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Nerve Growth Factor serum concentrations in healthy volunteers: physiological variance and stability

Nerve Growth Factor serum concentrations in healthy human volunteers: physiological variance and stability

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Abstract

Human nerve growth factor (NGF) serum concentrations have been measured in a healthy sample of 126 participants by a modified highly sensitive and specific two-site enzyme immunoassay. The measured NGF concentrations differ considerably from a normal distribution. The median NGF concentration was 19.68pg/ml with an interquartile range of 11.06 to 41.74pg/ml, which means that 50% of the NGF levels are in this range. In our healthy sample, we found no gender differences but a slight age-related decrease of NGF ($r = -0.1326$, $p = 0.1560$). Moreover intraindividual stability of NGF has been examined in 10 volunteers, where no significant changes of serum NGF concentrations have been detected over 4 weeks. This stability of our repetitive measurements over 4 weeks is possibly reflecting this neurotrophin to be an intraindividually solid marker at least in human serum.

Key words:

enzyme immunoassay, nerve growth factor, healthy volunteers, stability, physiological variance, age, gender

Nerve growth factor (NGF) is the best known neurotrophin, which not only acts on central cholinergic neurons as well as subsets of peripheral neurons, this neurotrophin might also maintain a balanced interplay between the nervous, immune and endocrine system [for review see 2]. As NGF is synthesized and stored by a variety of cells outside the nervous system, NGF may operate through multiple pathways to regulate physiological homeostasis and behavioural coping [for review see 1]. Neuroendocrine, neurochemical, and behavioural changes in the adult, which occur in most of the neuropsychiatric disorders, are known to be critically influenced by brain development and plasticity, both of them mediated by neurotrophins [for review see 5]. Correspondingly, several studies have examined human NGF serum and plasma concentrations in different psychiatric disorders and compared them to healthy controls. The mean human NGF serum and plasma values reported in these studies differ, and variations with age and gender are sometimes contradictory [3,4,6,7,12,13,14,15,16,17]. Thus, although neurotrophins are getting an attractive target for neuropsychiatric research, there are only few data concerning the NGF serum levels in healthy volunteers. In this study the physiological variance of NGF serum concentrations in a sample of 116 healthy controls has been measured and intraindividual changes of NGF serum concentrations in healthy volunteers have been examined for the first time.

116 healthy volunteers participated in this study. The investigated 48.3% female and 51.7% male subjects aged 21-71 years (44±13) and were recruited by newspaper advertisement. Any psychiatric disorder in their medical history and any history of cardiopulmonar or metabolic diseases led to exclusion from the study. After complete description of the study to the subjects, written informed consent was obtained.

In addition, we studied the intraindividual changes of NGF serum concentration in 10 physicians age ranging from 28 to 53 (33±6) years. These subjects were employees of the psychiatric department of the Free University of Berlin, whose good and stable state of health had been documented in the records of this institute for a period of several years. Blood has been taken on 3 following days, after 2 weeks and after 4 weeks at the same daytime (±30 minutes).

Blood for the NGF protein measurement was collected in tubes without additives. After centrifugation at room temperature (3500g/15min) the blood samples were stored at –70° until processing. Chemicals of analytical grade were purchased from Merck (Darmstadt, Germany). Each 140µl of serum was diluted with sample buffer and endogenous NGF was quantified by a highly sensitive and specific two-site enzyme immunoassay as described in detail previously [8,9,15]. Briefly black 96-well flat bottom immunoplates (Dynatech Laboratories, Inc.,

Virginia) were coated with 50µl per well of 1.0µg/ml monoclonal anti-mouse-β-NGF antibody 27/21 (Chemicon, Hofheim, Germany). Parallel wells were coated with mouse IgG_1 (MOPC 21, Sigma Chemie, Deisenhofen, Germany) for evaluation of non-specific signals. After 2h at room temperature (20°C) the plates were washed three times with 200µl per well of washing buffer. Unlike the previous protocol [8], this assay did not contain gelatin and the samples were incubated in the coated wells (50µl each) overnight at 4°C [9]. After an additional three washes the immobilized antigen was incubated with 0.75mU per well of monoclonal antibody 27/21 conjugated with β-D-galactosidase (Roche Diagnostica and Biochemicals, Germany) for 1.5h at room temperature. The plates were again washed with washing buffer for 1h, and then finally washed twice with substrate buffer. To start the enzyme reaction, 50µl substrate buffer containing 0.2mM 4-methylumbelliferyl-β-D-galactoside (Sigma Chemie, Deisenhofen, Germany) was added to each microwell. After overnight incubation at 4°C the enzyme reaction was stopped with 200µl per well of stopping buffer. The fluorescent reaction product, 4-methylumbelliferone, was measured in a microplate fluorometer (Labsystems Flouroskan II, Germany). NGF concentrations were determined from the regression line for the NGF standard (ranging from 0.25 to 1000pg/ml purified mouse 2.5 S NGF) incubated under similar conditions in each assay. The measured levels for NGF were corrected for mean recoveries of added mouse NGF (125pg/ml), which were determined in each assay. Determinations of recovery, specific and unspecific NGF binding, each involving quadruplicate fluorescence determinations, were run for each serum sample.

The Kolmogorov-Smirnov test showed that the NGF level is not a normally distributed trait. The measured NGF concentrations differ considerably from a normal distribution ($D=0.423;p<0.01$) and even a logarithmic transformation does not remove the skewness completely ($D=0.147;p<0.01$;figure1).

Hence results are presented primarily as medians and interquartile ranges of the measured values, additionally means and standard deviations (SD) of the logarithmically transformed values are mentioned. Group differences were tested by non-parametric tests. Homogeneity of distributions of NGF levels between males and females were tested by means of Mann-Whitney test.

Intraindividual variations in NGF levels were analyzed by means of a Bland and Altmann plot, in which the differences between successive measurements are plotted against the mean of these two measurements. Furthermore differences in the distribution of NGF levels across five measurements was analyzed by means of Friedman's analysis of variance for statistical significance.

NGF serum concentrations revealed a large physiological variance (figure

1). The measured NGF concentrations varied from 6.44pg/ml to 3589pg/ml with the next highest observed concentration of NGF being 440.91pg/ml, underlining the outlier nature of the 3589pg/ml observation. The median NGF concentration was 19.68pg/ml with an interquartile range of 11.06 to 41.74pg/ml, which means that 50% of the NGF levels are in this range. Only 10% of observed NGF levels were greater than 164.16pg/ml. Mean and standard deviation of the logarithmically transformed levels were 1.396±0.442pg/ml. There were no statistically significant differences in the distribution of NGF concentrations between males and females (p=0.802;figure 1).

Although overall there was a slight tendency for NGF levels to decline with age (Spearman's r =-0.1326), this correlation was statistically not significant (p=0.1560). The number of observations with NGF concentrations >100pg/ml was slightly increasing with age.

To test the intraindividual stability of serum NGF the variation of NGF concentrations between the five measurement occasions in 10 healthy volunteers has been measured. There were no significant changes of NGF serum concentrations between the five occasions (p=0.2616;figure 2). Mean NGF levels were 47.7±16, 41.88±10, 39.42±2, 43.83±20, 45.55±13. They did not significantly change (Friedman test 0.259). The Bland and Altmann plot (figure 2) showed that the mean of differences between successive measurements was close to zero (-0.72) and only 4 observations were not in the range of the mean ±2 SD. This homogeneity was underlined by a correlation of r=0.6926 (p<0.001;Spearman´s r) between successive measurements.

This is the second study, where the range of NGF serum concentrations in a large number of healthy subjects has been investigated [17]. For the first time we also investigated the intraindividual changes of NGF serum concentrations in healthy volunteers over the period of one month.

Normally the NGF values in healthy controls referred to in the literature include only small groups of subjects [3, 4, 7, 11, 12, 14, 15, 16]. In these studies the reported means of NGF are different and variations with age and gender are contradictory [3,4,6,7,12,13,16,17].

In our sample NGF serum concentrations differ between individuals, and 7.7% of the healthy participants revealed relatively high NGF serum concentrations (>100pg/ml). This non-parametric distribution of NGF serum concentrations could explain the differing mean NGF

serum concentrations referred from the literature. The mean NGF serum and plasma concentrations of several studies with maximal 25 healthy controls amounted to 3.8±1.7pg/ml [4]; 20pg/ml [6], 36 to 72pg/ml [3], 66±18pg/ml [16], 51.68±5.94pg/ml [12], 57.3±96.6pg/ml [14], 94.87±8.63pg/ml [7] and 110.4±152.1pg/ml [15].

A slight age-related decrease of NGF has previously been discussed by others [17], which has also been found in our present investigation. To our knowledge only one study examined the range of NGF serum concentrations in healthy participants in a large number of 157 blood donors, who were not further described. In this study mean NGF levels were 194±25pg/ml [17]. In this sample no statistically significant variations with age appeared, but the NGF level was significantly lower in females (112±31pg/ml) than in males (243±35pg/ml) [17]. In our sample of healthy volunteers there were no gender related differences according to NGF values, which is not in line with two contradicting reports, where NGF serum concentrations in females were either significantly lower [17] or higher [16] when compared to males. However, the repetitive measurements of NGF serum concentrations over 4 weeks are possibly reflecting this neurotrophin to be an intraindividually solid marker.

In conclusion, NGF serum concentrations in healthy humans are not normally distributed and there are about 10% outliers, who reveal relatively high NGF concentrations (>100pg/ml). We hypothesize these high NGF concentrations to reflect an increased vulnerability to several neuropsychiatric [11, for review see 10] or allergic diseases [4], indeed they could also be genetically determined; the latter issue has not been studied yet.

Acknowledgments

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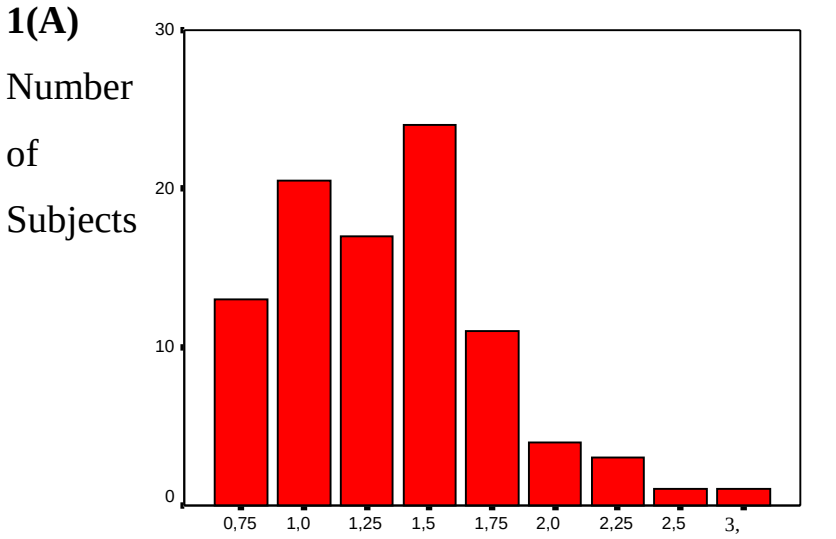
Figure 1. Human nerve growth factor (NGF) serum concentrations revealed a large physiological variance (n=116). **(A)** Physiological Variance of NGF serum concentration in all healthy participants.

1(A)



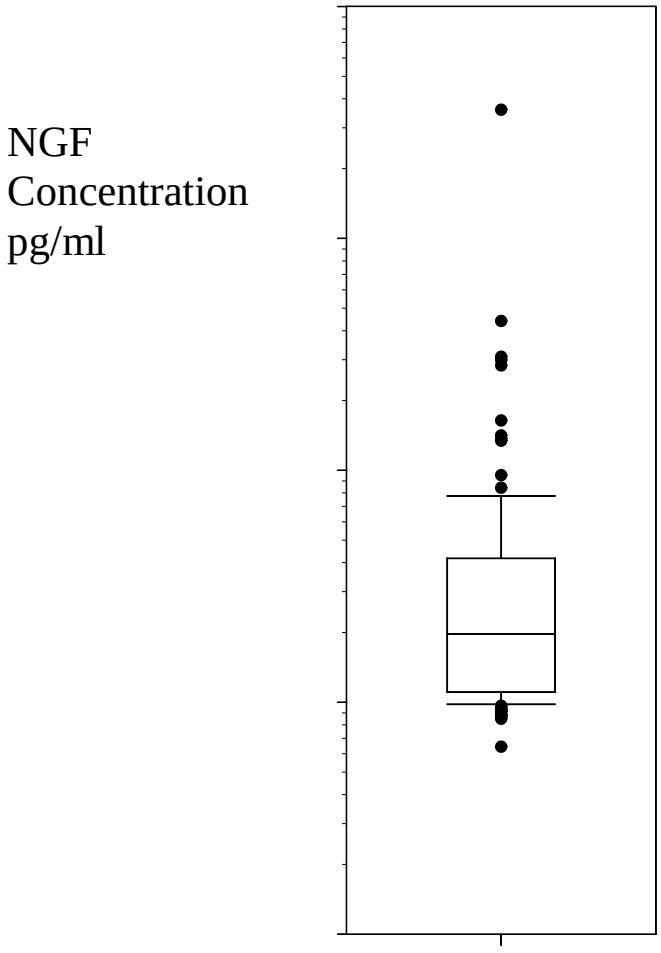


Figure 1. (B) Box plot of NGF levels. The mean NGF concentration in all participants was 71.37± 335pg/ml, the median concentration was 19.68pg/ml. Mean logarithmically transformed NGF levels are 1.396±0.442pg/ml.

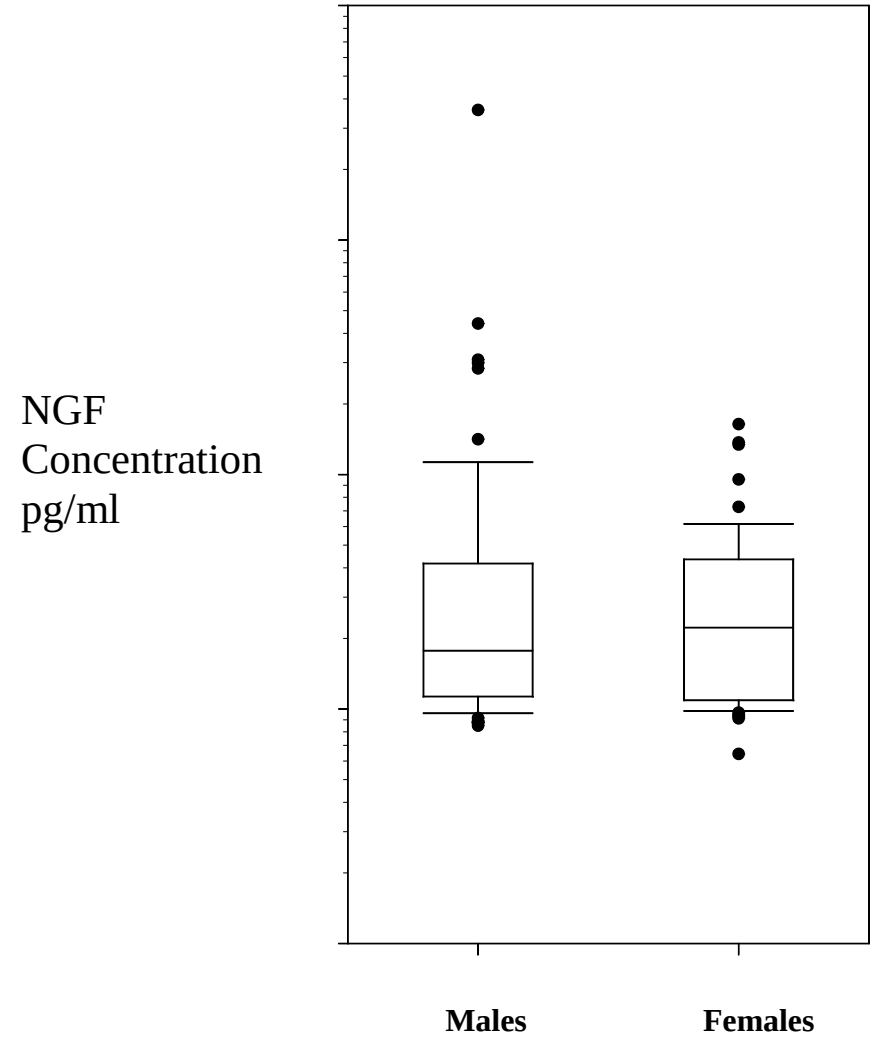


Figure 1. (C) Box plot of the gender difference of NGF serum concentrations shows no statistically significant differences in the distribution of NGF concentrations between males (n=60;mean level: 106.89±464pg/ml) and females (n=56; mean level: 33.31±33pg/ml)(p=0.802).

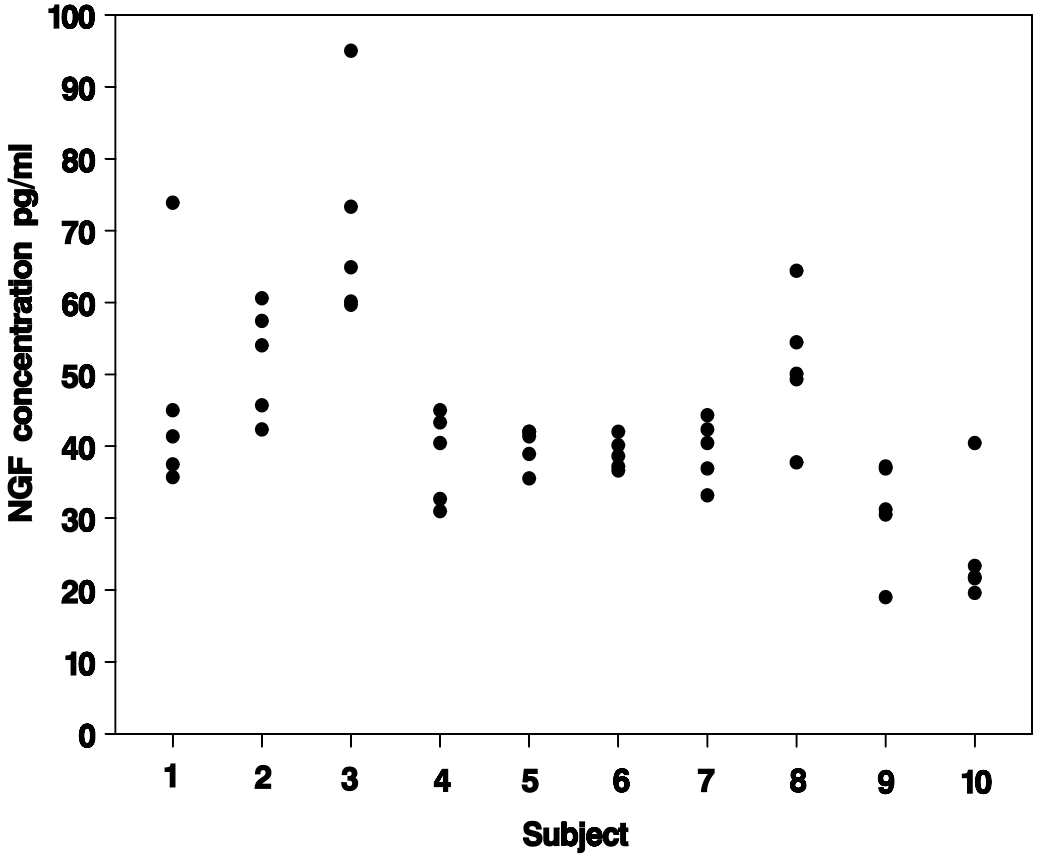


Figure 2. (B) The Bland and Altmann plot shows that the mean of differences between successive measurements is close to zero (-0.72) and only 4 observations are not in the range of the mean ± 2 SD. This homogeneity is underlined by a correlation of r=0.6926 ($p<0.001$; Spearman's r) between successive measurements.

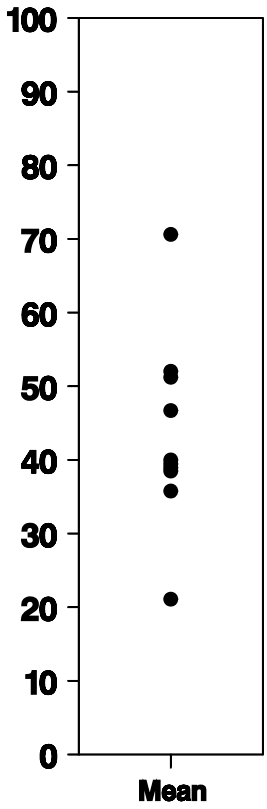


Figure 2 (A) Intraindividual nerve growth factor (NGF) serum concentration changes in 10 physicians. Mean NGF levels were 47.7 (±16,3); 41.88 (±10.54); 39.42 (±2.02); 43.83 (±20.61); 45.55 (±13.11). The variation of NGF concentrations between the five measurement occasions was statistically not significant (p = 0.2616).