Plasma brain-derived neurotrophic factor daily variations in men: correlation with cortisol circadian rhythm

Silvia Begliuomini, Elena Lenzi, Filippo Ninni, Elena Casarosa, Sara Merlini, Nicola Pluchino, Valeria Valentino, Stefano Luisi[1], Michele Luisi and **Andrea R Genazzani**

Division of Gynecology and Obstetrics, Department of Reproductive Medicine and Child Development, University of Pisa, Pisa 56100, Italy
[1]Division of Obstetrics and Gynecology, Department of Pediatric Obstetrics and Reproductive Medicine, University of Siena, Siena 53100, Italy
(Correspondence should be addressed to A R Genazzani; Email: a.genazzani@obgyn.med.unipi.it)

Abstract

Expression and secretion of neurotrophins, including brain-derived neurotrophic factor (BDNF), are regulated also by neuronal activity. Data available in the literature suggest that BDNF central levels are influenced by light and dark. Diurnal changes of BDNF mRNA and protein contents have been demonstrated in the rat central nervous system. Based on these pieces of evidence, we investigated the hypothesis of a possible diurnal variation of BDNF circulating levels in human males. Moreover, we looked for a possible correlation with cortisol circadian rhythm, since both BDNF and cortisol are implicated in the maintenance of cerebral functions. In this study, 34 healthy young male volunteers were included. Five blood samples were drawn from each subject thrice in a month at regular 4-h intervals (0800, 1200, 1600, 2000, and 2400 h). BDNF and cortisol were measured in all samples. BDNF was determined by ELISA method. Our results show that plasma BDNF levels, as well as cortisol levels, are significantly higher in the morning when compared with the night ($P<0{\cdot}001$), with a trend of constant decrease during the day. Furthermore, plasma BDNF and cortisol are positively correlated (Spearman index $=0{\cdot}8466$). The present study is the first to demonstrate the presence of a diurnal rhythm of BDNF in humans. Moreover, the correlation found out between BDNF and cortisol circadian trend allows us to speculate that these two factors may be physiologically co-regulated, in order to maintain the homeostasis of integrated cerebral activities.

Journal of Endocrinology (2008) **197,** 429–435

Introduction

Brain-derived neurotrophic factor (BDNF) is a member of the neurotrophin family expressed in many areas of the adult mammalian brain. Its biological action is mediated by the specific tyrosine kinase receptor trkB (Tapia Aranciba *et al.* 2004).

BDNF is recognized to play an important role in growth, differentiation, and survival of neurons during brain development (Bothwell 1995, Lewin & Barde 1996), as well as in adulthood (Con Over & Yancoupouolos 1997, Lu & Figurov 1997). BDNF has also been shown to play an important role in activity-dependent synaptic plasticity in the hippocampus (Kang & Schumann 1995, Korte *et al.* 1995), produce a lasting enhancement of synaptic efficacy in the dentate gyrus (Messaoudi *et al.*1998), and enhance glutamatergic synaptic transmission in hippocampus cell cultures through a presynaptic mechanism (Li *et al.* 1998). It is possible that these effects may, in turn, enhance specific learning and memory processes and help to reduce cognitive deficits connected with aging and neurodegenerative disorders (Howells *et al.* 2000, Michalski & Fahnestock 2003).

It is well established that neuronal activity regulates BDNF mRNA expression. Sensorial stimuli are able to influence BDNF mRNA levels, as demonstrated by experiments based on light stimulation, in both developing and adult rats (Castrèn *et al.* 1992). Moreover, physical activity has been shown to increase BDNF mRNA in rat cerebral cortex and hippocampus (Neeper *et al.* 1991). Thus, the hippocampal BDNF expression largely depends on the neuronal excitation/inhibition balance (Castrèn *et al.* 1992), even if it appears to be also affected by corticosteroid hormones that seem to down-regulate it (Schaaf *et al.* 1998).

In recent years, many experimental studies in rats and mice indicate an endogenous cyclical change in central BDNF and trkB expressions within 24 h (Bova *et al.* 1998, Schaaf *et al.* 2000*a*, Dolci *et al.* 2003), although the implication of these circadian oscillations still remains unclear.

The suprachiasmatic nucleus (SCN) of the hypothalamus contains an endogenous oscillator that is the primary biological clock in mammals (Hastings 1997). Although the mechanisms underlying the endogenous clock rhythmicity are not yet fully characterized, recent findings suggest that BDNF may be involved in the light-regulated circadian pacemaker of the central nervous system (CNS; Liang *et al.* 2000).

First evidence for the presence of BDNF in human circulation emerged a decade ago (Rosenfeld *et al.* 1995).

DOI: 10.1677/JOE-07-0376
0022–0795/08/0197–429
Online version via http://www.endocrinology-journals.org

Since then many studies investigated the various sites of BDNF production in humans, in both neuronal and non-neuronal cells (Donovan *et al.* 1995, Yamamoto *et al.* 1996, Gielen *et al.* 2003). As there are no studies at present in the literature investigating a possible BDNF circadian rhythm in humans, we studied the BDNF levels throughout 24 h in healthy men, in order to detect the possible relative changes in plasma BDNF protein.

Additionally, we looked for a possible interplay between BDNF and cortisol, the circadian physiological rhythm of which is well recognized in human plasma, with a zenith in the morning and a large decrease during the day. For this purpose, we studied whether there are any similarities or divergences between plasma BDNF and cortisol physiological circadian behavior.

Materials and Methods

Subjects

Thirty-four young healthy male volunteers were recruited for this study. Their age was between 20 and 33 years (mean ± S.D. = 25·6 ± 3·2), with a body mass index (BMI) between 20·5 and 28·3 (mean ± S.D. = 23·9 ± 1·9).

Prior to enrollment, participating subjects gave their written informed consent. The study was approved by the Ethics Committee of the Faculty of Medicine of the University of Pisa. Each subject was 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, or anti-inflammatory drugs, and no mood or behavior disturbances were referred at the time of enrollment.

Protocol

In order to investigate circadian BDNF variations, blood from each subject was collected every 4 h for a total of five samples in 24 h. At 0800 h, after overnight fasting, the first blood sample was drawn from the cubital vein of each subject into EDTA-coated tubes (Vacutest Kima s.r.l., Arzergrande, Italy). Subsequently, blood sampling was repeated at 1200, 1600, 2000, and 2400 h. The tubes were kept on ice and, after collection, blood samples were immediately centrifuged at 4 °C (2500 ***g*** for 15 min). Afterward, plasma was aliquoted and stored at −80 °C until assay. For each subject, sampling was repeated thrice in a month in order to analyze possible intra-individual variations in BDNF and cortisol circadian changes.

BDNF assay

Plasma levels of BDNF were determined with an ELISA method (BDNF Emax Immunoassay System, Promega, Madison, WI, USA), after appropriate dilution of samples (1:4) using block and Sample buffer, according to the manufacturer's instructions.

Briefly, 96-well flat-bottom immunoplates (Iwaki) were coated with anti-BDNF mAb and incubated at 4 °C overnight. After blocking by non-specific binding with block and 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 pAb. The last incubation required the addition of Anti-IgY-HRP 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 M, 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 purified BDNF).

The entire procedure was performed using a semi-automated Basic Radim Immunoassay Operator (BRIO-Radim, Pomezia, Italy) equipped with a microplate reader of optical density. A computer system linked to the BRIO analyzed the final results and expressed them in pg/ml.

Cortisol assay

Plasma concentration of cortisol was determined by a specific commercially available RIA kit (Radim).

The sensitivity of the assay was 0·9 μg/l. The intra- and inter-assay coefficients of variation were 2·6 and 8·0% respectively.

Parameters used and statistical analysis

Plasma BDNF levels were expressed in pg/ml, whereas cortisol was expressed as μg/l. All data are reported as mean ± S.D. Statistical analysis was carried out using GraphPad Prism 4.0 (San Diego, CA, USA). A Friedman test was performed, followed by a *post hoc* analysis with Dunn test. Percentages of decrease at each time point with respect to basal value (1200, 1600, 2000, and 2400 vs 0800 h) were calculated for both BDNF and cortisol. Finally, the correlation index (Spearman correlation coefficient) between BDNF and cortisol trends was calculated, based on the total 34 subjects × three samples × five time points.

Results

Intra-individual variability in BDNF and cortisol measurements

We checked for possible intra-individual variations in BDNF and cortisol measurements by repeating blood samples thrice in a month for each subject (one blood sample every 10 days). The mean ± S.D. of the three blood samples for each subject was calculated; subsequently, the means and standard deviations for each time point were computed, as reported in Tables 1 and 2.

Table 1 Intra-individual variability in brain-derived neurotrophic factor (BDNF) measurement: standard deviations of the means calculated for each subject×three blood samples at each time point

	0800 h	**1200** h	**1600** h	**2000** h	**2400** h
Subject 1	8·287792	15·86579	23·81197	26·12897	5·186842
Subject 2	121·2239	22·10611	24·59885	7·518643	11·42483
Subject 3	15·55635	14·13695	11·30133	12·9508	6·757465
Subject 4	11·37292	8·835723	4·68615	6·369458	8·778952
Subject 5	11·06662	8·538345	14·02581	13·78514	7·989994
Subject 6	17·89786	29·58485	42·42641	12·38669	1·001665
Subject 7	23·45854	22·18738	12·55083	12·30867	10·56456
Subject 8	107·3352	19·31942	16·59187	4·978956	7·18401
Subject 9	134·4917	11·45528	17·44735	7·60548	10·17988
Subject 10	14·40417	12·45913	4·942671	18·44641	26·45751
Subject 11	3·260368	10·59827	6·863672	18·40734	5·494543
Subject 12	10·98287	9·106225	7·218726	12·99654	4·513314
Subject 13	9·87269	10·61508	7·493998	7·076722	5·271622
Subject 14	16·78779	6·655073	9·696907	14·94925	8·425556
Subject 15	11·3377	10·91375	13·88488	5·896609	24·0211
Subject 16	8·861151	18·5262	6·409368	9·298925	1·868154
Subject 17	28·93095	12·00125	9·457272	6·413267	6·236185
Subject 18	13·2714	5·146844	6·819335	4·856954	7·672679
Subject 19	7·992496	8·006872	13·6504	8·43386	10·04092
Subject 20	13·99893	2·419366	9·551963	6·856384	12·25126
Subject 21	7·1631	10·92337	6·128621	8·764131	5·892368
Subject 22	15·12481	2·757716	4·259499	12·87478	8·608717
Subject 23	15·79335	8·016441	4·309292	5·38145	4·853864
Subject 24	12·75003	2·285461	7·979348	5·54617	3·459769
Subject 25	6·646804	8·822131	14·52859	12·6891	1
Subject 26	8·248636	2·357965	2·088061	4·562163	9·026627
Subject 27	5·444722	4·427565	16·1397	11·04596	4·2
Subject 28	15·27874	7·399324	16·34044	6·244998	13·25255
Subject 29	23·69578	14·26266	4·041452	9·455334	3·026549
Subject 30	1·907878	4·880915	17·55192	3·219213	18·21208
Subject 31	10·15332	20·71545	11·95031	17·22585	13·09313
Subject 32	11·88108	10·05037	19·20104	10·10149	9·034932
Subject 33	10·44031	15·06088	12·38184	17·38534	9·016282
Subject 34	13·31015	6·978777	10·43264	14·6186	9·9985
Mean	22·00676811	11·1005	12·08125	10·49352	8·646953

BDNF circadian variations

The highest BDNF level was found early in the morning (827·0±178·3), with a progressive decrease during the day (Fig. 1). In particular, in the second blood sample, drawn at 1200 h, plasma BDNF levels were significantly lower when compared with BDNF morning circulating levels ($P<0·001$). The BDNF levels further decreased during the day, so that values detected in the afternoon (1600 h) and the evening (2000 h) were significantly lower than those measured at 2400 h ($P<0·001$). The lowest concentration was achieved at midnight (214·4±44·3) ($P<0·001$ vs 0800 h, $P<0·001$ vs 1600 h, $P<0·001$ vs 2000 h).

Statistical results of the Friedman test and the difference in rank sum are reported in Table 3.

Cortisol circadian variations

We detected the highest plasma cortisol levels early in the morning (226·5±53·1). Then, plasma cortisol showed a progressive decrease during the day, reaching a nadir at 1200 h (61·9±14·0; $P<0·001$ vs 0800 h; Fig. 1b). In particular, 1600-h cortisol values were significantly lower than the morning values ($P<0·001$ vs 0800 h). Moreover, cortisol plasma concentrations measured at 2000 h were significantly lower than those detected at 1200 h ($P<0·001$) and, analogously, 2400 h cortisol levels were significantly lower than those measured at 1600 h ($P<0·001$).

Statistical results of the Friedman test and the difference in rank sum are reported in Table 4.

BDNF/cortisol correlation

In order to strengthen the observation of a similarity in BDNF and cortisol decreasing trend, we calculated the percentage of decrease in both BDNF and cortisol with respect to the maximum value detected in the morning. The correspondence between BDNF and cortisol circadian decreasing trend was attested by the determination of the Spearman index (0·8466) that was calculated based on the total 34 subjects×three times a month×five tests per day.

Table 2 Intra-individual variability in cortisol measurement: standard deviations of the means calculated for each subject×three blood samples at each time point

	0800 h	**1200** h	**1600** h	**2000** h	**2400** h
Subject 1	15·57337	16·92306	8·650434	3·204684	1·800926
Subject 2	4·331282	9·277392	6·213158	2·468468	1·12645
Subject 3	1·414214	6·17333	1·081665	1·473092	1
Subject 4	10·58301	3·869108	0·64291	1·552417	2
Subject 5	8·035131	5·245951	3·019934	8	1·2
Subject 6	10·00017	9·497895	3·380828	1·664332	2·605763
Subject 7	11·32608	4·801389	8·900187	3·3	2·260531
Subject 8	1·553491	0·61101	2	1·053565	1·209683
Subject 9	7·636753	3·605551	2·882707	1·266228	1·931321
Subject 10	3·132092	3·241913	3·100538	1·36504	1·800926
Subject 11	3·671512	2·986637	2·9	2·847806	1·656301
Subject 12	1·30767	1·915724	2·421432	2·358672	2·847806
Subject 13	1·9	4·092676	2·233831	1·802776	1·410674
Subject 14	3·567913	1·769181	2·271563	1·389244	0·832666
Subject 15	3·994997	2·93087	5·031898	1·571623	2·389561
Subject 16	6·846167	3·464823	2·402082	1·637071	2·487971
Subject 17	7·707464	5·663038	3·557152	2·55147	2·905168
Subject 18	6·317436	2·1	1·868154	2·570992	2·828427
Subject 19	9·266607	2·98161	3·482815	1·417745	2·868798
Subject 20	2·351595	2·628688	3·251154	1	0·9
Subject 21	2·426932	2·95973	1·473092	1·664332	1·571623
Subject 22	8·870738	3·031501	4·06325	1·819341	1·342882
Subject 23	3·987894	2·433105	1·604161	1·274101	2·066398
Subject 24	2·511971	2·042058	1·9	1·571623	1·852026
Subject 25	1·414214	2·260531	2·165641	2	1·637071
Subject 26	0.960902	1·442221	1·835756	1·30767	1·56205
Subject 27	2·828427	3·207803	1·587451	3·098925	2·475884
Subject 28	4·033609	4·65224	1·7	0.916515	2·066398
Subject 29	6·413267	1·664332	1·708801	1·777639	2·042058
Subject 30	1·5	1·03923	3·973663	1·266228	2·554082
Subject 31	7·697402	10.3769	5·798276	10.32037	3·619853
Subject 32	8·217664	6·533758	8·835723	3·292416	2·787472
Subject 33	11·59483	7·470609	9·597395	1·530795	2·622975
Subject 34	7·893668	9·1	5·216321	4·517743	3·642801
Mean	5·613778	4·470408	3·551529	2·378027	2·056075

Discussion

The primary purpose of the present study was to investigate the presence of a possible circadian rhythm in BDNF circulating levels in humans.

In our previous study, we found that BDNF circulating levels are closely related to the sex hormonal status, pointing out the key role played by sex gonadal hormones in the modulation of expression and production of BDNF (Begliuomini *et al.* 2007). However, it is reasonable to suppose that changes in the plasma BDNF levels are not only endocrine based, but also influenced by other factors, such as neurotransmitters, sensorial stimuli, and physical activity.

Even though no studies have been yet published about circadian oscillations of BDNF in humans, many pieces of experimental evidence in rats corroborate the hypothesis of a circadian BDNF rhythm. Light:darkness cycles could in some way influence BDNF expression by modulating the cerebral circadian pacemaker localized in the SCN of the hypothalamus. It has been shown that both BDNF mRNA and protein, as well as trkB receptor, present rhythmic variations in rat CNS (Bova *et al.* 1998, Berchtold *et al.* 1999). Bova *et al.* (1998) and Berchtold *et al.* (1999) analyzed the expression of BDNF mRNA in the rat hippocampus and frontal cortex and they both observed that the highest BDNF mRNA levels were reached during the dark hours (activity period), while the lowest BDNF levels were detected during the light hours (rest period).

The present results show that plasma BDNF in human males presents a characteristic trend during the day; in our study population, the highest BDNF concentrations were detected in the morning, followed by a substantial decrease throughout the day and the lowest values were observed at midnight. Evidently, this decline in BDNF during the day may be ascribed to a circadian secretory model. In fact, it has been shown that BDNF presents a very short half-life in plasma ($t_{1/2}$=0·92 min; Poduslo & Curran 1996), and then it is conceivable that BDNF is secreted with a pulsatory circadian rhythm, featured by a progressive reduction in the amplitude of the pulses throughout the day.

Furthermore, our results point out that the daily trend of plasma BDNF is very similar to the trend of cortisol. Cortisol

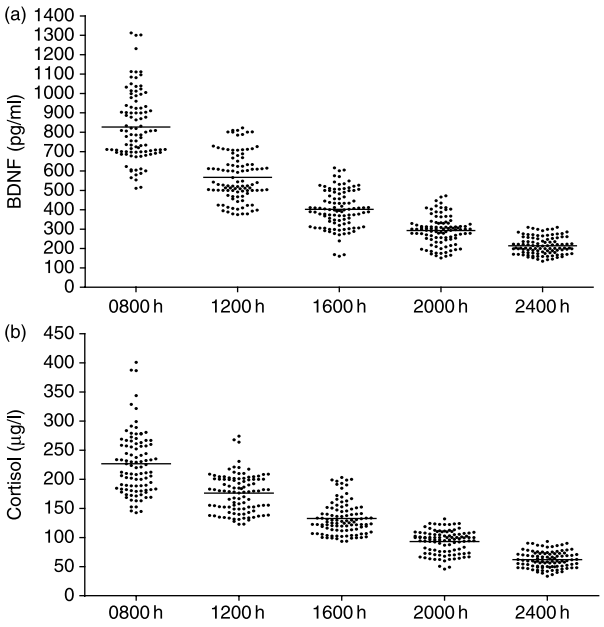

Figure 1 Scatter plot representing the decreasing trend of (a) plasma BDNF (pg/ml) and (b) cortisol (µg/l) throughout the day in healthy males.

is the most important glucocorticoid in humans and, besides its well-known effects on metabolism, bone, and blood pressure, it has been recognized to play a key role in the homeostasis of the CNS. The pulsatile secretion of cortisol in humans has been well established; its pulses have frequencies in the range of 60–90 min. Regulation of cortisol secretion depends on the integrity of the hypothalamic–pituitary–adrenal axis. In physiological conditions, hypothalamic CRF stimulates the pituitary to produce adrenocorticotrophin (ACTH) that, in turn, has a stimulatory effect on cortisol production by the adrenal cortex. Cortisol exerts a negative feedback with a long-loop mechanism, so that high circulating cortisol levels are associated with low CRF levels

Table 3 Results of *post hoc* analysis of brain-derived neurotrophic factor (BDNF) circadian rhythm by the means of Dunn's test

	Difference in rank sum	*P* value
Dunn's test		
0800 vs 1200 h	88·00	<0·001
0800 vs 1600 h	189·0	<0·001
0800 vs 2000 h	285·0	<0·001
0800 vs 2400 h	378·0	<0·001
1200 vs 1600 h	101·0	<0·001
1200 vs 2000 h	197·0	<0·001
1200 vs 2400 h	290·0	<0·001
1600 vs 2000 h	96·00	<0·001
1600 vs 2400 h	189·0	<0·001
2000 vs 2400 h	93·00	<0·001

Value of Friedman test is 370.8 ($P<0.0001$).

Table 4 Results of *post hoc* analysis of cortisol circadian rhythm by the means of Dunn's test

	Difference in rank sum	*P* value
Dunn's test		
0800 vs 1200 h	61·5	>0·05 (NS)
0800 vs 1600 h	169·5	<0·001
0800 vs 2000 h	271·0	<0·001
0800 vs 2400 h	368·0	<0·001
1200 vs 1600 h	108·0	<0·001
1200 vs 2000 h	209·5	<0·001
1200 vs 2400 h	306·5	<0·001
1600 vs 2000 h	101·5	<0·001
1600 vs 2400 h	198·5	<0·001
2000 vs 2400 h	97·0	<0·001

Value of Friedman test is 371.3 ($P<0.0001$).

and vice versa (Young *et al.* 2004). Disruption in cortisol rhythm has been observed in many pathological conditions, such as major depression, sleep, and mood disorders, as well as in acute or chronic psychophysical stress conditions (Chrousos & Gold 1992, Erickson *et al.* 2003).

Since a down-regulation by corticosteroid hormones on BDNF mRNA and protein in the rat has been reported (Schaaf *et al.* 1998, 2000*b*), we verified whether there was a relationship in humans between cortisol and BDNF plasma levels in physiological conditions.

The present results underline a positive correlation between plasma BDNF and cortisol daily trend, thus suggesting a possible co-regulation of the expression of these two compounds. Additionally, the similarity in the circadian variations of BDNF and cortisol allows us to hypothesize that glucocorticoid and neurotrophic tone may have a synergic role in the homeostasis of cerebral functions.

In pre-clinical studies, it has been shown that endogenous or exogenous corticosterone, which represents the main glucocorticoid in the rat, suppresses central BDNF mRNA and peptide expressions (Schaaf *et al.* 1998). This might be related to the inhibition of BDNF synthesis mediated by the activation of mineralocorticoid and glucocorticoid receptors (Schaaf *et al.* 2000*b*). On the other hand, the role of corticosterone in regulating the BDNF levels is still a subject of debate. In fact, it has been shown in the animal model that stress can decrease hippocampal BDNF, also in adrenalectomized rats, suggesting that corticosterone, ACTH, and CRF are not the only elements of stress response contributing to the observed decrease in the BDNF levels in rats (Smith *et al.* 1995).

Our results, demonstrating a consensual diminishing trend of BDNF and cortisol plasma levels in a physiological 24-h cycle, seem to enter in conflict with studies on the rat hippocampus. Our data suggest a fairly consistent co-regulation of plasma BDNF and cortisol, mediated by an unknown mechanism, rather than a down-regulation of BDNF by corticosteroids.

Alternatively, the hypothesis of a down-regulation of BDNF by corticosteroids could be sustained if we consider the delay between the BDNF mRNA expression and the subsequent process of translation, synthesis, and release of BDNF plasma protein. From this point of view, it would be reasonable to suppose that high levels of cortisol may inhibit the BDNF mRNA expression in the CNS, but this phenomenon cannot be immediately observed at the peripheral level.

However, it has recently been demonstrated that chronically enhanced cortisol induces an augmentation in BDNF in primates (McMillan *et al*. 2004), thus constituting a compensatory mechanism in response to cortisol-induced neurotoxicity. These discrepancies may also be influenced by a differential distribution of the glucocorticoid and mineralocorticoid receptors in rats and humans (Sanchez *et al*. 2000).

In conclusion, our results demonstrate that, in physiological conditions, circulating BDNF in humans presents a characteristic daily variation that is closely similar to the cortisol circadian rhythm. In our opinion, this may be explained by the presence of an individual internal balance that assures a homeostasis between protective and insulting factors at the neuronal level. The correct functioning of this compensatory mechanism may be responsible for maintaining appropriate levels of activity in the hippocampus and other brain areas.

Acknowledgements

This work was partially supported by a grant from the Fondazione Cassa Risparmio di San Miniato, San Miniato, Italy. The authors would also like to thank Dr Massimetti for statistical advice and Dr Monteleone for linguistic revision. The authors declare that there is no conflict of interest that would prejudice the impartiality of this scientific work.

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Received in final form 30 January 2008
Accepted 15 January 2008
Made available online as an Accepted Preprint
15 January 2008