

# Influence of endogenous and exogenous sex hormones on plasma brain-derived neurotrophic factor

S.Begliomini<sup>1</sup>, E.Casarosa<sup>1</sup>, N.Pluchino<sup>1</sup>, E.Lenzi<sup>1</sup>, M.Centofanti<sup>1</sup>, L.Freschi<sup>1</sup>, M.Pieri<sup>1</sup>, A.D.Genazzani<sup>2</sup>, S.Luisi<sup>3</sup> and Andrea R.Genazzani<sup>1,4</sup>

<sup>1</sup>Department of Reproductive Medicine and Child Development, Division of Gynecology and Obstetrics, University of Pisa, Pisa, Italy, <sup>2</sup>Department of Obstetrics and Gynecology, University of Modena, Modena, Italy and <sup>3</sup>Department of Pediatric Obstetrics and Reproductive Medicine, Division of Obstetrics and Gynecology, University of Siena, Siena, Italy

<sup>4</sup>To whom correspondence should be addressed at: Department of Reproductive Medicine and Child Development, Division of Gynecology and Obstetrics, University of Pisa, Via Roma 35, 56100 Pisa, Italy. Tel.: +39 050 503985; Fax: +39 050 553410; E-mail: a.genazzani@obgyn.med.unipi.it

**BACKGROUND:** Brain-derived neurotrophic factor (BDNF) is a mediator of neuronal plasticity and influences learning, memory and cognitive behaviour. The aim of this study is to assess plasma BDNF variations according to hormonal status. **METHODS:** A total of 60 subjects were included: 20 fertile ovulatory women, 15 amenorrhoeic women and 25 postmenopausal women. Blood samples were collected after overnight fasting. For 5 out of the 20 fertile women, samples were collected every 2 days throughout the whole menstrual cycle. Following basal evaluation, 10 out of 25 postmenopausal women were administered a hormone replacement therapy (HRT) and reevaluated after 6 months of treatment. Plasma BDNF concentrations were measured by enzyme-linked immunosorbent assay. In fertile women, estradiol (E<sub>2</sub>), progesterone and gonadotrophins were also assessed. **RESULTS:** In fertile women, luteal phase levels of plasma BDNF were significantly higher than follicular phase levels ( $P < 0.001$ ). BDNF increased from early follicular phase up to Day 14 of the cycle, reaching a pre-ovulatory peak, similar to E<sub>2</sub>. A second rise took place during mid-luteal phase, with a peak on Day 24. Amenorrhoeic subjects, as well as postmenopausal women, showed significantly lower plasma BDNF levels compared with fertile females ( $P < 0.001$ ). BDNF was positively correlated with E<sub>2</sub> and progesterone and negatively correlated with menopausal age. HRT restored BDNF levels to those present in fertile women during the follicular phase. **CONCLUSIONS:** Plasma BDNF levels are influenced by hormonal status. Modifications in BDNF circulating levels during the menstrual cycle suggest a potential role for gonadal sex hormones (E<sub>2</sub> and progesterone) in regulating neurotrophin expression.

*Key words:* brain-derived neurotrophic factor/hormone replacement therapy/menopause/ovarian cycle/sex hormones

## Introduction

Brain-derived neurotrophic factor (BDNF) is a member of the neurotrophin family (NT) and is abundantly expressed in the central and peripheral nervous system, particularly in the hippocampus, cerebral cortex (especially in the temporal and occipital area, insula, motor and sensitive cortex) and amygdala (Murer *et al.*, 2001). Its chemical structure is that of a small homodimeric protein (P.M. 28 kDa), containing a cystine cluster (*cystine knot motif*) and a  $\beta$ -antiparallel strand that confer a 3D extended configuration, which is typical of other growth factors [transforming growth factor- $\beta$ 2, platelet-derived growth factor, vascular endothelial growth factor (VEGF)], thus suggesting a probable common evolution (Van Kesteren *et al.*, 1998).

BDNF is known to cross the blood-brain barrier in both directions, and a physiologically relevant amount of circulating

BDNF might derive from neurons and glia cells of the central nervous system (Pan *et al.*, 1998; Karege *et al.*, 2002a,b). Although it was originally described in the nervous system, BDNF has been shown to be expressed in a variety of non-neuronal cells (Yamamoto *et al.*, 1996). In fact, potential additional sources are represented by vascular endothelial and smooth muscle cells (Donovan *et al.*, 1995; Nakahashi *et al.*, 2000). Moreover, evidences suggest that elements of the immune system, such as activated macrophages and lymphocytes, could contribute to BDNF production (Gielen *et al.*, 2003).

BDNF is already present in fetal life and its expression rises to a maximal level after birth, promoting neuronal outgrowth and differentiation in neonates (Con Over and Yancopoulos, 1997). In adults, it plays a predominant functional role: it has been shown to induce long-lasting

changes in synaptic plasticity, neurotransmitter and neuropeptide production and excitability (Kang and Schuman, 1995; Li *et al.*, 1998; Carter *et al.*, 2002) and to play a key role in learning, memory and behaviour (Hall *et al.*, 2000; Egan *et al.*, 2003).

BDNF is also present in human plasma and, since platelets represent a major storage site of BDNF in peripheral blood, serum levels are higher than plasma levels. It has been shown that plasma concentrations decrease significantly with age or weight gain, whereas platelet or serum levels do not seem to be altered (Lommatzsch *et al.*, 2005). Modifications in platelet BDNF have been reported in women throughout the menstrual cycle (Lommatzsch *et al.*, 2005): studies have shown higher levels during the luteal phase than in the follicular phase. This may be due to the fact that a relevant amount of this protein derives from the endometrium, since experimental studies have reported that BDNF mRNA and protein are present in the uterus of rodents (Lommatzsch *et al.*, 1999).

Several studies have shown an altered BDNF production and secretion in a variety of diseases. Neurodegenerative disorders such as Alzheimer's and Parkinson's diseases seem to be associated with decreased levels of BDNF in the brain (Connor *et al.*, 1997; Parain *et al.*, 1999), and low serum levels of BDNF are demonstrated in major depression (Karege *et al.*, 2002a,b), schizophrenia (Toyooka *et al.*, 2002) and eating disorders such as bulimia and anorexia nervosa (Nakazato *et al.*, 2003; Monteleone *et al.*, 2005). In contrast, subjects affected by epilepsy show an increase in central and peripheral BDNF levels (Ernfors *et al.*, 1991).

Modifications in rat hippocampus BDNF levels during the estrous cycle and the effect of estradiol (E<sub>2</sub>) replacement after ovariectomy on BDNF mRNA and protein levels, suggest a strong correlation between BDNF functional role and reproductive system (Gibbs, 1999; Scharfman *et al.*, 2003; Singh *et al.*, 2005).

Recently, BDNF has been shown to be present in the follicular fluid, both in normally cycling women (Seifer *et al.*, 2003) and in women undergoing ovulation induction for IVF (Seifer *et al.*, 2002). Furthermore, BDNF levels in follicular fluid seem to be up-regulated by gonadotropin stimulation, since BDNF levels were 27 times higher in follicular fluid of women undergoing ovulation induction when compared with normally cycling women (Seifer *et al.*, 2002).

In the last years, BDNF assays have become an attractive target for neuropsychiatric research in humans. At present, there are no studies in the literature which evaluate the time course of plasma BDNF levels in specific cohorts of women, intra-individual variations of plasma BDNF throughout the menstrual cycle, or the influence of a hormonal replacement therapy (HRT) on plasma BDNF.

Consequently, the aim of the present study is to assess BDNF plasma concentrations in normal female subjects according to hormonal status (menstrual cycle, amenorrhoea and menopause).

Finally, validation of the assay for BDNF determination in plasma was also investigated and discussed.

## Materials and methods

### Subjects

Sixty females, either volunteers or outpatients of our Gynaecological Department, were included in the present study. They were subdivided into the following three groups:

- (i) Fertile women ( $n = 20$ ), aged 20–40 years, with body mass index (BMI) between 20 and 23;
- (ii) Amenorrhoeic women ( $n = 15$ ), aged 21–42 years, with BMI 18.5–24; amenorrhoea of at least 6 months;
- (iii) Postmenopausal women ( $n = 25$ ), aged 48–70 years, with BMI 21–25.

Prior to enrollment, participating subjects gave their written informed consent and were asked to answer a questionnaire regarding age, weight, height, chronic diseases, current illness, regular medication, allergies or a family history of endocrinological, psychiatric or neurological diseases. Physical examination and routine laboratory tests were performed, and they disclosed no abnormalities. None of the subjects was taking psychoactive medications, hormone therapies (including oral contraceptives) or anti-inflammatory drugs, and no mood or behaviour disturbances were referred at the moment of the enrollment.

All fertile women had regular menstrual cycles ( $28 \pm 2$  days, mean  $\pm$  SD), and ovulation was ascertained by assessment of Day 21 plasma progesterone levels (values  $10.0 \text{ ng ml}^{-1}$  were considered ovulatory). The study was approved by the Ethics Committee of the Medical Faculty of the University of Pisa.

### Protocol

After overnight fasting, a blood sample was drawn from the cubital vein of each subject in EDTA-coated tubes (Vacutest Kima s.r.l., Arzergrande, Italy) between 7.30 and 9.00 a.m. in order to minimize the effects of a possible circadian variation of plasma BDNF concentrations, as previously suggested in the literature (Solum and Handa, 2002).

The tubes were kept on ice and, after collection, blood samples were immediately centrifuged at  $4^\circ\text{C}$  (2500  $g$  for 15 min). Plasma was aliquoted and stored at  $-80^\circ\text{C}$  until assay.

Five of the 20 ovulatory women were tested every 2 days throughout a whole menstrual cycle. In the other normally menstruating women blood samples were drawn during the follicular phase (Days 6–8) and during the luteal phase (Days 20–24).

In amenorrhoeic subjects, three blood samples were drawn 10 days apart in order to minimize possible intra-individual variations.

After the first blood sampling, 10 postmenopausal women started receiving a combined sequential oral HRT [estradiol valerate (E<sub>2</sub>V)  $2 \text{ mg day}^{-1}$  for 16 days, followed by E<sub>2</sub>V  $2 \text{ mg day}^{-1}$  + levonorgestrel  $0.075 \text{ mg day}^{-1}$  for 12 days] and they underwent a second sampling after 6 months of treatment.

### BDNF assay

Plasma levels of BDNF were determined with an enzyme-linked immunosorbent assay (ELISA) method (BDNF Emax Immunoassay System, Promega, USA), after appropriate dilution of samples (1:4) using Block & Sample Buffer, according to the manufacturer's instructions. Briefly, 96-well flat bottom immunoplates (Iwaki) were coated with Anti-BDNF monoclonal antibody and incubated at  $4^\circ\text{C}$  overnight. After blocking by non-specific binding with Block & Sample Buffer, standards and samples were added to the plates and incubated and shaken for 2 h at room temperature. Subsequently, after washing with TBST wash buffer, plates were incubated for 2 h with Anti-Human BDNF polyclonal antibody. The last incubation

**Table I.** Recovery of brain-derived neurotrophic factor (BDNF, pg/ml) from human plasma after dilution

Plasma dilution	Expected BDNF	Measured BDNF	Recovery (%)
Undiluted	—	1281.5	—
1:2	640.75	616.80	96.1
1:4	320.37	299.80	93.6
1:8	160.18	144.76	90.4
1:16	80.09	68.50	85.5
1:32	40.04	31.86	79.5

required the addition of Anti-immunoglobulin Y-horse-radish peroxidase conjugate. In the last step of the assay, TMB One solution was added in order to develop the colour. After stopping the reaction with HCl 1 N, the absorbance was read at 450 nm on a microplate reader and BDNF concentrations were determined automatically according to the BDNF standard curve (ranging from 7.8 to 500 pg ml<sup>-1</sup> purified BDNF).

The entire procedure was performed by using a semi-automated Basic Radim Immunoassay Operator (BRIO-Radim, Italy) equipped with a microplate reader of optical density. A computer system linked to the BRIO analysed the final results and expressed them in picogram per millilitre.

#### *E<sub>2</sub>, progesterone, LH and FSH assay*

Plasma concentrations of E<sub>2</sub>, progesterone, LH and FSH were determined by specific commercially available radioimmunoassay kits (Radim, Pomezia, Italy).

The sensitivity of the assays were 10 pg ml<sup>-1</sup> for E<sub>2</sub>, 0.12 ng ml<sup>-1</sup> for progesterone, 0.20 mIU ml<sup>-1</sup> for LH and 0.18 mIU ml<sup>-1</sup> for FSH. The intra- and inter-assay coefficients of variation were, respectively, 2.1% and 4.2% for E<sub>2</sub>, 4.2% and 7.8% for progesterone, 2.4% and 3.3% for LH and 1.97% and 4.1% for FSH.

#### *Parameters used and statistical analysis*

Plasma BDNF levels are expressed in pg/ml. All data are reported as mean ± SD. Data obtained were analysed by one-way analysis of variance, as appropriate. Differences between single pairs of groups were compared by means of the Bonferroni test. A correlation index (Pearson Index) was computed in order to investigate the connection between plasma BDNF and sex steroids all through the cycle.

## Results

### *Methodology*

Recovery of two (low and high) doses of exogenous BDNF was assessed in plasma: mean recovery (±SD) of BDNF was 91.3 ± 8.8% at 60 pg ml<sup>-1</sup> (n = 15) and 94.0 ± 10.5% at 125 pg ml<sup>-1</sup> (n = 15). The sensitivity of the assay, expressed as a minimal amount of BDNF distinguishable from the zero sample with 95% probability was 15.6 pg ml<sup>-1</sup>, and the intra- and inter-assay coefficients of variation were 6.0% (n = 21) and 8.5% (n = 25), respectively.

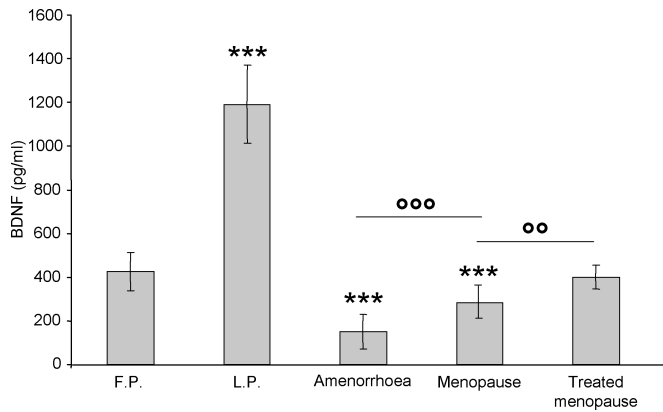
The antiserum employed is highly specific, showing <3% cross-reactivity with other related neurotrophic factors (nerve growth factor, NT-3 and NT-4) at 100 ng ml<sup>-1</sup>, as specified by the manufacturer's notes.

To validate the BDNF assay in plasma, serial dilutions of a high-concentration plasma sample were checked for parallelism, producing the results reported in Table I.

Finally, to check the validity of EDTA as anticoagulant for plasma BDNF assay, we performed the following test: after overnight fasting (8.00 a.m.) two blood samples have been drawn from the cubital vein of five healthy volunteers subjects. The first sample was collected in EDTA tube (containing 1.8 mg ml<sup>-1</sup> EDTA) and the second one was collected in heparinized tube (containing 250 U ml<sup>-1</sup> heparin). After collection, blood samples were immediately centrifuged at 4°C (2500 g for 15 min), and plasma was aliquoted. In order to test the interference of EDTA or heparin with the BDNF assay, two doses of heparin (low and high) have been added to EDTA plasma, and two doses of EDTA (low and high) have been added to heparinized plasma. Immediately after, ELISA BDNF assay was performed (BDNF Emax Immunoassay System, Promega), as already described above. Results of our test are presented in Table II. Our results show that heparin seems to interfere with the measurement of plasma BDNF. In fact, by adding heparin in EDTA plasma, BDNF concentration falls (with a mean decrease of 40%) to approximately the same values measured in heparinized plasma samples. Conversely, the addition of EDTA to heparinized plasma does not produce any significant change in BDNF measurement (mean increase 6.8%). This outcome allows us to confirm that EDTA is the most suitable anticoagulant in our assay.

**Table II.** Evaluation of EDTA and heparin (Hep) interference in plasma BDNF measurement (BDNF values are expressed in pg/ml)

	EDTA plasma	EDTA plasma + Hep 50 µl	EDTA plasma + Hep 200 µl
Subject 1	2006	1617	1600
Subject 2	1202	704	703
Subject 3	776	363	333
Subject 4	911	507	457
Subject 5	377	232	223
	Hep plasma	Hep plasma + EDTA 50 µl	Hep plasma + EDTA 200 µl
Subject 1	1117	1253	1414
Subject 2	713	733	798.83
Subject 3	361.2	383	410
Subject 4	553	594	616
Subject 5	254	268.6	284



**Figure 1.** Brain-derived neurotrophic factor (BDNF) plasma levels in all groups analysed. FP, follicular phase; LP, luteal phase. (\*\*\*)  $P < 0.001$  versus FP and  $^{\circ\circ}P < 0.01$ ,  $^{\circ\circ\circ}P < 0.001$  between groups). Data are mean  $\pm$  SD.

We concluded that BDNF plasma assay is valid for human plasma measurement.

### Clinical results

#### Fertile women

In general, fertile women showed significantly higher plasma BDNF levels during the luteal phase in comparison to those found in the follicular phase ( $P < 0.001$ ) (Figure 1).

In particular, in the subgroup of five women who were followed up closely during a whole menstrual cycle, we obtained the following results (Figure 2): BDNF levels were lowest in the early follicular phase (Days 1–8) (from  $292.8 \pm 77.4$  on the second day to  $541.8 \pm 113.7$  on Day 8,  $P < 0.01$ ), then rose significantly from Day 10 of the menstrual cycle, reaching a peak value on Day 14, ( $P < 0.001$  versus Day 8); after ovulation (Days 16–18), BDNF levels tended to decrease (without nevertheless reaching statistical significance) and subsequently rose again in the mid-luteal phase (Days 20–24), reaching a second peak on Day 24 ( $1186.4 \pm 190.6$ ); finally,

we observed a marked fall in BDNF at the end of menstrual cycle, with Day 28 levels reaching values similar to those observed in the early follicular phase ( $P < 0.001$  versus Day 24).

Plasma BDNF variations correlated with those of sex steroids throughout the menstrual cycle, as demonstrated by the correlation index reported in Table III. Moreover, a global correlation index was also calculated, showing that BDNF was positively correlated with both  $E_2$  and progesterone ( $r = 0.8027$ ,  $P < 0.001$  and  $r = 0.7342$ ,  $P < 0.01$ , respectively).

#### Amenorrhoeic women

As represented in Figure 1, women who were amenorrhoeic for at least 6 months showed the lowest BDNF plasma levels ( $150.7 \pm 80.7$ ) in comparison to all the other subjects involved in the study ( $P < 0.001$  versus fertile women in follicular phase,  $P < 0.001$  versus postmenopausal women).

#### Postmenopausal women

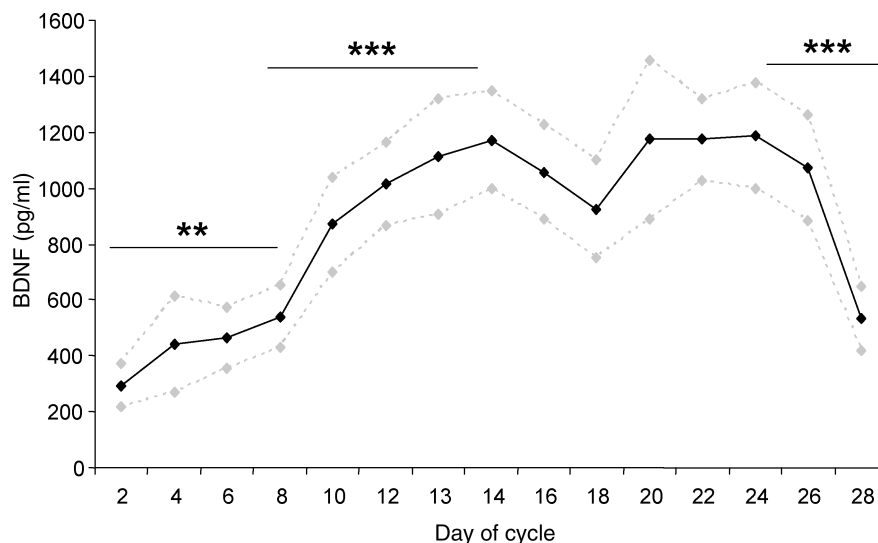
BDNF plasma levels of postmenopausal women were lower in comparison to follicular phase levels of fertile women ( $280.6 \pm 116$ ,  $P < 0.001$  versus follicular phase, see Figure 1).

We noticed that there was an age-related decrease in BDNF plasma levels: in fact, aged women showed the lowest concentrations ( $r = -0.87$ ,  $P < 0.001$ ). Moreover, BDNF circulating levels showed a negative correlation ( $r = -0.86$ ,  $P < .001$ ) with menopausal age (Figure 3).

After 6 months of HRT treatment, BDNF plasma levels increased significantly ( $P < 0.01$ ) to values present in fertile women during the follicular phase (see Figure 1).

### Discussion

At present, most of the studies about BDNF available in the literature analyse serum BDNF concentration as the index of BDNF peripheral levels.



**Figure 2.** Trend of plasma BDNF during menstrual cycle, measured in 5 of the 20 fertile ovulatory women. Significant difference between evaluation times  $^{**}P < 0.01$ ,  $^{***}P < 0.001$ .



**Table III.** Correlation index ( $r$ ) between plasma BDNF and sex steroid concentrations all through menstrual cycle

	Subject 1	Subject 2	Subject 3	Subject 4	Subject 5
BDNF versus E <sub>2</sub>	0.8542***	0.7469**	0.7128**	0.7297**	0.6144*
BDNF versus Progesterone	0.6944**	0.7874**	0.668**	0.6026*	0.6087*

\* $P < 0.05$ .\*\* $P < 0.01$ .\*\*\* $P < 0.001$ .

Nevertheless, in our approach to BDNF study, we preferred to analyse plasma BDNF concentration, in order to avoid possible inter-individual variations connected with physiological and/or pathological alterations of platelets count, since platelets are known to be one of the major human BDNF storage site.

Discordant opinions and inconsistent data are available in the literature about the accurate procedure to test plasma BDNF levels. One of the most debated issue is about the choice of the most suitable anticoagulant, since it has been

hypothesized that some molecules (for example EDTA) might produce, in some way, a release of BDNF from platelets during plasma extraction (Lommatzsch and Virchow, 2006).

Therefore, in order to overcome these methodological worries, we checked the validity of EDTA as an anticoagulant. We showed that plasma BDNF levels in heparinized plasma are systematically lower than those in EDTA plasma. The addition of a low- or a high-dose heparin to EDTA plasma samples produces a significant BDNF decline (with a mean decrease of 40%). Conversely, BDNF levels are not significantly altered by the addition of EDTA to heparinized samples. This phenomenon suggests that heparin, but not EDTA, could in some way interfere with BDNF assay. On the other hand, since BDNF levels are much higher in EDTA plasma than in heparinized plasma, we cannot exclude that some BDNF in EDTA plasma could be platelet-derived. In this view, both heparin and EDTA present some limitations when used as anticoagulant in plasma BDNF assay.

However, regardless of the discrepancy in absolute BDNF values between our results and other data found out in literature (Lommatzsch *et al.*, 2005), it is essential to consider that our study is focused on the variations of plasma BDNF in accordance with various hormonal conditions. Therefore, the central feature is the comparison between groups and the study of the trend of BDNF throughout the ovarian cycle.

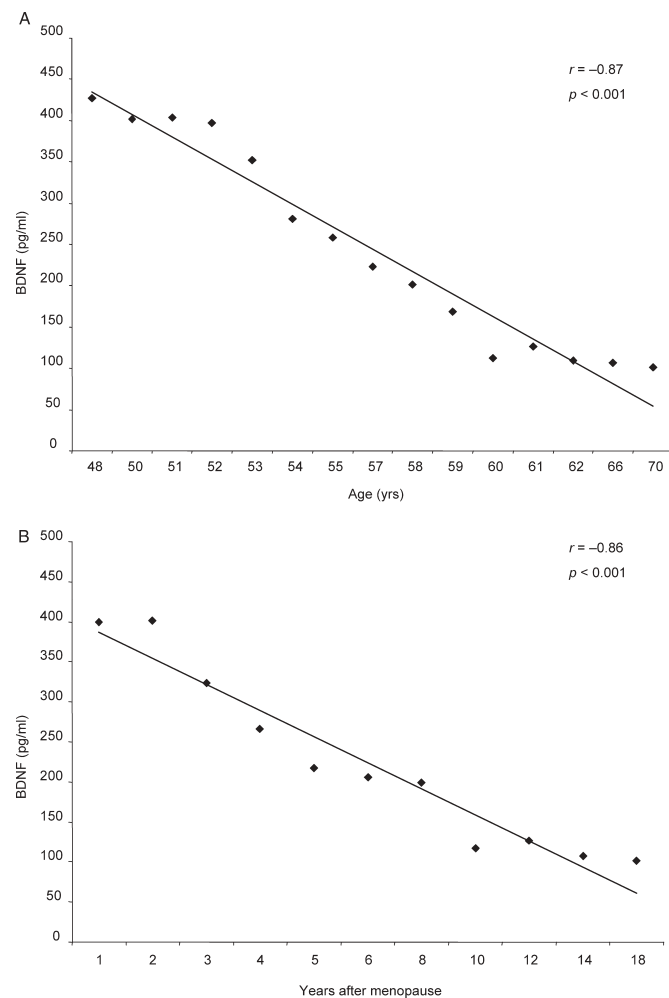
The aim of the present study is to investigate physiologic changes in BDNF circulating levels according to hormonal status. In particular, we were looking for a possible relationship between plasma BDNF and hormonal variations during menstrual cycle, as well as modifications in plasma BDNF related to hormonal deficiency (amenorrhoea, menopause).

Several studies report serum BDNF levels in healthy human subjects and, in most cases, these are control subjects of studies that evaluate the impact of psychiatric diseases (such as depression or schizophrenia) on BDNF central and peripheral levels. A study in a large cohort of elderly individuals ( $n = 516$ ; 70–103 years old) has been recently published (Ziegenhorn *et al.*, in press), confuting the hypothesis that serum BDNF levels are related to mental disorders such as depression and dementia.

However, no systematic evaluation of hormonal influence on BDNF circulating levels emerges from any studies, and inter- and intra-individual variations of peripheral BDNF levels related to hormonal environment have never been investigated in detail.

Our data show a close relationship between hormonal status and neurotrophic milieu. In fact, women with regular ovulatory cycles present higher BDNF levels when compared with amenorrhoeic or postmenopausal women ( $P < 0.001$ ).

It is interesting to notice that both amenorrhoea and menopause are associated with low plasma BDNF concentration, thus suggesting a predominant role of sex steroid hormones in the regulation of neurotrophin expression. Actually, a link between sex hormones (in particular estrogen) and BDNF production and activity has already been suggested. Experimental studies on the animal model showed that estrogen treatment of ovariectomized rats improved learning acquisition and memory performances (O'Neal *et al.*, 1996; Daniel *et al.*,

**Figure 3.** Representation of the negative correlation between plasma BDNF levels and chronological age (A), and between plasma BDNF levels and years after menopause (B).

1997). The well-known protective influence exerted by estrogens on cognitive functions may be mediated by BDNF. Further evidence that BDNF may mediate the effects of E<sub>2</sub> in the hippocampus comes forth from studies carried out on cultured developing hippocampal neurons: E<sub>2</sub> increases dendritic spine number, through a BDNF-dependent mechanism (Segal and Murphy, 2001).

It has been observed that E<sub>2</sub> and BDNF have similar effects on central nervous system functions and activities (and in particular on hippocampal-related functions). In fact, both E<sub>2</sub> and BDNF produce an enhancement of glutamate-mediated transmission (Woolley, 1998; Lu, 2003), modulation of NMDA receptors (Adams *et al.*, 2004), facilitation of memory (Luine, 1997; Tyler *et al.*, 2002) and increased dendritic spine and spine synapse numbers (McEwen *et al.*, 2001; Segal and Murphy, 2001; Tyler *et al.*, 2002).

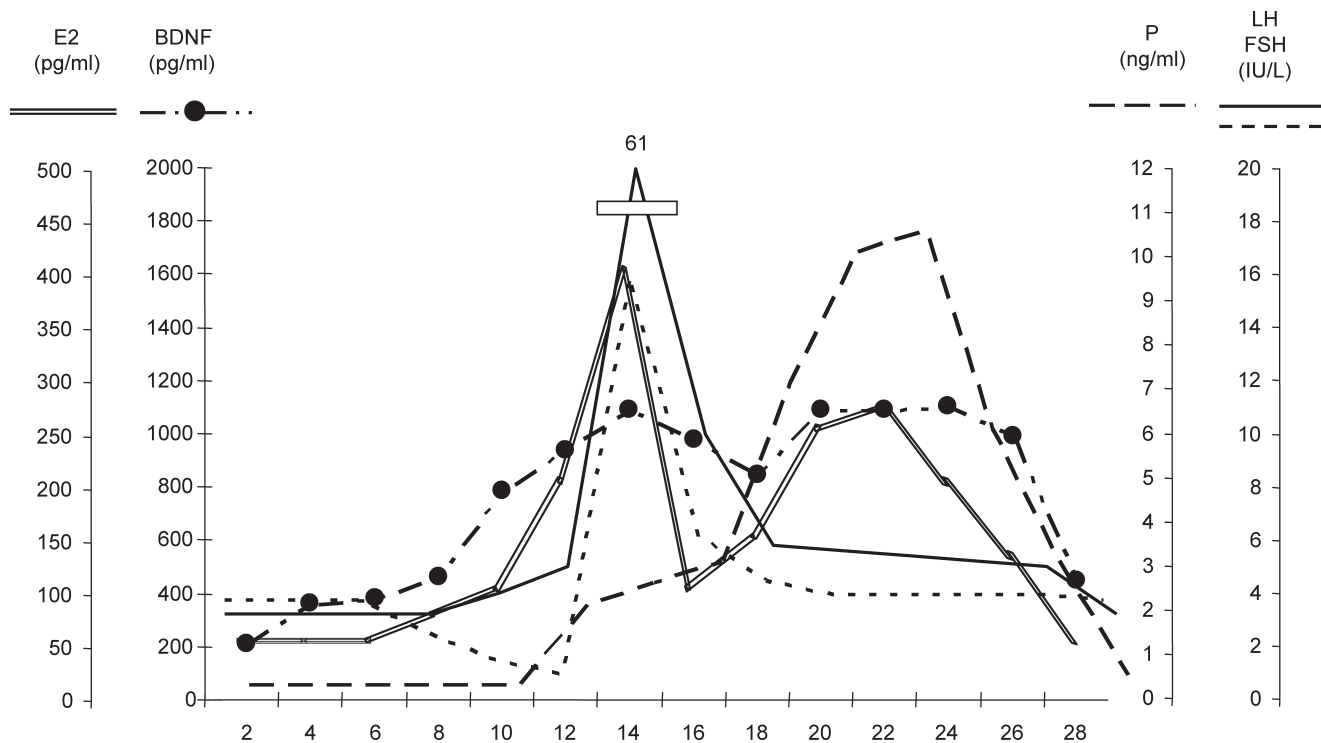
Furthermore, our results allow us to assert that a significant fall in BDNF levels not only occurs with chronological age, as already affirmed in the literature (Lommatzsch *et al.*, 2005) but also with menopausal age. In fact, we found a strong negative correlation ( $r = -0.86$ ,  $P < 0.001$ ) between plasma BDNF levels and the number of years since menopause. Therefore, in this view, women who experience an earlier menopause may have detrimental effects from the longer deficiency of BDNF, with respect to women who enter menopause later on in life. These data are not lacking clinical implications: in fact, this could at least in part explain the increased incidence of psychiatric illness (most of all depression) and neurodegenerative diseases (such as Alzheimer's and Parkinson's disease) after the menopause, since BDNF has been shown to be

involved in such diseases (Connor *et al.*, 1997; Parain *et al.*, 1999; Karege *et al.*, 2002a,b).

One of the oldest and most debated issues in HRT is its ability to improve cognitive functions in postmenopausal women (Genazzani *et al.*, 2005; Prelevic *et al.*, 2005). The efficiency of a 6-month oral HRT in restoring BDNF levels to those present in fertile women may be one of the mechanisms underlying this phenomenon.

By testing five fertile women with regular ovulatory cycles every 2 days, we demonstrated changes in BDNF levels according to menstrual cycle phase, suggesting a close relationship between this factor and sex steroids. In particular, one can observe how the fluctuations of BDNF and E<sub>2</sub> are extraordinarily similar: both are very low in the very first days of the cycle, then rise until Day 14, reaching a peak just before ovulation, approximately just a few hours before the LH surge. In effect, recent studies have suggested a role for BDNF in the complex mechanism which leads to ovulation, probably under the direct influence of E<sub>2</sub>. BDNF may be considered a valid marker of follicular maturation (Seifer *et al.*, 2002, 2003, 2006).

After this 'pre-ovulatory peak', both E<sub>2</sub> and BDNF show a trend to decrease up to Days 16–17 of the menstrual cycle. Concurrently, progesterone starts to increase, due to its secretion by the corpus luteum, under the influence of LH. During the mid-luteal phase, a new significant rise in plasma BDNF levels occurs (Days 20–24). Even if the precise origin of plasma BDNF remains to be elucidated, we can suppose that the high plasma BDNF levels observed in the luteal phase may be related, at least in part, to a local



**Figure 4.** Trend of plasma BDNF, gonadotrophin (LH, FSH), estradiol (E<sub>2</sub>) and progesterone (P) during menstrual cycle, measured in 5 of the 20 fertile ovulatory women.

production. This source may be the corpus luteum, seeing its capability to synthesize and release other growth factors such as VEGF (Fraser *et al.*, 2005; Tropea *et al.*, 2006). Another hypothesis is that BDNF may be produced at least in part, by the endometrium, since it has been reported that the rat endometrium expresses both BDNF protein and mRNA (Krizsan-Agbas *et al.*, 2003). Obviously, because of the small number of women studied, and because our experimental design was not planned to explain the origin of circulating BDNF increase during the luteal phase, further studies will be done to explain it Figure 4.

In conclusion, we can hypothesize that the follicular rise in circulating BDNF levels and consequent pre-ovulatory peak is an estrogen-related phenomenon. Since it has been shown that human ovaries—specifically granulosa cells—are able to produce BDNF (Feng *et al.*, 2003; Seifer *et al.*, 2006), it is conceivable that high levels of estrogen can induce, either directly at ovarian level through a paracrine mechanism of modulation or at central level with a long loop, an increase of BDNF production and release.

For the first time, the pattern of BDNF levels throughout ovarian cycle has been investigated in detail.

It has been recently shown (Lommatzsch *et al.*, 2006) that pregnancy is accompanied by a decrease in serum BDNF, and levels remain low after childbirth. This phenomenon has been interpreted as a potential increased risk for the development of mood disorders in post-natal period, in line with the hypothesis that a deficiency in BDNF may contribute to the pathogenesis of depressive disturbances.

According to the close link between sex hormone variations and BDNF, in the near future, it would be interesting to understand if BDNF plays a role in the pathogenesis of other hormone-related disorders such as premenstrual syndrome and postmenopausal depression.

Certainly, further studies are needed to improve our knowledge about this nerve growth factor, not only in reproductive medicine.

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