	−0.276	0.150	−0.570	0.018	−1.838	.066
Manjarrez-Gutierrez et al, 2009[8]	−3.607	0.772	−5.120	−2.095	−4.675	.000
Sublette et al, 2011[12]	−0.115	0.253	−0.611	0.381	−0.453	.650
Maes and Rief, 2012[7]	−0.862	0.280	−1.411	−0.313	−3.076	.002
Pinto et al, 2012[11]	−0.202	0.573	−1.325	0.921	−0.352	.724
Xu et al, 2012[9]	−1.014	0.293	−1.589	−0.438	−3.454	.001
Present Study	−0.297	0.165	−0.621	0.027	−1.795	.073
Total	−0.627	0.098	−0.819	−0.436	−6.407	.000



B. Funnel Plot of Standard Error by Hedges *g*

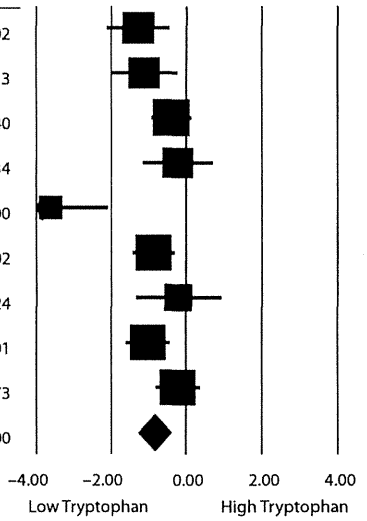


(continued)

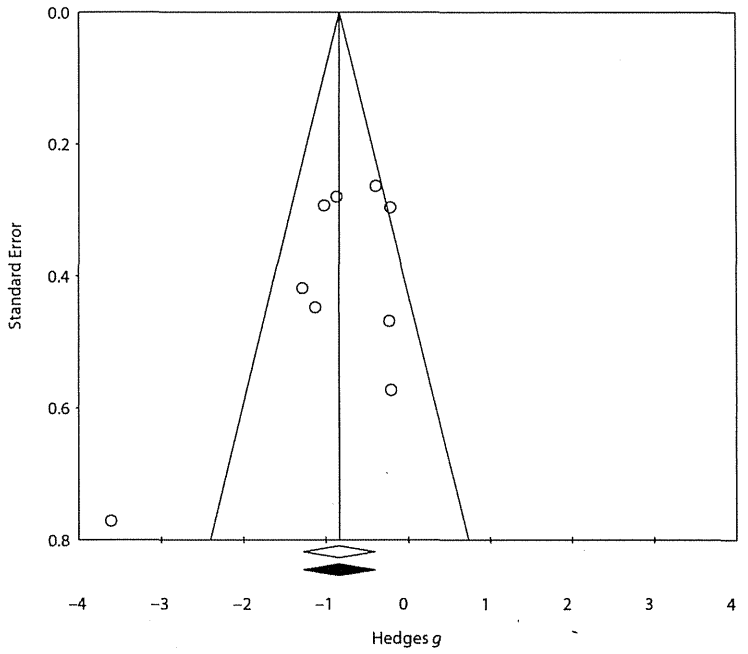
Figure 2 (continued). Forest Plots and Funnel Plots of Meta-Analysis[a]

C. Forest Plot of Meta-Analysis Using Psychotropic Drug–Free Patients of Our Subjects and Previous Studies

Study	Hedges *g*	Standard Error	Lower Limit	Upper Limit	*Z* Value	*P* Value
DeMyer et al, 1981[41]	−1.274	0.419	−2.095	−0.453	−3.040	.002
Joseph et al, 1984[39]	−1.117	0.448	−1.995	−0.239	−2.492	.013
Mauri et al, 1998[31]	−0.389	0.264	−0.906	0.128	−1.475	.140
Song et al, 1998[30]	−0.223	0.469	−1.142	0.695	−0.476	.634
Manjarrez-Gutierrez et al, 2009[8]	−3.607	0.772	−5.120	−2.095	−4.675	.000
Maes and Rief, 2012[7]	−0.862	0.280	−1.411	−0.313	−3.076	.002
Pinto et al, 2012[11]	−0.202	0.573	−1.325	0.921	−0.352	.724
Xu et al, 2012[9]	−1.014	0.293	−1.589	−0.438	−3.454	.001
Present Study	−0.213	0.297	−0.794	0.368	−0.718	.473
Total	−0.836	0.220	−1.268	−0.404	−3.790	.000



D. Funnel Plot of Standard Error by Hedges *g*

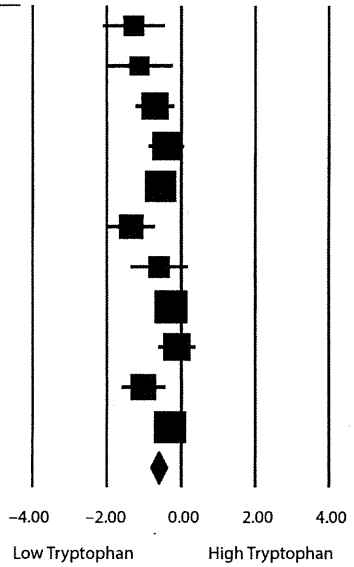


[a]The forest plots describe statistical data and effect size of each study, and the result of quantitative synthesis. Black squares depict effect size, and horizontal bars indicate 95% confidence interval. The funnel plots, which were made to examine the presence of publication bias, depict the effect size against the standard error of individual studies. Black circles represent potentially missing trials that were imputed based on the trim-and-fill method. The white rhombus represents the point estimate for plasma tryptophan concentration effect based on published trials. The black rhombus represents the new point estimate for the effect size of plasma tryptophan when publication bias was adjusted by means of the trim-and-fill method.

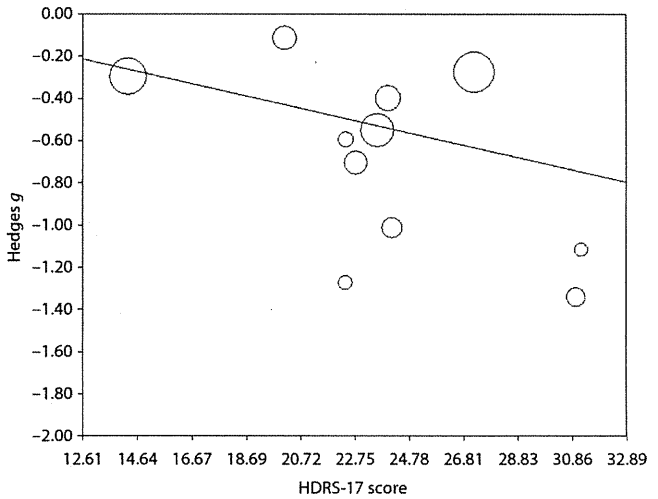
Figure 3. Forest Plot and Scatter Plot of Meta-Regression on HDRS-17 Scores and Effect Size (Hedges *g*)[a]

A.

Study	Hedges *g*	Standard Error	Lower Limit	Upper Limit	*Z* Value	*P* Value
DeMyer et al, 1981[41]	−1.274	0.419	−2.095	−0.453	−3.040	.002
Joseph et al, 1984[39]	−1.117	0.448	−1.995	−0.239	−2.492	.013
Anderson et al, 1990[38]	−0.705	0.259	−1.212	−0.198	−2.726	.006
Moller, 1993[32]	−0.400	0.238	−0.866	0.066	−1.683	.092
Maes et al, 1995[27] *	−0.551	0.182	−0.907	−0.195	−3.035	.002
Hoekstra et al, 2001[29]	−1.341	0.316	−1.961	−0.721	−4.238	.000
Mauri et al, 2001[28]	−0.595	0.388	−1.356	0.166	−1.532	.126
Myint et al, 2007[10]	−0.276	0.150	−0.570	0.018	−1.838	.066
Sublette et al, 2011[12] *	−0.115	0.253	−0.611	0.381	−0.453	.650
Xu et al, 2012[9]	−1.014	0.293	−1.589	−0.438	−3.454	.001
Present Study	−0.297	0.165	−0.621	0.027	−1.795	.073
Total	−0.603	0.114	−0.827	−0.379	−5.277	.000



B.



[a]The forest plot of 11 comparisons for meta-regression shows statistical data and effect size on each trial and result of quantitative synthesis (A). Scatter plot and regression line depict the result of meta-regression analysis. Those circles represent each trial (B). Our selected method for estimating was "method of moments," which is a mixed-effects model rather than fixed-effect model, for carrying out this meta-regression.
*As described in the Meta-Analytic Method section, values of subgroups of patients (n, SD, mean) were united into one group.
Abbreviation: HDRS-17 = Hamilton Depression Rating Scale 17-item version.

concentrations than controls, suggesting that MDD is associated with low plasma concentration in our Japanese sample, which is in accordance with the results of the meta-analysis.

The initial meta-analysis on the total subjects indicated a heterogeneity and a publication bias. The heterogeneity may have resulted from differences in demographic and clinical characteristics, including medication across the studies. After adjustment of the publication bias, the effect size became somewhat lower (Hedges *g* of −0.45).

When the meta-analysis was performed only for patients without psychotropic medication, the obtained effect size (Hedges *g* of −0.84, ie, a large effect size) became substantially higher than that in the total subjects, suggesting that the observed difference in tryptophan concentration between patients and controls is not attributable to medication.

Rather, medication may have reduced the difference between patients and controls.

With regard to the possible correlation between depression severity and plasma tryptophan levels, we obtained no evidence for such a correlation in our Japanese sample. In the meta-regression analysis, however, we found a small but significant correlation between severity and plasma tryptophan. The failure to detect the correlation in our sample might be due in part to the small effect and that the majority of our subjects were medicated.

There might be several mechanisms underlying the association between MDD and decreased plasma tryptophan levels. Recent studies have suggested the stress- and inflammation-related mechanisms. There are enzymes to degrade tryptophan to kynurenine: tryptophan 2,3-dioxygenase (TDO) and IDO. TDO is highly expressed in the liver and activated by glucocorticoids (ie, cortisol in humans).[43,44] In line, both patients and control subjects who were administered dexamethasone, a synthetic glucocorticoid, showed lower plasma tryptophan concentrations.[24] Many studies, including ours, demonstrated that patients with MDD show hypercortisolism.[45,46] Therefore, increased enzymatic activity of TDO due to hypercortisolism is a mechanism underlying the observed reduction in plasma tryptophan levels in patients with MDD.

IDO may also play a role, since proinflammatory cytokines induce IDO activation,[47,48] and cytokine levels are elevated in MDD patients.[49] In line, a drastic fall of plasma tryptophan was observed in patients with inflammatory disorder and in those patients receiving immunotherapy.[3] Indeed, the immune system activation by hepatitis C virus infection or chronic interferon-α administration increases prevalence of MDD.[50,51] Moreover, we found higher interleukin-6 levels in cerebrospinal fluid (CSF) of MDD patients compared with controls,[52] suggesting neuroinflammation in at least a portion of the patients. In the brain, IDO is expressed in astrocytes and microglial cells. In astrocytes, kynurenine is converted to kynurenic acid, which has a neuroprotective effect by antagonizing glycine coagonist site of *N*-methyl-D-aspartate (NMDA) receptor.[53] In microglial cells, by contrast, kynurenine is predominantly converted to quinolinic acid or 3-hydroxykynurenine, which have a neurotoxic effect through agonizing the NMDA receptor.[53] Therefore, inflammation-induced activation of IDO and microglial cells might be another mechanism.

Since tryptophan is an essential amino acid, it is also possible that the dietary intake of tryptophan might be decreased in patients with MDD. The tryptophan depletion procedure is known to precipitate low mood and other symptoms of depression in vulnerable subjects and there is some evidence that tryptophan loading is effective as a treatment for depression (reviewed by Parker and Brotchie[54]). However, there is little information on the dietary tryptophan intake in depressed patients. In a population-based prospective study of 29,133 men in Finland whose intake of amino acids was calculated from a diet history questionnaire, there was no significant association between reduced dietary intake of tryptophan and depressed mood.[55] However, a possibility remains that tryptophan intake may be specifically important for depressive symptoms in persons with a diagnosed depressive disorder, as opposed to depressive symptoms within a general population. Further studies are warranted to see whether the dietary intake contributes to the observed decrease in plasma tryptophan levels in MDD.

There are several limitations in the study. We measured only total tryptophan level, so we could not address whether free tryptophan levels were different between the MDD patients and controls. In our case-control study, the measurement of plasma tryptophan level was done in the "real world" setting, ie, we did not control for fasting, time of sampling, or medication. The majority of previous studies controlled fed status (ie, overnight fasting). With respect to timing of sampling, there was no significant difference in the timing of measurement between the 2 groups (data not shown). The majority of our subjects were medicated. Benzodiazepines increase free tryptophan concentration in rat serum,[56] although conflicting negative results have also been reported in humans.[57] Antidepressants such as citalopram decrease TDO activity,[58] which may have increased plasma tryptophan level in medicated MDD patients. Therefore, medication is likely to have minimized rather than exaggerated the difference in plasma tryptophan level between our patients and controls. This is consistent with the results of our meta-analysis. Our cross-sectional study precludes us from elucidating the cause-effect relationship between low plasma tryptophan and the development of MDD. In addition, plasma tryptophan concentration may not be an index for brain tryptophan level.[59] To examine brain tryptophan levels, analyses of CSF tryptophan levels in MDD patients are currently underway. In the meta-analysis, we did not search for the literature outside of the PubMed database, which may have caused us to miss some studies included in other databases.

In conclusion, in spite of these limitations, the present study clearly indicated that MDD is associated with lower plasma tryptophan levels. Although the majority of previous studies were from Western populations, results of our case-control study are in accordance with those of Western studies regardless of differential lifestyle and dietary habits. If there is any correlation between plasma tryptophan level and depression severity, the effect size would be small.

Drug names: citalopram (Celexa and others), diazepam (Diastat, Valium, and others), imipramine (Tofranil and others).

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Potential conflicts of interest: None reported.

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Increased protein and mRNA expression of resistin after dexamethasone administration

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Abstract:	Background: Synthetic glucocorticoids such as dexamethasone are widely used to treat a variety of inflammatory and autoimmune conditions but may induce adverse events including hyperglycemia. To shed light on the effect and action mechanism of dexamethasone, we examined the alterations of gene expression levels caused by dexamethasone. Methods and Results: Microarray analysis was performed on whole blood collected from 24 physically healthy subjects at baseline and after dexamethasone administration. The expression levels of resistin mRNA were found to be significantly increased after the dexamethasone administration. In a separate sample of 12 subjects, we examined plasma resistin protein levels and found that they were increased after dexamethasone administration. Furthermore, the plasma mRNA and protein levels of resistin were significantly higher in individuals who carried the A allele of RETN single nucleotide polymorphism rs3219175 than in those who did not carry the allele. There was no significant interaction between the genotype and dexamethasone administration. No significant

	correlation was found between plasma levels of cortisol and resistin. Conclusions: Consistent with previous studies, the genotype of RETN rs3219175 was a strong determinant of resistin levels. The present study showed that oral administration of dexamethasone increases the protein and mRNA levels of resistin irrespective of the rs3219175 genotype.

Endocrine Care

Increased protein and mRNA expression of resistin after dexamethasone administration

Running title: Dexamethasone alters resistin expression

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Abstract

Background: Synthetic glucocorticoids such as dexamethasone are widely used to treat a variety of inflammatory and autoimmune conditions but may induce adverse events including hyperglycemia. To shed light on the effect and action mechanism of dexamethasone, we examined the alterations of gene expression levels caused by dexamethasone.

Methods and Results: Microarray analysis was performed on whole blood collected from 24 physically healthy subjects at baseline and after dexamethasone administration. The expression levels of resistin mRNA were found to be significantly increased after the dexamethasone administration. In a separate sample of 12 subjects, we examined plasma resistin protein levels and found that they were increased after dexamethasone administration. Furthermore, the plasma mRNA and protein levels of resistin were significantly higher in individuals who carried the A allele of *RETN* single nucleotide polymorphism rs3219175 than in those who did not carry the allele. There was no significant interaction between the genotype and dexamethasone administration. No significant correlation was found between plasma levels of cortisol and resistin.

Conclusions: Consistent with previous studies, the genotype of *RETN* rs3219175 was a strong determinant of resistin levels. The present study showed that oral administration of dexamethasone increases the protein and mRNA levels of resistin irrespective of the rs3219175 genotype.

Keywords: microarray analysis; gene expression; single nucleotide polymorphism

Introduction

Glucocorticoids exert various biological effects and regulate numerous physiological processes including metabolic, immunologic, cardiovascular, and endocrine activities. Synthetic glucocorticoids are used therapeutically to suppress allergic, inflammatory, and autoimmune disorders. Due to their nonselective biological activities, however, the use of glucocorticoid drugs can lead to unwanted side effects such as hyperglycemia, weight gain, adrenal insufficiency, osteoporosis, glaucoma, and cataracts [1]. Furthermore glucocorticoids with high mineralocorticoid activity may exert excess mineralocorticoid effects such as fluid retention and potassium depletion.

Dexamethasone is a synthetic glucocorticoid with high glucocorticoid activity and negligible mineralocorticoid effects. It is widely used for the treatment of a variety of inflammatory and autoimmune conditions. Although generally safe and effective, adverse events associated with glucocorticoid actions may occur in some patients. Further understanding of the mechanism through which dexamethasone induces various effects is required.

Previous studies showed that a large number of genes are up- or down-regulated by dexamethasone administration in a cell-type specific way [2]. Galon et al [3] showed that expression levels of several genes were altered in human peripheral blood mononuclear cells when treated with dexamethasone. They revealed that dexamethasone administration induced the expression of cytokine, chemokine, and complement family members while repressing the expression of adaptive immune-related genes. Other studies showed alterations of gene expression induced by dexamethasone in a variety of human cells such as hepatocytes [4], glomerular podocytes [5], and mesenchymal stem cells [6].

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To shed light on the effect and action mechanism of dexamethasone, we examined the alterations of gene expression levels caused by oral administration of dexamethasone in human subjects. To our knowledge, only one study has previously examined the effects of systemic administration of dexamethasone on gene expression in human peripheral blood. Menke et al [7] examined whole blood gene expression before and 3 hours after oral dexamethasone administration and revealed various genes acutely regulated by dexamethasone. In the present study, we investigated the late *in vivo* effect of dexamethasone on gene expression by examining the overnight change in overall gene expression levels induced by dexamethasone administration.

The results of the microarray analysis in the present study showed that the expression levels of resistin were significantly higher after dexamethasone administration. Then we went on to examine plasma protein levels of resistin in a separate sample. Furthermore, since previous studies reported that the single nucleotide polymorphism (SNP) rs3219175 of resistin gene (*RETN*) influences the gene [8] and protein [9] expression levels of resistin, we examined whether there is an interaction between the genotype of this SNP and dexamethasone administration on mRNA expression and plasma protein levels of resistin.

Materials and Methods

Subjects

RNA microarray analysis was carried out at baseline and after dexamethasone administration (post-DEX) in 24 subjects (14 men and 10 women, mean age ± standard deviation = 39.9 ± 7.8 years). A separate sample of 12 subjects (6 men and 6 women, 39.6 ± 10.5 years) was examined for baseline and post-DEX plasma resistin

protein levels. All subjects were biologically unrelated Japanese who were recruited from the outpatient clinic of the National Center Hospital, National Center of Neurology and Psychiatry, Kodaira, Tokyo, Japan or through advertisements in free local information magazines and by our website announcement. Most of the subjects participated in our previous studies [8,10], one of the aims of which was to examine the gene expression levels of those with psychiatric disorders. Therefore, approximately half of the subjects were diagnosed with a psychiatric disorder, but all were physically healthy and without clinically significant systemic disease (e.g., malignant disease, diabetes mellitus, hypertension, renal failure, or endocrine disorders), based on self-reports, at the time of assessment. The study protocol was approved by the ethics committee at the National Center of Neurology and Psychiatry, Japan. After description of the study, written informed consent was obtained from every subject.

Microarray methods

Baseline and post-DEX gene expression levels in whole blood were measured using the venous blood samples collected on two days that were 41.8 ± 42.7 (mean ± standard deviation) days apart. Baseline level was examined with blood collected at 1000 h, and post-DEX level was examined with blood collected at 1500 h with 1.5 mg of dexamethasone administered orally at 2300 h the previous day. Venous blood was collected in PAXgene tubes (Qiagen, Valencia) and was incubated at room temperature for 24 hours for RNA stabilization. RNA was extracted from whole blood according to the manufacturer's guidelines by using the PAXgene Blood RNA System Kit (PreAnalytix GmbH, Hombrechtikon, Switzerland). The RNA was quantified by optical density readings at A260nm by using

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the NanoDrop ND-1000 (Thermo Scientific, Rockford). Gene expression analysis was performed using Agilent Human Genome 4 × 44 K arrays (Agilent Technologies, Santa Clara). Raw signal data were analyzed by the GeneSpring GX software (Agilent Technologies). Data were filtered according to the expression level for quality control to eliminate genes that were below the 20th percentile threshold. The expression value of each gene was normalized to the median expression value of all genes in each chip. After quality control, 31,287 probes were included in the analysis.

Plasma protein measurement

All venous blood samples were collected at 1000 h on two consecutive days, with dexamethasone 0.5 mg administered orally on the first day at 2300 h. The samples were immediately transferred on ice, centrifuged at 3000 × g, aliquoted, and stored at -80°C until they were assayed. Plasma resistin protein levels were determined using a commercially available immunoassay kit (Abnova, Taiwan) according to manufacturer's instructions. The mean minimum detectable dose of the kit was 1 ng/ml. All samples were assayed in duplicate on the same plate. The mean intra-assay coefficient of variation was 6.96%. Plasma levels of cortisol were measured by radioimmunoassay at SRL Corporation (Tokyo, Japan). The detection limit for cortisol was 1.0 μg/dl.

Genotyping

Genomic DNA was prepared from the venous blood according to standard procedures. The rs3219175 polymorphism was genotyped using the TaqMan 5'-exonuclease allelic discrimination assay. Thermal cycling conditions for polymerase chain reaction (PCR) were 1 cycle at 95°C for 10 minutes followed by 50 cycles of

92°C for 15 seconds and 60°C for 1 minute. The allele-specific fluorescence was measured with ABI PRISM 7900 Sequence Detection Systems (Applied Biosystems, Foster City).

Statistical analysis

Baseline and post-DEX gene expression levels in microarray data were compared using the paired *t*-test. Bonferroni correction with the number of probes analyzed was used to adjust for multiple testing. Because the plasma resistin levels were not normally distributed, they were log-transformed to pass the Shapiro-Wilk normality test ($P = 0.93$ for baseline levels and $P = 0.66$ for post-DEX levels after transformation). A repeated measures analysis of variance (ANOVA) was performed with dexamethasone administration as within-subjects factors and genotype as between-subjects factors. The results of the repeated measures ANOVA were also confirmed by nonparametric tests using Wilcoxon signed-rank test for comparison between baseline and post-DEX resistin levels and Mann-Whitney test for comparison between resistin levels in individuals who carried the A allele of rs3219175 (A carriers) and who did not (non-A carriers). Since there was only one individual homozygous for the A allele, AA and GA genotypes were combined in the analysis. The effects of age and body mass index (BMI) on resistin levels were examined by Pearson's correlation coefficient (r). Mann-Whitney test was used to compare resistin levels between men and women and also between those with and without psychiatric disorders. All statistical analyses were performed using IBM SPSS Statistics Version 21 (IBM SPSS, Tokyo, Japan). All statistical tests were two-tailed, and $P < 0.05$ indicated statistical significance.

Results

Table 1 shows the clinical characteristics of the A carriers and non-A carriers of *RETN* rs3219175. No significant difference in clinical characteristics was observed between the two groups. Table 2 lists 4 probes which showed a significant change in gene expression levels in whole blood after dexamethasone administration (Bonferroni-corrected $P < 0.05$). The highest significance was obtained for the probe of *RETN*.

Figure 1A shows the baseline and post-DEX gene expression levels of resistin and the rs3219175 genotype of each subject. Figure 2 shows the mean fold change after dexamethasone administration. A repeated-measures ANOVA with dexamethasone administration as a within-subjects factor and genotype as a between-subjects factor yielded a significant within-subjects effect [$F(1, 22) = 94.0$, $P < 0.0001$] and a significant between-subjects effect [$F(1, 22) = 147.3$, $P < 0.0001$]. There was no significant interaction effect between the genotype and dexamethasone administration [$F(1,22) = 0.13$, $P = 0.72$]. Nonparametric tests also showed significant effects of dexamethasone administration and genotype. Wilcoxon signed-rank test revealed a significant increase in the gene expression levels of resistin after dexamethasone administration ($Z = 4.286$, $P < 0.0001$). Mann-Whitney test showed that the expression levels of resistin were significantly higher in A carriers of rs3219175 than in non-A carriers ($Z = 4.157$, $P < 0.0001$ for both baseline and post-DEX).

Figure 1B shows the baseline and post-DEX plasma protein levels of resistin and the rs3219175 genotype of each subject. Mean baseline plasma levels of resistin in A carriers and non-A carriers were 7.9 ± 2.3 ng/ml and 3.9 ± 1.0 ng/ml, respectively. Mean post-DEX plasma levels of resistin in A carriers and non-A carriers

were 8.5 ± 3.6 ng/ml and 2.4 ± 0.9 ng/ml, respectively. A repeated-measures ANOVA with dexamethasone administration as a within-subjects factor and genotype as a between-subjects factor yielded a significant within-subjects effect [$F(1,10) = 7.4$, $P = 0.021$] and a significant between-subjects effect [$F(1,10) = 14.2$, $P = 0.004$]. There was no significant interaction effect between the genotype and dexamethasone administration [$F(1,10) = 1.6$, $P = 0.23$]. Nonparametric tests also showed significant effects of dexamethasone administration and genotype. Wilcoxon signed-rank test revealed significant increase in the plasma resistin levels after dexamethasone administration ($Z = 2.275$, $P = 0.023$), and Mann-Whitney test showed that the plasma levels of resistin were significantly higher in A carriers than in non-A carriers ($Z = 2.722$, $P = 0.004$ for baseline and $Z = 2.562$, $P = 0.009$ for post-DEX).

Mean baseline plasma levels of cortisol in A carriers and non-A carriers were 11.0 ± 4.4 µg/dl and 12.4 ± 5.1 µg/dl, respectively. Mean post-DEX plasma levels of cortisol in A carriers and non-A carriers were 2.8 ± 4.1 µg/dl and 2.4 ± 2.9 µg/dl, respectively. Plasma cortisol levels were significantly decreased after dexamethasone administration ($Z = 3.059$, $P = 0.002$). No significant difference in plasma cortisol levels was observed between A carriers and non-A carriers ($Z = 0.481$, $P = 0.70$ for baseline and $Z = 0.243$, $P = 0.82$ for post-DEX). No significant correlation of baseline or post-DEX cortisol levels with baseline or post-DEX resistin levels was observed.

There was no significant correlation of age with baseline or post-DEX mRNA or protein levels (data not shown). There was no significant correlation of BMI with baseline or post-DEX mRNA or protein levels (data not shown). There was no significant difference between men and women in baseline or post-DEX mRNA or protein

levels (data not shown). There was no significant difference between those with and without psychiatric disorders in baseline or post-DEX mRNA or protein levels (data not shown).

Discussion

The gene expression microarray analysis using the whole blood samples of human subjects revealed that mRNA expression of resistin was increased the day after dexamethasone administration. A consistent finding was obtained with plasma protein levels of resistin in a separate sample group. To our knowledge, the present study is the first to show that oral administration of dexamethasone significantly increases the blood levels of resistin in human subjects.

A few studies have examined the influence of oral glucocorticoid administration on resistin levels in human subjects. Menke et al [7] compared gene expression profiles in peripheral blood before and 3 hours after oral administration of dexamethasone. Their study revealed various genes that were upregulated or downregulated by dexamethasone; however, resistin was not included in the list of significantly regulated genes. Tanaka et al [11] showed that serum resistin level decreased after 3 weeks of prednisolone therapy in patients with systemic autoimmune diseases. The varying results between studies may be partly due to difference in duration after glucocorticoid administration in which the expression levels were examined. Menke et al [7] examined the acute effects of dexamethasone administration while the present study examined the later effects. Tanaka et al [11], on the other hand, examined the effects of chronic administration of glucocorticoid.

Several lines of evidence indicate involvement of resistin in inflammation in humans. According to Lehrke et al [12], circulating

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resistin levels increase 8-16 hours after lipopolysaccharide injection in healthy subjects. The expression of human resistin in macrophages is induced in response to proinflammatory cytokines such as interleukin-1β (IL-1β), IL-6, and tumor necrosis factor alpha (TNF-α) [13]. Therefore, inhibition of cytokine expression by dexamethasone [14] may result in decreased expression of resistin, as reported by Tanaka et al [11].

The present study showed a conflicting result that resistin expression is increased by dexamethasone. Some studies, however, have also shown findings in line with our results. A recent study reported that resistin protein secretion from human monocyte-enriched mononuclear cells increased after *in vitro* exposure to dexamethasone for 24 hours [15]. Lewandowski et al [16] reported a nearly significant (P = 0.051) increase in serum levels of resistin 24 hours after the oral dexamethasone suppression test in obese subjects. A study in neonatal rats showed that dexamethasone administration caused an increase in serum resistin levels [17]. Positive correlation between saliva cortisol concentration and plasma resistin levels reported by Weber-Hamann et al [18] also suggests an association between increased glucocorticoid activity and elevation of resistin levels. On the other hand, our results showed no association between plasma cortisol and resistin levels. The mechanism of the increase in resistin remains to be investigated. It may be in response to the decrease in insulin sensitivity [19] or enhancement in adipocyte differentiation [20] induced by dexamethasone.

Table 3 shows the alterations of resistin levels in human samples induced by glucocorticoid administration reported in present and previous studies. Although data are insufficient to determine the dose and time effect of glucocorticoid on resistin