

Associations between brain-derived neurotrophic factor and estradiol in women's saliva

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Abstract

OBJECTIVE: The aim of this study was to assess the relationship between brain-derived neurotrophic factor (BDNF) and sex hormones (estradiol [E₂] and progesterone), using saliva samples obtained from healthy women.

METHODS: Forty female dental hygienist students were divided into groups according to being in the follicular phase or luteal phase. Saliva BDNF, E₂, and progesterone levels were measured using a sandwich ELISA system. The correlation between these factors was analyzed using Spearman's index, and fluctuations of these levels in the whole menstrual cycle were investigated classifying the subjects by every 4 days according to the phase of their menstrual cycle.

RESULTS: Saliva BDNF variations strongly correlated with saliva E₂ levels in the follicular phase ($r=0.721$, $p=0.000$) and luteal phase ($r=0.770$, $p=0.000$). The correlation coefficient showing the relationship between progesterone and BDNF levels in the luteal phase ($r=0.371$, $p=0.157$) was lower than that in the follicular phase ($r=0.631$, $p=0.001$). Moreover, the fluctuation of BDNF levels in the menstrual cycle followed a similar pattern to that of E₂.

CONCLUSIONS: We found that saliva BDNF and E₂ levels were closely related in healthy young women. In particular, for first time, that correlation was investigated throughout the menstrual cycle. Monitoring of saliva BDNF may yield insight into women's reproductive and mental health.

INTRODUCTION

Brain-derived neurotrophic factor (BDNF) is a member of the neurotrophin family (NT), which includes nerve growth factor (NGF), NT-3, -4/5, -6, and -7. BDNF is abundantly expressed in the central and peripheral nervous system, and, in particular, in the hippocampus (Saruta *et al.* 2010). Hippocampal BDNF plays an important functional role in learning and memory (Hall *et al.* 2000). Although it was originally identified in the nervous system, BDNF is now known to be expressed in a wide variety of non-neural cells, such as the lacrimal glands (Ghinelli *et al.* 2003), heart (Scarlsbrick *et al.* 1993), and retina (Seki *et al.* 2003) in rodents, as well as in the testis (Muller *et al.* 2006), lung (Ricci *et al.* 2004), and endothelial cells (Nakahashi *et al.* 2000) in humans. We previously reported that BDNF expression in the rat submandibular gland is up-regulated by chronic stress, and localization of BDNF mRNA and protein was observed in salivary acini cells in humans (Saruta *et al.* 2012). In recent years, immunohistochemical and *in vitro* studies have demonstrated BDNF expression in reproductive organs, including the ovaries (Kawamura *et al.* 2005), in particular in the granular cells (Domingue *et al.* 2011), and the endometrium (Krizsan-Agbas *et al.* 2003). Moreover, it was found that the ovaries specifically expressed a BDNF receptor, TrkB (Kawamura *et al.* 2009). These reports suggested that there is a close association between BDNF and the reproductive system in women.

Moreover, changes in plasma BDNF levels have been reported to occur in women throughout the menstrual cycle: studies have shown higher levels of BDNF during the luteal phase than in the follicular phase (Cubeddu *et al.* 2011). Hormone replacement therapy (HRT) is able to increase BDNF levels from menopausal levels to approximately those observed in the follicular phase (Begliomini *et al.* 2007). Furthermore, estradiol (E₂) is induced by BDNF in neural cells of the rat brain, including those in the hypothalamus and hippocampus (Scharfman & MacLusky 2008). E₂ plays an important role in the modification of intra-tissue (Scharfman & MacLusky 2008) and plasma levels of BDNF (Begliomini *et al.* 2007). Interestingly, the BDNF profile of premenstrual syndrome is characterized by a low plasma level in the luteal phase, despite increases in E₂ levels (Cubeddu *et al.* 2011). Measurement of plasma BDNF levels during the menstrual cycle may have important implications in women's health and reproduction.

The sensitivity and precision of the saliva E₂ assay are comparable to those of assays of serum E₂ for assessing changes in hormone levels (Lu *et al.* 1999). The measurement of E₂ and progesterone concentrations in saliva has become increasingly common over the past several years (Dame *et al.* 1989). The measurement of saliva E₂ and progesterone levels during the post-awakening period could provide information regarding the hypothalamus-pituitary-ovary axis response to awak-

ening (Ahn *et al.* 2011). In addition, using ELISA and western blotting, we have demonstrated that human saliva contains BDNF. Saliva BDNF concentrations in males tended to be lower than those in females (Saruta *et al.* 2012), resembling the profile of plasma BDNF (Lommatzch *et al.* 2005). To date, there are no studies evaluating the relationship between BDNF and sex hormones in saliva.

In recent years, analysis of saliva has become an attractive sampling approach. The collection of saliva is easier than venipuncture and can readily be repeated at frequent intervals (Ahn *et al.* 2011). These advantages allow for a better assessment of the diurnal rhythm-mediated secretion of sex hormones and related factors, such as BDNF (Ahn *et al.* 2011). Thus, saliva testing may contribute to the monitoring of women's health.

Consequently, the aim of the present study was to assess correlations between BDNF and E₂ as well as progesterone in the saliva of healthy female subjects.

MATERIALS AND METHODS

Subjects

The study was approved by the Ethics Committee of the Kanagawa Dental University, graduate school of Dentistry. Forty females, volunteer dental hygienist students, were included in the present study. They were divided into the following two groups: fertile women in the follicular phase (n=24), aged 21.0±3.5 y (mean±SD, range 19–32 y), with a body mass index (BMI) 19.4±1.5 (range 16.2–21.9), and fertile women in the luteal phase (n=16), aged 23.0±3.9 (range 19–31 y), with a BMI 19.6±1.6 (range 16.8–22.0). Each group was assessed both in the follicular phase (days 0–14) and in the luteal phase (days 15–28) of the menstrual cycle. Menstrual phase was determined based on self-reporting.

Prior to enrollment, participating subjects gave their written informed consent and they were asked to answer a questionnaire regarding age, weight, height, and menstrual cycles. Subjects with oral infective diseases, current illness, under medication, or with allergies, or a family history, or overweight (BMI ≥25.0) were excluded from this study. The mean menstrual cycles of all subjects lasted 27.9±4.8 days.

Saliva collection

For 5 min prior to sample collection, subjects did not consume any food or drink, or brush their teeth. All samples were collected between 1 and 4 pm, within a 10-min period, to minimize any possible effect of diurnal variation. All saliva samples were collected using the Salivette (Sarstedt Co. Ltd., Nümbrecht, Germany) absorbent method according to the manufacturer's instructions. Briefly, participants were instructed place a cotton roll on the floor of the mouth for 5 min, or until the cotton was fully saturated with saliva, and then spit the cotton into the Salivette tube, which was subsequently stored on ice. The tubes were centrifuged at

626 × g for 15 min at 4 °C and were then stored at –80 °C until required for use. Upon thawing, the samples were centrifuged once more to ensure complete debris removal, using the same conditions.

BDNF assay

Human BDNF was detected by sandwich ELISA according to the manufacturer's instructions (CYT306; Millipore Corp, Bedford, MA, USA). All assays were performed in F-bottom 96-well plates (Nunc, Wiesbaden, Germany). Tertiary antibodies were conjugated to horseradish peroxidase and color was developed with tetramethylbenzidine and measured at 450/570 nm. The BDNF content was quantified against a standard curve; the detection limit of <4 pg/mL was calibrated with known amounts of human recombinant BDNF. All samples were tested twice, and the mean was calculated. Cross-reactivity to related NTs (NGF, NT-3, and NT-4) was less than 3%. Intra- and inter-assay coefficients of variation were 3.7% and 8.5%, respectively. Concentrations were expressed as pg/mL (Goto *et al.* 2012).

E₂ and progesterone assays

The saliva ELISA kit for measuring free 17β-estradiol and progesterone were used according to the manufacturer's instructions (IBL, Hamburg, Germany) (Rijn *et al.* 2011). The lowest detectable levels of E₂ and progesterone that could be distinguished from the zero standard were 0.4 pg/mL for E₂ and 3.8 pg/mL for progesterone, at the 95% confidence limit. The intra- and inter-assay coefficients of variation were 2.6% and 2.1% for E₂, and 4.7% and 5.3% for progesterone, respectively.

Parameters and statistical analysis

ELISA data are reported as a mean ± SD. Statistical analysis was carried out using SPSS software (version 17.0; SPSS Inc., Chicago, IL, USA). We used the Mann-Whitney test to evaluate differences between follicular and luteal phase subjects for each parameter. A correlation index (Spearman's index) was computed in order to investigate the relationship among saliva BDNF levels and E₂ as well as progesterone levels. The *p*-values of 0.05 or less were accepted as significant.

RESULTS

Differences among parameters between follicular phase and luteal phase subjects

Data from subjects in the follicular phase or luteal phase, as determined by ELISA, are compared in Table 1. Although these levels tended to be higher in individuals in the luteal phase than those of subjects in the follicular phase, the differences were not significant.

Correlation index

There were positive correlations between saliva E₂ and progesterone levels for subjects in the follicular phase and for individuals in the luteal phase, represented in

Tab. 1. Differences between follicular phase and luteal phase.

	Follicular phase		Luteal phase	
	Mean	SD	Mean	SD
BDNF	8.510	8.657	13.106	14.255
E ₂	7.264	4.485	8.254	5.297
Progesterone	60.407	46.090	87.258	49.550

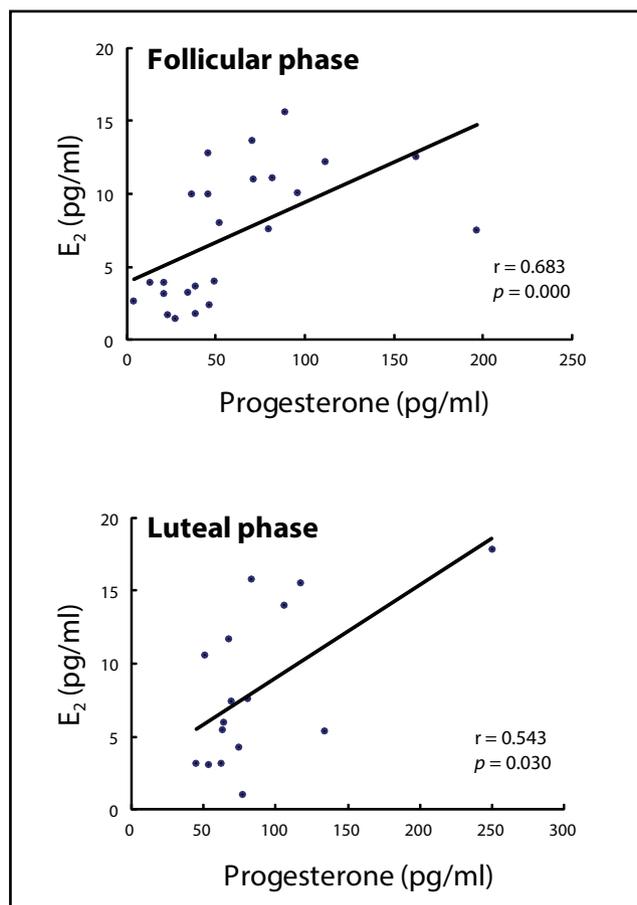


Fig. 1. Scatterplot showing the relationship between saliva E₂ levels and saliva progesterone levels of subjects in the follicular phase and those in the luteal phase.

Figure 1. Saliva BDNF variations strongly correlated with saliva E₂ changes in the follicular phase and in the luteal phase (Figure 2). Although saliva BDNF and progesterone levels correlated in individuals in the follicular phase, no correlations were noted in individuals in the luteal phase, as shown in Figure 3.

Variation of saliva BDNF, E₂, and progesterone during menstrual cycle

By classifying subjects by every 4 days according to the phase of their menstrual cycle, we investigated the variations in saliva BDNF and sex hormone levels during the whole of the menstrual cycle (Figure 4). BDNF levels were found to show two peaks: one lower peak

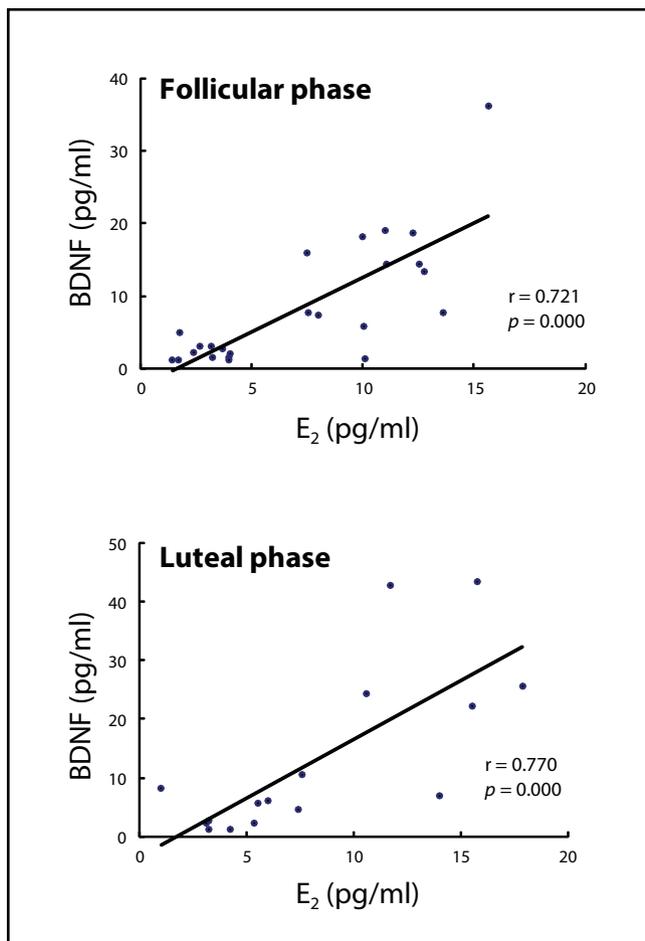


Fig. 2. Scatterplot showing the relationship between saliva BDNF levels and saliva E₂ levels in subjects in the follicular phase and those in the luteal phase.

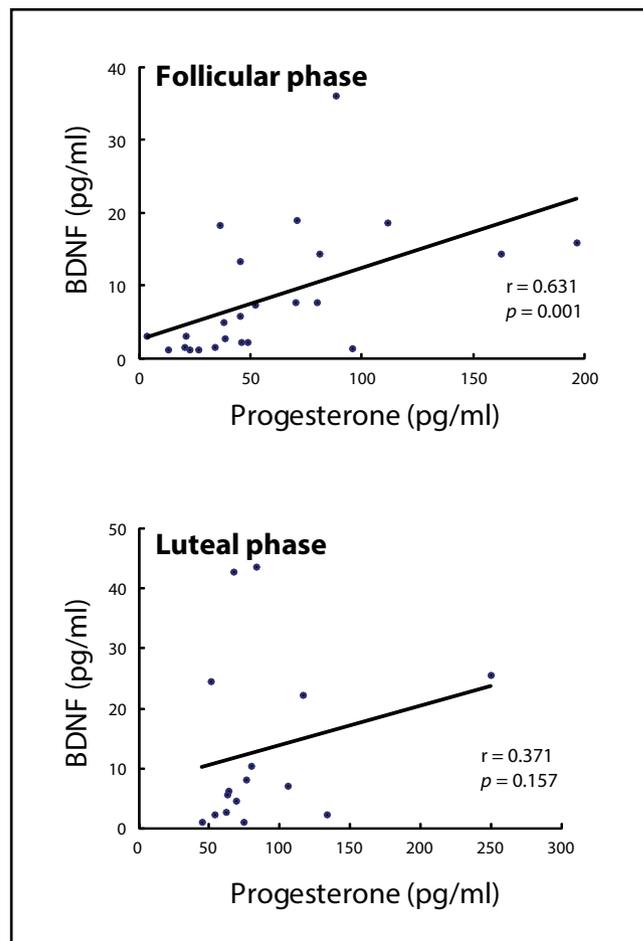


Fig. 3. Scatterplot showing the relationship between saliva BDNF levels and saliva progesterone levels of subjects in the follicular phase and those in the luteal phase.

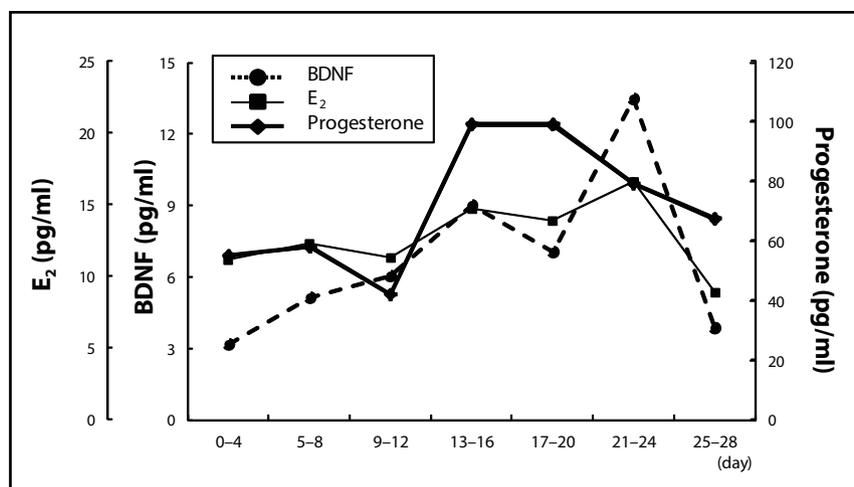


Fig. 4. Changes in saliva BDNF, E₂, and progesterone levels during the menstrual cycle in young healthy women. Average levels for every 4 days of the cycle are presented.

at the 13th–16th day (14.916±7.702) and one tall peak at the 21st–24th day (22.309±29.990) during a normal menstrual cycle. Similar to BDNF, E₂ levels also showed two peaks (13th–16th day: 8.858±4.863; 21st–24th day:

10.007±8.154). Moreover, the levels of progesterone peaked at the 13th–20th day (13th–16th day: 98.591±52.624, 17th–20th day: 98.835±67.356).

DISCUSSION

Saliva E₂ and progesterone concentrations tended to be higher in subjects in the luteal phase than in those in the follicular phase, but these differences, as determined using an ELISA system, were not statistically significant. In addition, there were significant correlations between E₂ and progesterone levels in the saliva of both groups of subjects. Lu *et al.* reported that

the sensitivity and precision of saliva E₂ and progesterone assays were comparable with assays of blood E₂ and progesterone for assessing changes in hormone levels (Lu *et al.* 1999). It is well-known that blood and

saliva concentrations of E_2 and progesterone are related (Celec *et al.* 2009; Dame *et al.* 1989). Since sex hormone levels, including those of E_2 and progesterone, are highest in blood during the luteal phase (Cubeddu *et al.* 2011; Okifji & Turk 2006), our results can be attributed to the increase of E_2 and progesterone concentrations in blood. If the number of subjects had been sufficient, the difference between levels of individuals in the follicular and luteal phase may have been significant.

We found that there was a strong linear correlation between BDNF and E_2 levels in saliva. Experimental studies on animal models showed that estrogen treatment of ovariectomized rats improved learning acquisition and memory performance (O'Neal *et al.* 1996). Indeed, E_2 increased the dendritic spine number through a BDNF-dependent mechanism (Segal & Murphy 2001). In humans, it has been reported that there is a positive relationship between plasma BDNF and E_2 levels during the menstrual cycle, based on a study of five healthy women, with a correlation index ranging from 0.6144 to 0.8542 (Begliuomini *et al.* 2007). Interestingly, BDNF levels were increased by HRT (including E_2) in subjects showing a low plasma BDNF level, such as amenorrhoeic women and postmenopausal women (Begliuomini *et al.* 2007). HRT also improved cognitive functions in postmenopausal women (Genazzani *et al.* 2005; Prelevic *et al.* 2005). These previous studies and our results established that estrogens and BDNF were reciprocally and positively involved in various situations.

Moreover, when we investigated the fluctuations in the levels of BDNF and sex hormones during the menstrual cycle, the fluctuation pattern of BDNF was similar to that of E_2 . Sex hormone levels in blood differ in terms of their peaks during the menstrual cycle: E_2 is elevated in the pre-ovulatory and middle luteal phase, whereas progesterone is increased during the middle luteal phase (Begliuomini *et al.* 2007). Interestingly, in the present study, reflecting the changes in blood levels, saliva E_2 levels showed two peaks (13th–16th and 21st–24th day), although saliva progesterone levels differed from the blood levels, because it was not examined consecutively throughout the whole menstrual cycle in all subjects. Thus, our findings indicated the probability that changes in saliva BDNF levels are associated with those of E_2 during the menstrual cycle. This suggests that measurement of saliva BDNF may be useful in monitoring reproductive health in women.

On the other hand, the source of saliva BDNF has never been investigated in detail, although saliva clearly contains BDNF protein, as determined by western blotting and ELISA analysis. Saruta *et al.* reported that BDNF mRNA and protein are expressed in the acini cells of the human salivary gland (Saruta *et al.* 2012). In addition, human salivary gland tissue expresses estrogen receptor (*ER*) mRNA, as determined by RT-PCR (Leimola-Virtanen *et al.* 2000), but does not express E_2 . Additionally, HRT can improve both the quantity and

the quality of salivary gland function (Laine & Leimola-Virtanen 1996). It is thought that E_2 plays crucial roles in the salivary gland. It is possible that saliva BDNF may be induced and/or released from acini cells, mediated by E_2 -ER interaction, and is not only derived from blood.

In addition, the saliva BDNF levels of subjects in the follicular phase tended to be lower than those in the luteal phase; thus, it was reasonable that a decrease in saliva BDNF was induced by a decrease in blood E_2 levels. Thus, measurement of saliva BDNF may be useful to determine the effects of HRT as a marker of increased blood BDNF.

The relationship between progesterone and BDNF levels in subjects in the follicular phase was significant, but this correlation was non-significant in the luteal phase. In recent years, it has been shown that progesterone attenuated the E_2 -mediated increase in the levels of BDNF and the BDNF receptor, TrkB, in cultured hippocampal slices (Aguirre & Baudry 2009). Progesterone probably suppresses BDNF expression (Bimonte-Nelson *et al.* 2004). However, the interplay between BDNF and progesterone remains controversial (Cubeddu *et al.* 2011). Our results also suggest that BDNF levels are strongly correlated with E_2 levels, rather than with progesterone levels in saliva, which supports the previous report by Begliuomini *et al.* (2007).

Several studies have shown that there are associations between a decrease in blood BDNF and neurological diseases, such as depression, Alzheimer's disease, and Parkinson's disease (Connor *et al.* 1997; Parain *et al.* 1999; Tsukinoki & Saruta 2012). In addition, individuals with premenstrual syndrome and mood disorder in the postnatal phase have been reported to demonstrate lower blood BDNF levels than healthy women (Cubeddu *et al.* 2011; Lommatzsch *et al.* 2006). Moreover, it has been shown that a long-term deficiency of BDNF, such as that observed during menopause, may pose a higher risk for the development of mental disorders (Begliuomini *et al.* 2007). BDNF seems to be strongly involved in neurological diseases related to the reproductive cycle of women. It would be interesting to determine the saliva BDNF level profiles in various diseases known to be associated with a low blood BDNF levels.

CONCLUSION

We found that saliva BDNF and E_2 levels were closely related in healthy young women. In particular, for first time, this relationship was investigated throughout the menstrual cycle, including the follicular phase and the luteal phase. Since blood BDNF levels may be modified during the menstrual cycle, depending on E_2 levels, measurement of the saliva BDNF- E_2 balance may be useful for monitoring reproductive function. Examining saliva BDNF levels may also provide useful information for assessing women's mental health, although further studies are needed to support this proposal.

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Declaration of Interest

The authors have no conflicts of interest to report.

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