The role of BDNF in depression : will the neurothrophin hypothesis sparkle on, long after the glitter of the firework is gone?

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CHAPTER 4

Serum BDNF concentrations in major depressive disorder: state-trait issues, clinical features and pharmacological treatment

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SIGNIFICANCE: This paper reveals that low serum BDNF concentrations are a state characteristic of depression and that this normalizes following natural remission and in the course of antidepressant treatment. Critically however is that we show that the effect-sizes on these associations are small and that normalization of serum BDNF concentrations is not necessarily associated with a relief of depressive symptoms.

ABSTRACT

Recent evidence supports *the neurotrophin hypothesis of depression* in its prediction that Brain-Derived Neurotrophic Factor (BDNF) is involved in depression. However, some key questions remain unanswered, including whether abnormalities in BDNF persist beyond the clinical state of depression, whether BDNF concentrations are related to the clinical features of depression and whether distinct antidepressants affect BDNF concentrations equally. We addressed these questions and investigated serum BDNF concentrations in 962 depressed patients, 700 fully remitted persons (> 6 months) and 382 healthy controls. We found serum BDNF concentrations to be low in antidepressant-free depressed patients relative to controls (effect size, Cohen's *d*) = 0.19, $P < 0.01$) and to depressed patients who were treated with an antidepressant ($d = 0.23$, $P < 0.01$). BDNF concentrations of fully remitted persons (whether unmedicated or treated with an antidepressant) were comparable to those of healthy controls. Analyzing the sample of antidepressant-free depressed patients showed that BDNF concentrations were unrelated to the core clinical features of depression such as its severity or having a first versus a recurrent episode. The to antidepressant use associated up-regulation of serum BDNF concentrations in depressed patients was confined to selective serotonin reuptake inhibitors ($d = 0.39$, $P < 0.01$) and St John's wort ($d = 0.63$, $P = 0.03$). Our results suggest that low serum BDNF concentrations are a state abnormality that is evident during depression and that normalizes during remission. Increases in serum BDNF concentrations during antidepressant treatment appear to be confined to some antidepressants and do not parallel clinical characteristics, such as the severity of depressive symptoms.

INTRODUCTION

Brain-Derived Neurotrophic Factor (BDNF) is a neurotrophin that has been linked to the viability of neurons in brain circuits that regulate emotion, memory, learning, sleep and appetite (Duman *et al*., 2000; Sutton and Schuman, 2006). The neurotrophin hypothesis of depression is based on these functions of BDNF and postulates that depression results from stress-induced decreases in BDNF expression and that antidepressants are efficacious because they increase BDNF expression (Duman *et al*., 1997; Duman and Monteggia, 2006). Consistent with this hypothesis are the findings that depression is accompanied by decreased central and peripheral concentrations of BDNF (Sen *et al*., 2008), and that antidepressants elicit an increase in BDNF concentrations in animal models for depression (Angelucci *et al*., 2005) and in depressed humans (Brunoni *et al*., 2008). Together with the latency of weeks before antidepressants become clinically effective (Nemeroff and Owens, 2002), these observations shaped the hypothesis that the efficacy of antidepressants depend on neuroadaptive changes that are brought about by changes in BDNF signaling (Duman and Monteggia, 2006).

Taken together, there is reason to believe that BDNF is involved in depression and in antidepressant action. Results inconsistent with the neurotrophin hypothesis, however, also have been reported. There are, for example, studies that did not detect alternations in BDNF in depressed persons or in the course of treatment with an antidepressant (Matrisciano *et al*., 2009). In addition, some questions remain unanswered so that the neurotrophin hypothesis is at best incomplete (Groves, 2007). A major question that needs to be answered is whether low BDNF concentrations persist beyond the clinical state of depression (Trajkovska *et al*., 2008). A second question is whether BDNF concentrations are related to the clinical features of depression, such as having a first versus a recurrent episode (Lee *et al*., 2007). Yet a third outstanding question is whether all classes of antidepressants affect BDNF concentrations equally. We therefore studied, cross-sectionally, serum BDNF concentrations of depressed patients, remitted depressed persons and never depressed persons. Our efforts had three concerns: (1) to compare serum BDNF concentrations of antidepressant-free and antidepressant treated current and fully remitted depressed patients and never depressed persons, (2) to explore the associations between some of the core clinical features of depression and serum BDNF concentrations and (3) to evaluate the association between the use of several distinct classes of antidepressants and serum BDNF concentrations.

METHOD

Patients and sample collection

Patients were from the Netherland Study of Depression and Anxiety (NESDA). Full details on the rationale, objectives and protocol of NESDA are described in Penninx *et al*. (2008). In brief, NESDA is a prospective cohort study (*N* = 2,981) that recruited patients in mental health care, primary care and in the general population. Included were persons with a depressive and/or an anxiety disorder, persons with a depressive and/or an anxiety disorder in remission and persons without a history of these disorders. Persons who were diagnosed with a psychotic disorder, bipolar disorder (type I and II), obsessive-compulsive disorder, or severe alcohol use disorder were not eligible. Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV) diagnoses (APA, 1994) were assigned on the basis of responses to the Composite International Diagnostic Interview 2.1 lifetime version (CIDI 2.1; Wittchen *et al*., 1991) that was administered by trained interviewers. At baseline, participants provided blood samples, underwent a medical examination and gave written informed consent for the study that was approved by the Ethical Committees of the participating institutes.

Our study enrolled 2,044 persons (68.6% of the NESDA sample). On the basis of the assigned diagnosis, antidepressant use and the availability of BDNF data, we created five groups: antidepressant-free

depressed patients (*n* = 541), antidepressant-treated depressed patients (*n* = 421), antidepressant-free remitted depressed persons (*n* = 539), antidepressant-treated remitted depressed persons (*n* = 161) and healthy persons who served as controls (*n* = 382). Depressed patients met the criteria for a depressive episode the last 6 months (*n* = 541). The majority of these patients had a current diagnosis of depression (*n* = 388), but some (*n* = 153) had a diagnosis of depression 1–6 months prior to baseline and did not fulfill all criteria in the past month. Persons who were in full remission of depression were diagnosed with major depression somewhere in their lives, but had been free of depression and anxiety for at least 6 months. Persons were included in the control group when they had: (1) no lifetime mood or anxiety disorders, (2) no documented family history of depression or anxiety and (3) a low score (< 14) on the Inventory of Depressive Symptoms (IDS; Rush *et al*., 1996).

Data on antidepressants use were acquired through drug container observation and self-report. Use of an antidepressant was defined as intake of minimally the daily dose as recommended by the WHO (2010) during the last month on at least 50% of the days. We coded for the use of selective serotonin reuptake inhibitors (SSRIs), serotonin-norepinephrine reuptake inhibitors (SNRIs), tricyclic antidepressants (TCAs), noradrenergic and specific serotonergic antidepressants (NaSSAs) and St John's wort (Hypericum perforatum). The duration of antidepressant use was expressed in months.

Clinical features of depression

All patients were characterized on the symptom severity of depression using the IDS (Rush *et al*., 1996). Patient samples were further characterized on having a first or a recurrent depressive episode, the presence of comorbid anxiety, the age at onset of depression, the recency of depression, the chronicity of depression and on the presence of suicide ideation. The CIDI (Wittchen *et al*., 1991) served as source of information on the presence of a first- or a recurrent depressive episode, the presence of a comorbid anxiety, age at onset of depression (*i.e.,* the age at which the first episode occurred) and the recency of depression (*i.e.,* fulfilling criteria in the past month versus fulfilling criteria in the past 6 months but not in the past month). Depression was considered chronic if symptoms had been present for more than 24 months during the last 5 years, which was assessed using the life chart method (Lyketsos *et al*., 1994). The scale for suicide ideation (Beck *et al*., 1997) was used to examine the presence of suicide ideation.

BDNF measurements

A measure of 50 ml of blood was withdrawn into vacuum tubes between 0730 and 0930 hours after an overnight fast. After blood collection, serum was separated and stored at -85 C˚ until it was assayed. BDNF protein concentrations were measured using the Emax Immuno Assay system from Promega according to the manufacturer's protocol (Madison, WI, USA), in one laboratory by one technician who was blinded for the clinical diagnoses of the participants. Undiluted serum was acid treated as this reliably increased the detectable amount of BDNF in a dilution-dependent way. Greiner Bio-One high affinity 96-well plates were used. Serum samples were diluted 100 times, and the absorbency was read in duplicate using a Bio-Rad (Hercules, CA, USA) Benchmark microplate reader at 450 nm. The intra- and inter-assay coefficients of variation were found to be within 3 and 9%, respectively. Four persons had BDNF serum concentrations that were below the reliable detection threshold of 1.56 ng ml. These values were set at the lower detection limit. Positive outliers (mean **>** 3 SD, *n* = 6) were trimmed to the mean + 3 *SD* value. There were no differences between persons with missing and non-missing BDNF with regard to gender (*P* = 0.71), age (*P* = 0.67) and diagnoses (*P* = 0.33).

Covariates

Potential variance due to gender, age and educational level was controlled for in all analyses. In addition, we controlled for body mass index (BMI), physical activity and smoking as these variables are associated with BDNF (Suwa *et al*., 2006; Rojas-Vega *et al*., 2006) and mood (Simon *et al*., 2006; Harris *et al*., 2006; Kendler *et al*., 1993). Data on weight and height were collected, and BMI was calculated (weight/height^2). Information on physical activity was gathered using the International Physical Activity Questionnaire (IPAQ; Craig *et al*., 1995) and expressed as the number of met-minutes (*i.e.,* the ratio of the amount of energy expenditure during activity to the energy expenditure at rest). Smoking status was dichotomized as current versus non-smoker. Time of the morning blood withdrawal and duration of serum storage were controlled for since BDNF concentrations vary according to variation on these variables (Begliuomini *et al*., 2008; Trajkovska *et al*., 2007).

Statistical analyses

All computations were performed in SPSS version 17.0 (SPSS, Chicago, IL, USA). BDNF values were controlled for basic covariates in all analyses. Effect sizes on pair-wise comparisons were presented as Cohen's *d*. (Cohen, 1988). A two-tailed *P*-level of 0.05 was used to determine statistical significance.

Analysis of variance (ANOVA) was used to compare BDNF concentrations of antidepressant-free depressed patients and antidepressant-treated depressed patients, antidepressant-free patients and antidepressant- treated persons who were in remission (**<** 6 months) and controls. Post-hoc tests were performed following a significant *F*-statistic using Tukey's test.

A multivariable regression analysis was used to identify whether the clinical features of depression were associated with BDNF concentrations. Regression was performed in patient groups in which mean BDNF concentrations deviated significantly from the control group. Pearson correlation coefficients between predictors and BDNF concentrations were also calculated. Basic covariates were entered in the first step of regression. In the second step, the clinical features of depression were entered. The regression model was fit using method enter. Tolerance of the predictors and normality of error variances was verified.

To establish whether the use of an antidepressant effected BDNF concentrations equally in current and remitted depression, a 2 (currently depressed versus depression in (full) remission) times 2 (antidepressants; yes versus no) ANOVA was performed. Potential antidepressant-specific associations between the use of SSRIs, TCAs, SNRIs, NaSSAs and St John's wort and BDNF concentrations were evaluated by contrasting BDNF concentrations of persons who used one of these agents against the mean BDNF concentration of the antidepressant- free persons. Analyses were repeated with the severity of depressive symptoms and the duration of antidepressant use as covariates.

RESULTS

Demographics and clinical features

Demographical and clinical features among the five groups are given in Table 1. ANOVA and χ^2 tests showed that, compared with controls, depressed and remitted persons were more likely to be female, to be older, to have received fewer years of education and to smoke. BMI was higher in current and remitted antidepressant-treated depressed persons compared with controls and to antidepressant-free depressed and remitted persons. The amount of physical activity was low in the antidepressant treated currently depressed group relative to the other groups. Post-hoc comparisons on demographical and clinical features between the current and remitted depressed groups are given in **Table 1 ↓**.

BDNF concentrations in persons with current or remitted depression and controls

An ANOVA model showed a main effect of diagnostic status on serum concentrations of BDNF ($F_{1,\ 1578}$ =

4.09, *P* = 0.01). Pair-wise comparisons (see **Figure 1 ↓**) indicated that serum BDNF concentrations were low in antidepressant-free depressed patients compared with controls (*d* = 0.19), antidepressant-free persons who were in full remission (*d* = 0.15), and antidepressant-treated depressed patients (*d* = 0.23). BDNF concentrations of antidepressant-free persons who were in full remission and depressed patients who were treated with an antidepressant were comparable to those of controls.

Table 1. Demographic- and clinical characteristics (mean ± *STD* or percentages) of participants by depression diagnosis (never, current and remitted) and antidepressant use (no versus yes AD)

	Controls (*n* = 382)	Depressed (*n* = 541)	Depressed Antidepressant (*n* = 421)	Remitted (*n* = 539)	Remitted Antidepressants (*n* = 161)	*P*-value
Female (%)	61.0	66.7	67.0	71.1	70.8	< 0.05
Age	45.7 ± 12.3	39.8 ± 12.6	42.6 ± 11.0	43.1 ± 12.9	45.4 ± 10.8	< 0.001 [A,B]
Education (years)	13.4 ± 3.3	11.9 ± 3.2	11.7±3.3	12.6 ± 3.1	12.1 ± 3.3	< 0.001
Body mass index	25.4 ± 4.6	25.5 ± 5.4	26.3±5.6	25.3 ± 4.6	26.6 ± 5.6	< 0.01 [A,B]
Physical activity [C]	3.7 ± 3.0	3.5±3.3	3.2±3.3	3.8 ± 3.1	3.1 ± 2.8	< 0.01 [B]
Smoker	16.5	38.7	46.0	35.5	34.3	< 0.001 [A]
Alcohol dependent	5.4	23.3	20.0	17.0	13.7	< 0.001
Depression severity	5.3 ± 3.5	29.6 ± 12.7	34.5 ± 13.1	16.8 ± 10.3	20.3 ± 10.6	< 0.001 [A,B]
Age of onset of MDD	NA	26.1 ± 12.3	27.4 ± 12.6	27.6 ± 12.2	28.2 ± 11.7	0.35
Chronic depression [D]	NA	27.5	38.3	11.1	18.7	< 0.001 [A,B]
>1 episode	NA	63.6	58.2	54.6	61.5	< 0.05 [A]
Comorbid anxiety [E]	NA	42.2	47.7	NA	NA	< 0.05
Suicide ideation	NA	22.4	29.3	5.2	6.2	< 0.001 [A]
Antidepressant medication						
SSRI	NA	NA	62.7	NA	65.8	0.27
SNRI	NA	NA	16.4	NA	13.0	0.06
TCA	NA	NA	8.1	NA	13.0	0.19
NaSSA	NA	NA	8.6	NA	2.5	< 0.05
St John's wort	NA	NA	4.3	NA	5.6	0.32
Duration of use [F]	NA	NA	7.5 ± 4.9	NA	10.9 ± 3.5	< 0.001

Abbreviations: NaSSA, noradrenergic and specific serotonergic antidepressant; SNRI, serotonin and norepinephrine reuptake inhibitor; SSRI, selective serotonin reuptake inhibitor; TCA, tricyclic antidepressant

[A] Indicates a significant difference between the antidepressant treated and antidepressant free current depressed groups

[B] Indicates a significant difference between the antidepressant treated and antidepressant free remitted depressed groups

[C] Mean met-minutes (that is ratio of energy expenditure during activity to energy expenditure at rest) divided by 1000

[D] Symptoms were considered chronic if they were present for at least 24 months during the last 5 years

[E] Included social phobia, panic disorder with and without agoraphobia, agoraphobia and generalized anxiety disorder

[F] Duration of use is expressed in number of months

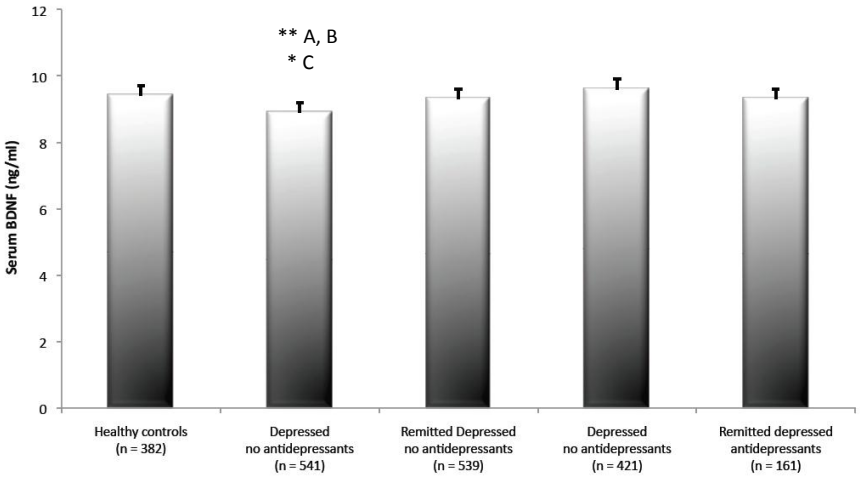

Figure 1. Plotted are mean serum BDNF concentrations by diagnosis and antidepressant status. Error bars reflect the *SEM*.

Serum BDNF concentrations are low in antidepressant-free depressed patients compared with controls (A: *d* = 0.19), antidepressant-free remitted persons (B: *d* = 0.15) and antidepressant-treated depressed patients (C: *d* = 0.23).

BDNF and the clinical features of depression

The exploration of the association between the clinical features of depression and serum BDNF was restricted to the antidepressant free currently depressed group, as BDNF concentrations in this group were low relative to controls. Pearson's correlation coefficients indicated that female gender and being in the early remission phase of depression (1–6 months) versus having a current episode were negatively associated with serum BDNF. Age, BMI, age at onset of depression and the presence of co-morbid anxiety were positively associated to serum BDNF (**Table 2 ↓**).

Basic covariates were entered in the first step of the multivariable regression analysis, followed by the clinical features that were entered in step two. Tolerance of the predictors was high (all > 0.70), indicating that our individual predictors were not redundant with one another. Error variances were normally distributed. Results of the first step showed that gender and age were significant predictors of BDNF concentrations. Women had lower concentrations of BDNF compared with men (ß = -0.10, *P* = 0.02) and older patients had higher concentrations of BDNF (ß = 0.11, *P* = 0.002) compared with younger patients. Results of the second step showed that none of the clinical features (listed in **Table 2 ↓**) was significantly associated with serum BDNF. Gender and age preserved its significance. BMI emerged as a significant (positive) predictor of serum BDNF. **Table 2 ↓** presents the results of the second step of the regression analysis.

BDNF and the use of antidepressants

A 2 (currently depressed versus depression in (full) remission) times 2 (antidepressant use; yes versus no) ANOVA showed that diagnostic status interacted with antidepressant use ($F_{1, 1578}$ = 4.19, *P* = 0.03), indicating that the use of an antidepressant during a depressive episode was associated with higher BDNF concentrations, whereas in the remission phase, the use of an antidepressant did not show such an association (see also **Figure 1 ↑**). Main effects of diagnostic status and antidepressant use were not observed. To uncover potential differences between various classes of antidepressants, we compared BDNF concentrations of depressed patients who used SSRIs, SNRIs, TCAs, NaSSAs or St John's wort among each other and those of antidepressant-free depressed patients. This analysis was restricted to the currently depressed group as the effect of the use of an antidepressant on serum BDNF concentrations was confined to this group. In this group, 67% (*n* = 282) used antidepressant for longer than 12 weeks. We observed a main effect of group ($F_{5, 941}$ = 4.29, *P* < 0.001). Post-hoc comparisons showed that, relative to not using an antidepressant, the use of SSRIs (*d* = 0.39) and St John's wort (*d* = 0.63) was associated with high

concentrations of BDNF. The use of a NaSSA was associated with low concentrations of BDNF relative to SSRI (*d* = 0.54) and St John's wort (*d* = 0.85) use. Analyses were run with and without co-varying for the severity of depressive symptoms and for the duration of antidepressant use. These analyses revealed a similar pattern of results. Furthermore, serum BDNF concentrations were unrelated to treatment duration (*r* = -0.02, *P* = 0.65), which might suggest that our findings were not driven by the duration of antidepressant use.

Table 2. Results of correlation and multivariable regression analyses of demographical and clinical characteristics with serum levels of BDNF in antidepressant free depressed patients (*n* = 541)

	r [A]	*B*	*95% CI B*	β	*P-value*
Gender (1 = male, 2 = female)	−0.13**	−0.65	−1.24 to −0.06	−0.10	0.03
Age (continuous, years)	0.17**	0.03	0.01 to 0.06	0.11	0.04
Education (continuous, years)	−0.04	−0.01	−0.09 to 0.08	−0.005	0.91
BMI (continuous)	0.13**	0.06	0.01 to 0.10	0.09	0.04
Physical activity (continuous per week)	−0.02	−0.001	−0.01 to 0.01	−0.009	0.83
Smoker (1=no, 2=yes)	−0.02	−0.07	−0.04 to 0.02	−0.02	0.66
Time of Blood withdrawal (continuous) [B]	−0.04	−0.004	−0.12 to 0.02	−0.04	0.23
Duration of serum storage (continuous)	0.02	0.14	−0.40 to 0.68	0.02	0.62
Current (= 1) vs. early remitted (= 2)[C]	−0.11*	−0.15	−0.50 to 0.25	−0.04	0.52
Severity (continuous)	0.03	−0.007	−0.04 to 0.02	−0.06	0.24
Single (= 1) vs. recurrent episode (= 2)	0.01	0.05	−0.56 to 0.66	0.007	0.88
Comorbid anxiety (1 = no, 2 = yes)	0.08*	0.31	−0.36 to 0.97	0.05	0.36
Age at onset (continuous)	0.14**	0.08	−0.04 to 0.18	0.07	0.21
Chronic depression (1 = no, 2 = yes)	0.07	0.19	−0.49 to 0.87	0.03	0.58
Suicide ideation (1 = no, 2 = yes)	0.06	0.59	−0.13 to 1.33	0.07	0.12

Abbreviations: BDNF, Brain-Derived Neurotrophic Factor; BMI, Body Mass Index; 95% *CI*, 95 percent *Confidence Interval*

[A] Univariate correlation with serum levels of BDNF; Pearson's *r* for continuous variables and Spearman's *ρ* for variables

[B] In minutes from 0600 hours

[C] The presence of a current (1 month) versus an early remission (1–6 months of remission) diagnosis

* Denotes statistical significance at *P* < .05

** Denotes statistical significance at *P* < .01

BDNF and the use of antidepressants

A 2 (currently depressed versus depression in (full) remission) times 2 (antidepressant use; yes versus no) ANOVA showed that diagnostic status interacted with antidepressant use ($F_{1, 1578}$ = 4.19, *P* = 0.03), indicating that the use of an antidepressant during a depressive episode was associated with higher BDNF concentrations, whereas in the remission phase, the use of an antidepressant did not show such an association (see also **Figure 1 ↑**). Main effects of diagnostic status and antidepressant use were not observed. To uncover potential differences between various classes of antidepressants, we compared BDNF concentrations of depressed patients who used SSRIs, SNRIs, TCAs, NaSSAs or St John's wort among each other and those of antidepressant-free depressed patients. This analysis was restricted to the currently depressed group as the effect of the use of an antidepressant on serum BDNF concentrations was confined to this group. In this group, 67% (*n* = 282) used antidepressant for longer than 12 weeks. We observed a main effect of group ($F_{5, 941}$ = 4.29, *P* < 0.001). Post-hoc comparisons showed that, relative to not using an antidepressant, the use of SSRIs (*d* = 0.39) and St John's wort (*d* = 0.63) was associated with high concentrations of BDNF. The use of a NaSSA was associated with low concentrations of BDNF relative to

SSRI (d = 0.54) and St John's wort (d = 0.85) use. Analyses were run with and without co-varying for the severity of depressive symptoms and for the duration of antidepressant use. These analyses revealed a similar pattern of results. Furthermore, serum BDNF concentrations were unrelated to treatment duration (r = -0.02, P = 0.65), which might suggest that our findings were not driven by the duration of antidepressant use.

DISCUSSION

Largely in accord with previous findings (Sen *et al*., 2008) and with the neurotrophin hypothesis of depression (Duman *et al*., 1997), our data showed that serum BDNF concentrations were low in antidepressant-free depressed patients compared with healthy controls. Our data further showed that BDNF concentrations were low in depressed patients who were not on antidepressant medication compared with antidepressant-free persons who were in full remission and that BDNF concentrations of this latter group were comparable to those of controls. Herewith, we establish as one of the first (Trajkovska *et al*., 2008) that low concentrations of BDNF in serum are a state characteristic of depression. In line with one study that reported low concentrations of BDNF in euthymic patients (Monteleone *et al*., 2008), we found that patients who were in early remission (1 – 6 months) had serum BDNF concentrations that were comparable to those of currently depressed patients. Thus, serum BDNF concentrations remain low after clinical improvement has set in. This could indicate that low concentrations of BDNF are a consequence of depressive symptoms that persist into early remission. Alternatively, the low concentrations of BDNF during early remission might also represent a scar of a depressive episode. These explanations could not be fully elucidated in the current study and longitudinal designs clearly need to be performed to understand this issue.

We were unable to replicate the earlier findings that a higher depression severity (Karege *et al*., 2002; Shimuzu *et al*., 2003) having a recurrent compared with a first episode of depression (Lee *et al*., 2007) and the occurrence of suicide ideation (Deveci *et al*., 2007; Kim *et al*., 2007) are accompanied by lower concentrations of BDNF. In fact, we even found that the early remission phase, which was accompanied by a lower symptom severity of depression (mean IDS scores were 22.4 ± 11.4 versus 32.4 ± 12.1 in early remitted and currently depressed patients respectively), was associated with somewhat lower BDNF concentrations compared with the current depressive state. The other clinical features (that is age at onset of depression, the presence of comorbid anxiety and the chronicity of depression) also were unrelated to serum BDNF in multivariable analyses. These findings, given the size of the current cohort, give us confidence in excluding the clinical features of depression as potential correlates of serum BDNF concentrations. This might be an important conclusion, as it hints that other (than specifically depression related) factors may be at play in the relative fall of BDNF concentrations during a depressive episode. Interestingly, being male and BMI were found to be positively associated with BDNF among antidepressant-free depressed patients. Although these findings were unsought, they parallel the results of some previous studies (Monteleone *et al*., 2005; Nakazato *et al*., 2003) and they give ground to interesting hypotheses. For example, as weight loss is a prime behavioral abnormality of depression (APA 1994) and often a residual symptom in early remission (Paykel 1985; Paykel *et al*., 1995) it could be that, alternations in BDNF concentrations are mediated by (transient) changes in eating behavior during, or in the aftermath of, a depressive episode. Likewise, weight gain is a documented side effect of antidepressant treatment (Kachur *et al*., 2005; Antilla and Leinonen, 2001) and thus the absence of weight loss could potentially explain the absence of a relative fall of BDNF in depressed patients during treatment with an antidepressant. Alternative factors that have been proposed to underlie the low concentrations of BDNF during depression are exposure to stressful life events. Two studies found that adverse life events are associated with lower

peripheral BDNF concentrations within a depressed and bipolar patient samples (Kauer-Sant'Anna *et al*., 2007; Grassi-Oliveira *et al*., 2008). Therefore, it seems worthwhile to integrate a wider range of variables, notably (early) adverse life events, but also genetic variants and their interactions with environmental variables (Gatt *et al*., 2009) in models that study the link between BDNF and depression.

In addition, we found that serum BDNF concentrations were higher in antidepressant-treated patients compared with patients who were antidepressant free. This finding largely is in accord with previous findings (Sen *et al*., 2008). We were able to expand previous findings by showing that the use of an antidepressant is associated with increased serum BDNF during a depressive episode but not during remission. This suggests that antidepressant-induced increases in BDNF occur in a disease state when BDNF functioning might be defective and not in remission when BDNF functioning is normalized. In addition, we found the increase in serum BDNF concentrations to be a specific associate of the use of SSRIs and St John's wort and not of the use of SNRIs, TCAs or NaSSAs. Although not directly confirmed, this finding might be explained by increased availability of extra-synaptic concentrations of serotonin. It is known that serotonin stimulates the expression of BDNF (Mattson *et al*., 2004; Martinowich and Lu, 2008). In line with this, we found the highest BDNF concentrations in patients who were treated with an agent that generally leads to an increase in the availability of serotonin, that is, SSRIs and St John's wort (Mann, 2005; Gaster and Holroyd, 2000). Furthermore, we found the lowest concentrations of BDNF in patients who were treated with agents that have little or no impact on the availability of serotonin, that is, NaSSAs (Kent, 2000; Antilla and Leinonen, 2001). Nevertheless, this antidepressant-specific finding seems at odds with the prediction of the neurotrophin hypothesis, stating that increases in BDNF concentrations are a key mediator for an antidepressant response to occur (Duman and Monteggia, 2006). According to this prediction, one might expect that antidepressants that are known to be about equally efficacious in the treatment of the symptoms of depression (Gaster and Holroyd, 2000; Kent 2000; Berton and Nestler, 2006) would have similar effects on serum BDNF concentrations. Yet another finding that seems hard to reconcile with the neurotrophin hypothesis is that the group of depressed persons who used antidepressants (for prolonged period and on a frequent base) had the highest BDNF concentrations, but also the highest symptom severity of depression. This suggests, to our belief that increases in peripheral BDNF concentrations do not parallel clinical effectiveness, or at least have no direct effects on depression characteristics such as its severity. Such a conclusion on the absence of direct effects could also be drawn on the findings that the severity of a depressive episode was unrelated to serum BDNF concentrations and that persons who were in early remission had similar concentrations of BDNF yet a marked lower depression severity as compared to currently depressed patients. Caution, however, is warranted when interpreting these findings because our patients were not randomly assigned to the various drugs (or no drug) conditions. Thus, our findings might be confounded by indication. An additional limitation of our study is that we relied on data that were collected in a single wave, precluding any form of causality. Furthermore, we measured serum concentrations of BDNF and assume that these measurements mirror the amount of BDNF in the brain. This assumption is validated on preclinical work that showed that cortical and peripheral concentrations of BDNF are correlated (Sartorius *et al*., 2009; Klein *et al*., 2010) but remains complicated, because in addition to neurons, several other tissues serve as sources of BDNF in serum (Karege *et al*., 2002). Various strengths of our study also seem evident and these include the use of multivariable techniques and the large sample size (that relates positive to all previous studies and to two previous meta-analyses (Brunoni *et al*., 2008).

In conclusion, we believe that our data indicate that low concentrations of BDNF in blood serum are a state characteristic of depression and thus an abnormality that is evident during the clinical state and the early remission phase of depression but not when the symptoms of depression are in full remission. Our findings further suggest that some of the core clinical features of depression are unrelated to serum

concentrations of BDNF. Finally, increases in serum concentrations of BDNF appear to be a specific pharmacological effect of a subset of antidepressants that does not parallel depression characteristics such as the severity of depression.