Growth Hormone Deficiency and Memory Functioning in Adults Visualized by Functional Magnetic Resonance Imaging

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Memory Performance and the Growth Hormone/Insulin-Like Growth Factor Axis in Elderly: A Positron Emission Tomography Study

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Key Words

Positron emission tomography · Growth hormone · Insulin-like growth factor I · Working memory · Regional cerebral blood flow · Cognition · Aging · Clinical neuroendocrinology · Prefrontal cortex · Premotor cortex

Abstract

The relationship between the growth hormone/insulin-like growth factor (GH-IGF)-I status and memory performance is studied in 24 elderly males and females, aged 75–85 years. Positron emission tomography (PET) was used to measure differences in regional cerebral blood flow during the performance of a delayed-non-match-to-sample (DNMTS) working memory task. Quality and speed of performance on the DNMTS task were measured separately for the easy items (3, 4 and 5 letters) and difficult items (6, 7 and 8 letters). Results were analyzed in two different groups based on the IGF-I level of the subjects (low or high IGF-I). Error rates on the working memory task were not different, but the high IGF-I group had shorter reaction times on the easy items. The high IGF-I group showed a significantly greater increase in cerebral blood flow in the left premotor cortex (easy items) and left dorsolateral prefrontal cortex (difficult items) compared to the low IGF-I group. It is concluded that elderly with high IGF-I levels are capable of faster working memory performance and increased recruitment of task-associated prefrontal regions.

Introduction

Several factors have been identified as contributors of cognitive decline in aging – vascular, genetic and endocrine factors [1–3]. Aging is accompanied by a decline in several aspects of somatic functioning, such as a decreased lean body mass, reduced protein synthesis, reduced muscle strength, decreased bone mass and increased body fat mass [4]. These features are comparable with those of patients with growth hormone deficiency (GHD), suggesting a mediating role for the growth hormone/insulin-like growth factor I (GH-IGF-I) axis in the process of aging.

GH stimulates the liver to secrete IGF-I and is secreted from the anterior pituitary under the modulation of GH-releasing hormone (GHRH) and somatostatin. GH secretion and serum GH concentrations fall with age, both baseline values and in response to provocative stimuli, with a parallel decrease in serum concentrations of IGF-I

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[5]. IGF binding proteins (IGFBPs) are involved in the regulation of IGF-I bioavailability, but also seem to have their own receptors mediating IGF-I independent actions [6].

It is known that GH may cross the blood-brain barrier and that it is present in cerebrospinal fluid and brain tissues [7]. It is also known that GH releases IGF-I in the central nervous system. GH receptors are found in the human brain in the choroid plexus, pituitary, hippocampus, hypothalamus, putamen and thalamus [8]. Both animal and human studies show that the number of GH receptors throughout the brain declines with age [9]. IGF-I receptors are also found in the brain, in the hippocampal and parahippocampal area, the amygdala, cerebellum and prefrontal cortex [10]. In an animal study in rhesus monkeys, in situ hybridization showed that docking protein p62 Dok-1 (Dok-1) mRNA was expressed in all layers of the dorsolateral prefrontal cortex (DLPFC) and in all neuronal subregions of the hippocampal formation [11]. These expression patterns are very similar to those of the IGF-I receptor and suggest that Dok-1 could be among the downstream targets of IGF signaling in areas of the primate brain involved in learning and memory.

IGF-I levels were found to be correlated with cognitive function in unselected elderly subjects [12] and in healthy centenarians [13]. A prospective study on circulating IGF-I, IGFBP-3 and cognitive function in elderly showed that higher total IGF-I and total IGF-I to IGFBP-3 levels were associated with less cognitive decline over the next 2 years, as measured with the Mini Mental State Examination (MMSE) [14]. Serum IGF-I levels were associated with mental processing speed in healthy elderly subjects [15, 16]. These findings suggest that increasing IGF-I levels in elderly subjects with low IGF-I levels may prevent cognitive decline or even improve memory performance or memory speed. In one study in healthy elderly subjects the effect of growth hormone substitution (0.03 mg/kg of body weight three times a week for a period of 6 months) on cognitive function was investigated. An improved performance on the Trail B test (a motor tracking task measuring information processing, planning and cognitive flexibility) was found, but not on the Digit Symbol Substitution Test, measuring working memory, visual acuity, cognitive flexibility and motor speed.

Scores on the MMSE and Geriatric Depression Scale also remained unchanged [17]. A relation between GH-IGF-I and cognition has also been found in childhood-onset GH-deficient (CO-GHD) adults, exhibiting low GH and IGF-I levels. These patients had significant cognitive deficits compared with healthy controls, especially with respect to memory function. CO-GHD patients performed worse on an iconic memory task, a short-term memory and a long-term memory task [18]. Growth hormone replacement therapy in these patients improved short- and long-term memory performance within 1 year [19].

The exact mechanism behind the relation between GH-IGF-I and memory is not known yet. To elucidate this issue, positron emission tomography (PET) was used. This provides the opportunity to investigate brain function in a non-invasive manner. Regional cerebral blood flow (rCBF) was measured as an indirect marker of local neuronal activity using oxygen-15-labeled water ($H_2{}^{15}O$). The physical half-life of ^{15}O of 2.07 min permits repeated measurements of rCBF during the performance of a cognitive task. Data are available on PET scanning in the field of cognition but, as far as we know, not in relation to the GH-IGF-I axis. However, studies in rats have shown increased cerebral blood flow as well as improved memory performance after GH and IGF-I administration [20].

The present study was aimed to investigate the neurophysiological substrate of working memory function in elderly subjects with either high or low GH-IGF-I axis activity. Working memory can be characterized as the ability to keep a limited amount of information 'on line' (maintenance) for immediate use (manipulation).

Subjects performed a delayed-non-match-to-sample (DNMTS) working memory task whilst being scanned. This enabled the assessment of differences in memory performance and brain activity at various memory loads. Lesion studies in monkeys have shown that the DNMTS task is highly dependent on the ventromedial prefrontal cortex [21]. In humans, the DNMTS task has also been found to activate parietal and ventrolateral prefrontal cortex and, at a higher task load, DLPFC [22].

As is stated above, memory performance has been shown to be decreased and correlated with IGF-I levels in elderly. There is accumulating evidence that IGF-I plays an important role in protecting neurons and participates in neuronal growth and synaptic reorganization. As IGF-I receptors have been localized in the frontal cortex, which plays an important role in working memory, the protective role of IGF-I may also apply to forebrain cholinergic neurons [23]. Based on the generally accepted knowledge that age-related memory decline is associated with deterioration of cholinergic neurons, we formulated the hypothesis that working memory performance and the working memory-associated brain activity is different in subjects with low or high activity of the GH-IGF-I axis. It was hypothesized that elderly subjects with high IGF-I levels

would exhibit both a better working memory and a higher brain activity in brain areas associated with working memory (DLPFC and parietal cortex) than subjects with a low GH-IGF-I status. Superior working memory would be reflected by a better and/or faster memory performance and increased regional brain activity as measured with PET.

Methods

Study Population

Participants were Caucasian non-demented elderly participating in the Longitudinal Aging Study Amsterdam (LASA). LASA investigates determinants of physical, social, emotional and cognitive functions in aging people in a representative stratified sample of The Netherlands [24]. In 1,318 elderly subjects between 65 and 88 years of age, the relationship between cognitive and emotional functions and serum IGF-I was investigated [16]. In the present study, subjects from the highest and lowest IGF-I quartile who met the inclusion criteria (n = 69) were asked by telephone to participate in the present study. Inclusion criteria were: (1) age between 70 and 85 years, (2) MMSE score >24 (to exclude subjects with cognitive deficits) and (3) thyroid function within the normal range. Exclusion criteria were: (1) not being able to remain supine for at least 1 h and (2) neurological or psychiatric disorders. A total of 24 elderly (14 males and 10 females) were included in the study. The other 45 subjects of the 69 potential subjects could not participate for different reasons, such as poor health, fear for magnetic resonance imaging (MRI) or PET, claustrophobia, recent hospital stay or unwillingness to be immobile for 1 h. Education levels were defined as asking the subject for the highest educational level completed and were ranked on a scale from 1 (elementary school not completed) to 9 (university education). All subjects gave written informed consent. The study was approved by the Medical Ethical Committee of the VU University Medical Center and was conducted according to the principles of the Helsinki Declaration.

Procedure

Participants were instructed not to use caffeine or soporific drugs the night before and on the day of the study session and underwent neuroimaging in the afternoon. A 30-cm^3 blood sample was drawn at noon, prior to the PET procedure to measure IGF-I, IGFBPs and thyroid function. The blood samples were centrifuged (10 min, 3,500 rpm at 4°C) and serum was stored at –20°C until analysis. Both the research physician and the participants were unaware whether the subject belonged to the low or high IGF-I group.

Biochemical Data

Serum concentrations of TSH, freeT_4 and freeT_3 were measured according to standardized techniques with commercially available radioimmunoassays. Plasma IGF-I levels and IGFBP-3 levels were measured with commercially available assays (IGF-I, Chemoluminescentic; Nichols Institute Diagnostics, San Juan Capistrano, Calif., USA; IGFBP-3, Immunoradiometric assay; DSL, Webster, Tex., USA). The detection limits for IGF-I and IGFBP-3 were 6.0 nmol/l and 0.4 mg/l, respectively. The intra-assay coefficient of variation for IGF-I was 3% at serum IGF-I of 20 nmol/l. The inter-assay coefficients of variation were 6 and 8% at serum IGF-I of 33 and 7 nmol/l, respectively. The normal range for IGF-I in adults, aged >61 years, is 10.3–19.0 nmol/l (P5–P95). For IGFBP-3 the intra-assay coefficient of variation is 4% at serum IGFBP-3 of 5 mg/l and the inter-assay coefficient of variation is 7% at serum IGFBP-3 of 3.9 mg/l. Plasma IGFBP-4 levels were determined as described before [25]. A specific RIA determined IGFBP-2 levels, using an antiserum that was raised in a New Zealand rabbit against recombinant bovine IGFBP-2 (rbIGFBP-2; GroPep Ltd, Adelaide, Australia). The antiserum was obtained after immunization with 150 µg of protein in incomplete Freund's adjuvant. The assay buffer was composed of 50 m*M* sodium phosphate (pH 7.4), 10 m*M* ethylene-diamino-tetra-acetate (EDTA), 0.05% (w/v) Tween-20, 0.2% BSA and 0.02% NaN_3. Recombinant hIGFBP-2 (GroPep Ltd) was used as a standard (range 0.02–15 ng/tube) and [^{125}I]-rhIGFBP-2 as a tracer. Iodination of rhIGFBP-2 was achieved following the procedure described for IGFBP-4 [25]. The RIA incubation mixture consisted of 200 µl standard or diluted sample, 100 µl antiserum (final dilution in assay buffer: 1:80,000), and 100 µl tracer (~12,000 cpm). After equilibrium incubation for 40–47 h at 4°C in polystyrene tubes, 100 µl Sac-Cel solid-phase anti-rabbit IgG-coated cellulose suspension (Immunodiagnostic Systems, Boldon, UK) was added. Complexation was complete after 30 min at room temperature, 0.5 ml distilled water was added to the samples, which were subsequently centrifuged at 10,000 *g* for 4 min. Pellets were counted in a t-counter (Packard Instrument Co., Inc., Downers Grove, Ill., USA). Intra-assay variations (10 replicates) were 8.3, 4.9 and 4.5% at mean plasma levels of 2.4 nmol/l (73 µg/l), 8.2 nmol/l (256 µg/l) and 38.6 nmol/l (1,205 µg/l), respectively. The sensitivity of the assay was 0.003 nmol/l, i.e. 0.1 µg/l (absolute concentration).

Neuropsychological Assessment

Mood. POMS (Profile of Mood States) [26], a self-report questionnaire, was administered in which subjects rated their feelings over the previous 3 days. A shortened Dutch version of 32 items was used for measuring depression, tension, fatigue, anger and vigor. The rating (1–5) for each item was recorded. Mood scales were assessed to control for possible group differences in task performance due to differences in mood state.

Memory. The task performed during neuroimaging was a DNMTS task, adapted for the PET procedure in our laboratory. This is a working memory task specifically measuring verbal recognition memory. During the DNMTS task a series of letters is presented on a computer screen positioned at a distance of 0.5 m in front of the subject. Subjects were instructed to read and remember the series of letters and thereafter to choose the letter that they had not seen before out of 2 letters presented on the screen. Subjects were asked to indicate the position of the novel letter ('non-matching') by pressing the compatible button of a two-button system with their right forefinger (left button) or middle finger (right button). A schematic representation of the task is shown in figure 1. Eight runs were presented and 8 PET scans were performed, each run consisting of 3 series of letters. Before each run, subjects were informed (on the screen) whether the upcoming set of letters had to be remembered (activation condition) or not (baseline condition). During each run, subjects were first presented with a series of letters for 4 s, after which the screen was blank for a 6-second delay interval. After this delay, one of the letters together with a novel letter appeared on the screen for 7 s, and subjects were asked to indicate the novel letter. Thereafter the screen was blank for 3 s followed by the second and third series of

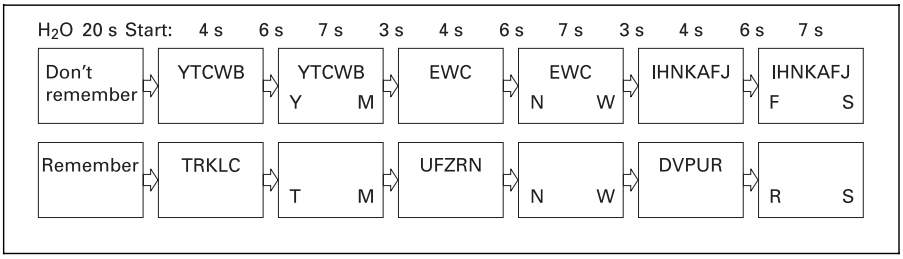

Fig. 1. Diagram of a baseline run (upper panel) of the DNMTS working memory task and an activation run (lower panel). The different screens were shown in succession. Baseline runs consisted of 3 series with different set sizes (i.e. 3, 5, 7 or 4, 6, 8 letters). Each activation run consisted of 3 series of letters of one set size to remember (3–8 letters) and all the runs were presented in random order.

letters. Different set sizes were presented in random order (3, 4, 5, 6, 7, or 8 letters) in each of the six activation runs, with each run consisting of only one set size. The two baseline runs each consisted of 3 series of letters of different length (i.e. 3, 5, 7 or 4, 6, 8) and the average of these 2 baseline runs provided a perceptual baseline condition, excluding the need for memory performance. In contrast to the memory condition, the stimuli were presented *continuously throughout* the 4-second familiarization period, the 6-second delay, and the 7-second novel/target stimulus presentation. As a result, rather than relying on memory, the subject could determine the correct answer perceptually. Main outcomes were the number of correct responses and reaction time (RT) in milliseconds. Task difficulty level was defined as easy (3, 4 or 5 letters to remember) versus difficult (6, 7 or 8 letters to remember). To get acquainted with the DNMTS task, subjects were presented with practice runs prior to the scanning session. Subjects were instructed to perform as accurate and fast as possible within the given time limits.

Neuroimaging

PET scans were acquired using a Siemens ECAT EXACT HR+ PET scanner (CTI/Siemens, Knoxville, Tenn., USA) in 3D mode with an axial field of view of 15 cm. Each subject underwent 8 PET scans and for each PET scan 450 MBq of $H_2{}^{15}O$ was injected intravenously in the antecubital vein as a bolus. Data acquisition per scan lasted 90 s and the intervals between successive $H_2{}^{15}O$ injections were approximate 9 min to allow for radioactive decay. The DNMTS task started 20 s after the injection of the $H_2{}^{15}O$ bolus. Attenuation correction was performed using a transmission scan acquired before the emission scans. From each subject a T_1-weighted structural MRI scan was acquired using a 1.5-Tesla, Siemens Magnetom Sonata MR scanner (MP-RAGE, magnetization prepared-rapid acquisition gradient echo, inversion time 300 ms, TR = 15 ms, TE = 7 ms, flip angle = 8°, voxel size 1 × 1 × 1.5 mm). These structural MRI scans were acquired to rule out structural abnormalities and for overlaying individual functional data. In addition, a mean brain image was created for these elderly subjects allowing for correct anatomic interpretation.

Image Processing

Images were reconstructed using an ordered subset-expectation maximization (OSEM) method, with 4 iterations and 16 subsets [27, 28], followed by motion-corrected attenuation correction [29] with the purpose to increase signal-to-noise ratio and to control for motion-related reconstruction artifacts. PET data were analyzed using Statistical Parametric Mapping (SPM99) software, developed by the Functional Imaging Laboratory, London (http://www.fil.ion.ucl.ac.uk). Images from each subject were realigned to correct for subject movement. Thereafter the images were spatially normalized to approximate Talairach space as defined by the SPM99 template, and re-sampled to 3 × 3 × 3 mm voxel size. Data were smoothed using a 10-mm full-width half-maximum (FWHM) Gaussian filter to increase signal-to-noise ratio. Next, PET data were analyzed using a linear regression model, and weighted contrasts were computed for task versus baseline (both for easy and difficult items) across groups as well as between groups. The main effects are reported at $p < 0.05$, corrected for multiple comparisons using the False Detection Rate (FDR) method [30], unless indicated otherwise. Group by task interactions were masked using the appropriate main effects, and are reported at $p < 0.001$ uncorrected, with a cluster size threshold of 5 voxels. Regions determined by Talairach coordinates for peak effects were verified using anatomical localization on the mean structural MRI created for the present study population.

Statistical Analysis

All analyses for demographic data, hormone values, mood scale scores and task performance were carried out by means of the Statistical Package for the Social Sciences (SPSS). Demographic variables were analyzed by means of a two-sample t-test. To compare the experimental groups with respect to the RT and accuracy of the DNMTS, we separately analyzed the easy memory sets (by averaging the data from 3, 4 and 5 letters) and difficult sets (by averaging the data from 6, 7 and 8 letters). As these data appeared not to be normally distributed, analyses were performed by means of a non-parametric test, i.e. the Mann-Whitney U test. Statistical tests for the DNMTS performance were one-tailed, based on the hypothesis that a higher IGF-I would enhance speed of processing and quality of performance. Statistical significance was set at the 0.05 level. Data are presented as means ± SD.

Results

Subject characteristics (demographic, biochemical, and psychometric data) are shown in table 1. One patient was excluded from analysis because of abnormalities on the structural MRI, suggesting a previous cerebral-vascular accident. Assignment to groups was based on IGF-I levels measured on the day of the PET scans. These IGF-I levels were not significantly different from those measured in 1999 (data not shown, p = 0.35), on which the selection of participants for the study was based. Only 2

Table 1. Subject characteristics (demographic, biochemical and psychometric data)

	IGF low	IGF high	p value
Subjects	13	10	
Male:female	5:8	8:2	0.05*
IGF-I, nmol/l	9.2 ± 2.3	17.5 ± 2.8	0.01*
IGFBP-3, mg/l	2.9 ± 0.8	3.8 ± 0.6	0.01*
Age, years	80.8 ± 2.3	78.6 ± 3.8	0.13
BMI, kg/m^2	28.2 ± 4.3	27.0 ± 2.8	0.44
MMSE	28.5 ± 1.6	28.6 ± 1.2	0.95
Education level	4.6 ± 1.3	5.5 ± 2.1	0.25
POMS depression	11.2 ± 5.1	10.2 ± 3.0	0.57
POMS tension	10.2 ± 2.7	9.9 ± 2.8	0.83
POMS fatigue	9.1 ± 3.8	8.7 ± 3.6	0.81
POMS anger	11.1 ± 6.3	10.2 ± 2.7	0.69
POMS vigor	18.9 ± 3.7	18.3 ± 2.3	0.69
TSH, mU/l	1.0 ± 0.8	1.3 ± 1.8	0.64
Free T_4, pmol/l	15.9 ± 1.8	15.7 ± 2.7	0.81
Free T_3, pmol/l	4.4 ± 0.7	4.7 ± 0.7	0.35
IGFBP-2, ng/ml	506 ± 245	404 ± 122	0.24
IGFBP-4, ng/ml	167 ± 40	173 ± 32	0.68

Values are means (± SD) unless otherwise stated (* p = 0.05).

IGFBP = IGF binding protein; MMSE = Mini Mental State Examination; POMS = Profile of Mood States.

Higher scores for POMS depression, tension fatigue and anger reflect a negative mood; higher scores for vigor reflect a better mood.

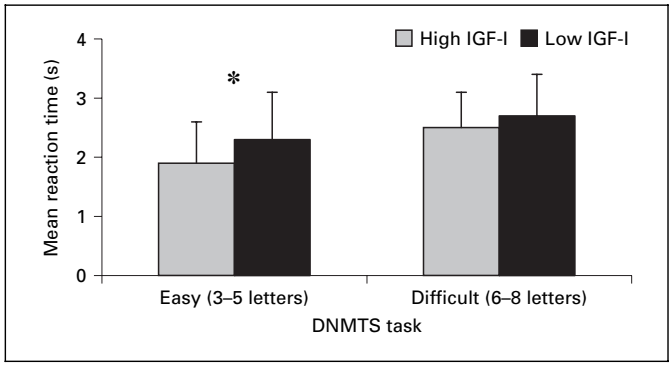

Fig. 2. Reaction time (in seconds) for the easy (averaged 3–5 letters) and difficult (averaged 6–8 letters) items of the DNMTS task in the low and high IGF-I group (* $p < 0.05$: low versus high IGF-I group).

subjects crossed over, 1 subject from the low to the high IGF-I group, the second the other way round. Mean age, BMI, education level and MMSE scores were similar for both subject groups (table 1). All participants were right-handed, except for 1 subject in the low and 3 subjects in the high IGF-I group who were ambidextrous. All subjects used their right hand to press the response buttons during the DNMTS task.

Biochemical Data

Thyroid function was within the normal range. The groups were significantly different in IGF-I and IGFBP-3, but not in IGFBP-2 and IGFBP-4 levels. Values are presented in table 1.

Neuropsychological Data

Regarding the performance on the DNMTS task, no group differences were found for either the total number of incorrect responses for easy items (3–5 letters) or for difficult items (6–8 letters). The number of incorrect responses during the DNMTS task for easy items was 0.4 ± 1.2 in the low IGF-I group and 0.4 ± 1.0 in the high IGF-I group. The number of incorrect responses for difficult items was 0.9 ± 1.5 in the low IGF-I group and 1.0 ± 1.3 in the high IGF-I group. With respect to the RTs averaged across easy items and across difficult items, we found significantly shorter RTs only for the easy items in the high IGF-I group (mean high IGF-I group = 1.9 ± 0.7 s, mean low IGF-I group 2.3 ± 0.6 s, $p < 0.04$) (fig. 2).

Imaging Data

The main effects for task (easy or difficult) versus baseline are listed in tables 2 and 3. Across subjects, task performance for easy (set size 3–5) items was associated with increased rCBF in left superior parietal and occipital cortex, and cerebellum; also at a slightly lower threshold ($p < 0.1$ corrected for multiple comparisons) increased rCBF was found in the left anterior prefrontal cortex, anterior cingulate cortex, left superior temporal cortex and bilateral motor cortex (fig. 3). Comparing task performance for difficult (set size 6–8) items versus baseline, increased rCBF was found in bilateral anterior and dorsolateral prefrontal cortex, left ventrolateral prefrontal cortex, as well as anterior cingulate cortex, left superior temporal cortex, motor cortex, right parietal cortex, occipital cortex and cerebellum (fig. 4). The reverse contrasts (baseline > task, both for easy and difficult items) did not show activations at the chosen threshold. Task by group interactions in favor of the high IGF-I group were identified only in left premotor cortex for easy items, and in left DLPFC for difficult items (table 4, fig. 5). No task by group interactions in favor of the low IGF-I group were found, either for easy items or difficult items.

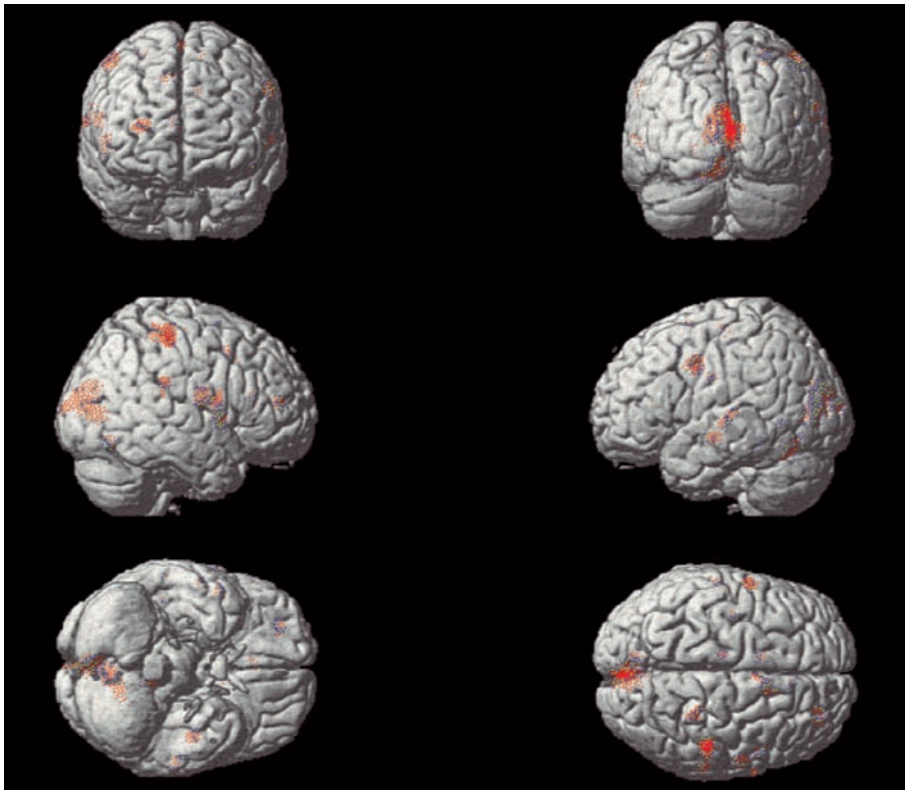

Fig. 3. Main effects of rCBF for easy items (3–5 letters) versus baseline across high and low IGF-I groups reported at $p < 0.05$, corrected for multiple comparisons using the FDR method.

Table 2. Significant rCBF differences during performance of easy items (3–5 letters to remember on the DNMTS task) versus baseline across IGF-I high and low groups (n = 23)

Region	Coordinates of peak voxel			BA	Z value
	X	Y	Z		
Left prefrontal anterior	−27	48	12	46/10	3.75*
Anterior cingulate	9	6	36	24	3.55*
	−6	12	48	24	3.42*
Left superior temporal	−63	−30	24	22	3.33*
Left superior parietal	−48	−30	57	7	4.04**
Motor/premotor	12	−18	60	6	3.37*
	−57	−6	18	6	3.71*
Occipital	−3	−78	3	18	4.98**
	12	−75	6	18	3.50*
	3	−81	21	18	4.11**
Cerebellum	3	−69	−12		4.40**
	9	−45	−12		3.74*

BA = Brodmann area.
* FDR corrected $p < 0.1$, cluster size threshold 5 voxels.
** FDR corrected $p < 0.05$, cluster size threshold 5 voxels.

Table 3. Significant rCBF differences during performance of difficult items (6–8 letters to remember on the DNMTS task) versus baseline across IGF-I high and low groups (n = 23)

Region	Coordinates of peak voxel			BA	Z value
	X	Y	Z		
Left prefrontal					
Anterior	−30	48	12	10	3.77
Dorsolateral	−54	39	15	6/8	3.90
Ventrolateral	−69	0	12	6	4.60
	−45	6	24	44	3.70
Right prefrontal					
Anterior	36	42	18	46/10	4.69
Dorsolateral	54	3	42	9	3.75
Anterior cingulate	−12	21	42	32	5.48
	−9	27	18	24	3.94
	12	9	36	32	3.76
Left superior temporal	−45	−36	−18	22	3.55
Right inferior parietal	51	−39	45	40	3.43
Motor/premotor	0	6	63	6	3.40
Occipital	−3	−78	3	18	4.47
	−9	−81	12	18	3.70
Cerebellum	6	−69	−15		3.44
	15	−69	−24		3.42

BA = Brodmann area. FDR corrected $p < 0.05$, cluster size threshold 5 voxels.

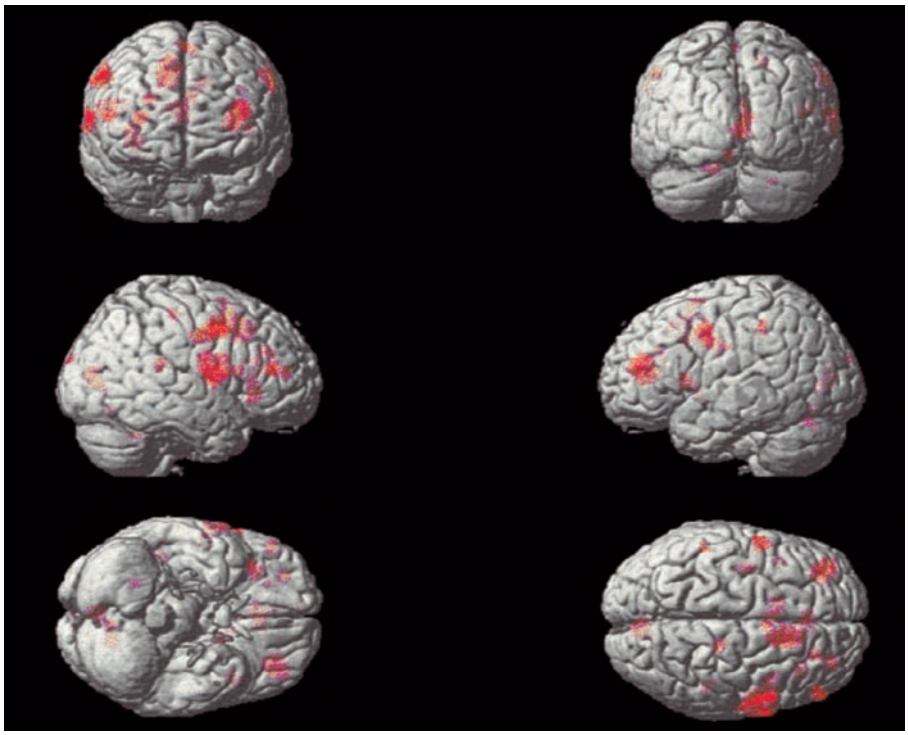

Fig. 4. Main effects of rCBF for difficult items (6–8 letters) versus baseline across high and low IGF-I groups, reported at $p < 0.05$, corrected for multiple comparisons using the FDR method.

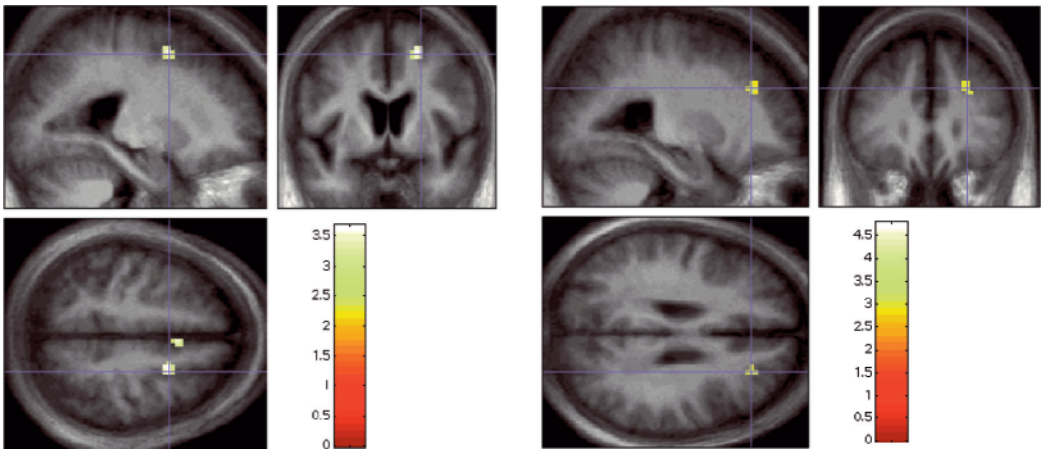

Fig. 5. Group (high IGF-I > low IGF-I) by task interaction effects shown on the created mean brain structural MRI scan. The left panel shows results for the easy task (3–5 letters) versus baseline, the right panel shows difficult items (6–8 letters) versus baseline. This image shows the areas that are more activated in subjects with high IGF-I levels than in those with low IGF-I levels for the easy and difficult conditions.

Table 4. Regions showing increased activity in high IGF-I subjects compared with low IGF-I subjects during performance of easy (3–5 letters) and difficult items (6–8 letters) versus baseline

Region	Coordinates of peak voxel			BA	Z value
	X	Y	Z		
Easy items versus baseline					
Left premotor	−21	6	54	6	3.58
	−3	12	54	6	3.31
Difficult items versus baseline					
Left dorsolateral prefrontal	−27	36	30	9	3.23

p uncorrected < 0.001, cluster size threshold 5 voxels. BA = Brodmann area.

Discussion

A relationship between the GH-IGF-I axis and cognition has been demonstrated in several studies using neuropsychological instruments. In the present study this relationship was studied in elderly normal subjects performing a working memory task during PET scanning. Healthy elderly males and females were included and based on their IGF-I level assigned to the group with the lowest or highest IGF-I quartile. T tests indicated that both groups had the same education level, MMSE and mood scale scores. Error rates on the DNMTS task were similar in both groups, indicating that the quality of working memory performance was not associated with IGF-I level. However, there was a significant difference in RTs on the DNMTS task between both groups. We found that memory processing is faster in subjects with higher IGF-I levels, but only for a small memory load. This finding is in accordance with previous data, showing a relationship between speed of information processing and circulating IGF-I levels in healthy elderly [15, 16].

A post-hoc analysis on the RT data from the baseline (perceptual) condition revealed that the high and low IGF-I groups had similar RTs on the DNMTS task. This indicates that both groups are not different with respect to perceptual-motor speed. The observed differences in speed between groups on the DNMTS task in the memory condition therefore cannot be attributed to differences in perceptual-motor speed, but point to a faster memory scanning process in the high IGF-I group.

The question arises why the high IGF-I group is faster only at a low memory load, that is the average of memory set sizes 3, 4 and 5. These results may be seen in the light of the reconsidered mental storage and processing capacity of 3–5 items. Instead of the magical number 7, the magical number 4 in working memory is supported by a large number of studies on memory capacity limits [31]. The capacity limit in working memory observed with a wide range of procedures suggests a mean memory capacity in adults of 3–5 chunks, whereas individual scores range from about 2 to 6 chunks. In addition, both processing and storage limits are supposed to be about 4 items each. The fundamental capacity limit coincides with conditions in which the chunks are held in the focus of attention, that is, the focus of attention appears to be capacity-limited. It may be argued that the working memory span is likely to be 4 rather than 7. Thus it may be assumed that, especially in our elderly subjects, the remembering of 5 or more letters is at the edge or exceeding the capacity limit of working memory so that manipulation of memory content by means of chunking and memorization is needed.

The present study shows that subjects with high and low IGF-I levels exhibit different patterns of relative brain activation during the performance of a working memory task. The groups, however, did not differ in the quality of memory performance, which may be due to ceiling effects: in spite of their highly advanced age, most subjects found the task easy to perform and had (nearly) perfect scores. Consequently, differences in working memory function between both groups could only be observed with regard to performance speed and brain activation patterns. Thus, we can conclude that whereas both groups show the same quality of performance, the low IGF-I group needs more time to reach this level of performance than the high IGF-I group.

With regard to rCBF, group differences in favor of the high IGF-I group were found in the left premotor cortex for easy items and left DLPFC for difficult items. As the premotor cortex is involved in the execution of complex behavior, the increased activity in this region may be related to the faster working memory processing of the high IGF-I group. The left DLPFC has been implicated in working memory performance in previous studies, particularly at increasing memory load or when tasks require manipulation of memory content (e.g., updating, as in the N-back task). A correlation between increased DLPFC activity in elderly subjects during performance of working memory tasks and task performance has been reported [32]. As manipulation processes are likely to occur for the higher memory loads, exceeding working memory capacity, our results indicating a larger rCBF in left DLPFC during higher memory loads are in line with these findings.

Across the two groups, increased task versus baseline activity in left parietal and occipital cortex for easy items was found, with additional activity in anterior cingulate cortex, premotor cortex, and left prefrontal cortex. For difficult items, more extensive, and bilateral, recruitment of prefrontal regions was seen. Indeed, it has been proposed that high-performing elderly subjects tend to recruit bilateral rather than left-lateralized prefrontal networks during encoding of verbal material, the so-called Hemispheric Asymmetry Reduction in Old age or HAROLD model [33]. The involvement of parietal, dorsolateral and ventrolateral prefrontal and anterior cingulate cortex in working memory is in agreement with numerous previous studies [see 34 for a review]. Specifically, parietal cortex has been associated with phonological storage, left ventrolateral prefrontal cortex with subvocal rehearsal [35], DLPFC with manipulation of working

memory contents [36] and anterior cingulate cortex with attention and response selection [37].

With regard to the HAROLD model, in the present study increased activity at higher task load was found only in left DLPFC. At a slightly lower threshold ($p = 0.0012$ uncorrected), however, increased activity in right DLPFC in high IGF-I subjects was also seen (data not shown). Therefore, the present data indicate that high IGF-I subjects were able to recruit prefrontal areas to a greater extent during a working memory task, which was not reflected by a higher quality of memory performance. Apart from possible different memorization strategies as mentioned above, it can be argued that the absence of more pronounced cognitive differences between groups may be due to instability of IGF-I levels over time. For the present study, inclusion of subjects was based on IGF-I levels measured in 1999, but group allocation was based on pre-scan levels. There was, however, a strong correlation ($p = 0.002$) between IGF-I levels measured in 1999 and in 2002. IGF-I serum concentrations, as a representative of GH secretion, are known to be stable during the day [38] and in elderly there is no sex difference in IGF-I levels. The reproducibility of IGF-I during repeated testing is high [39]. To ensure that individual fluctuations in IGF-I levels do not confound our data, we performed post-hoc analyses of the imaging and DNMTS data of the 21 subjects with stable IGF-I values between the first determination in 1999 and second IGF-I determination 3 years later. With respect to the imaging data, these analyses yielded virtually identical results (data not shown). In addition, our post-hoc analysis showed results for behavioral data as well: subjects with low IGF-I levels were significantly slower than those with high IGF-I levels with respect to the easy DNMTS items ($p = 0.04$). There was no difference between groups for the difficult DNMTS items. This indicates that our results cannot be attributed to instability of IGF-I levels.

We also investigated the possible role of gender, because the sex ratio differed between our two groups. However, analyses with sex as independent factor showed that performance and RTs on the DNMTS were similar in male and female subjects. In addition, post-hoc analyses of cerebral blood flow data for the 5 male subjects in the low IGF-I group compared with the 8 male subjects in the high IGF-I group revealed similar results as were obtained for all subjects (data not shown).

Finally, it should be noted that the regulation of IGF in the central nervous system is presumably more complex than was outlined in our Introduction, as cerebral IGF is also involved in response to tissue damage, ischemia, etc. We did not measure central nervous system IGF-I, but assumed that plasma IGF-I is a good marker of cerebral IGF-I concentration.

These data are, to the best of our knowledge, the first to relate memory, IGF-I levels and functional differences in brain activity. From other brain-imaging studies there is some evidence that female sex steroid hormones modulate brain metabolism and blood flow both during cognitive activity and baseline [see 40 for a review]. Estrogen replacement therapy in postmenopausal women increased rCBF in the temporal lobe during the resting state. Testosterone replacement has shown to enhance brain perfusion in the midbrain in hypogonadal men [41].

In conclusion, this $H_2{}^{15}O$ PET study shows that during a working memory task subjects with high IGF-I levels exhibit a faster memory performance and are able to recruit prefrontal areas to a greater extent than low IGF-I subjects. The main finding that a difference in IGF-I level in healthy elderly subjects can modulate rCBF activation patterns during a working-memory task clearly needs replication. Moreover, future studies should aim at controlling subjects' GH-IGF-I levels over time and to investigate whether an increase in IGF-I levels in low IGF-I subjects would result in increased performance and more extensive recruitment of prefrontal areas involved in working memory function. This knowledge is important in the light of counteracting negative implications of the aging process concerning cognitive functions, which may be related to reduced IGF-I levels.

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References

1 Piguet O, Grayson DA, Creasy H, Bennett HP, Brooks WS, Waite LM, Broe GA: Vascular risk factors, cognition and dementia incidence over 6 years in the Sydney Older Persons Study. Neuroepidemiology 2003;22:165–171.

2 Dik MG, Jonker C, Comijs HC, Bouter LM, Twisk JW, van Kamp GJ, Deeg DJ: Memory complaints and APOE-ε4 accelerate cognitive decline in cognitively normal elderly. Neurology 2001;57:2217–2222.

3 Tuppurainen M, Saarikoski S: Estrogens and aging. Adv Neurol 2003;91:101–106.

4 Rudman D: Growth hormone, body composition and aging. J Am Geriatric Soc 1985;33: 800–807.

5 Corpus E, Harman SM, Blackman MR: Human growth hormone and human aging. Endocr Rev 1993;14:20–39.

6 Collett-Solberg PF, Cohen P: The role of the insulin-like growth factor binding proteins and the IGFBP proteases in modulating IGF action. Endocrinol Metab Clin North Am 1996; 25:591–614.

7 Burman P, Hetta J, Wide L, Mansson JE, Ekman R, Karlsson FA: Growth hormone treatment affects brain neurotransmitters and thyroxine. Clin Endocrinol 1996;44:319–324.

8 Nyberg F: Growth hormone in the brain: Characteristics of specific brain targets for the hormone and their functional significance. Front Neuroendocrinol 2000;21:330–348.

9 Lai Z, Roos P, Zhai O, Olssen Y, Fholenhag K, Larsson C, Nyberg F: Age-related reduction of human growth hormone-binding sites in the human brain. Brain Res 1993;621:260–266.

10 Adem A, Jossan SS, d'Aggy R, Gillberg PG, Nordberg A, Winblad B, Sara V: Insulin-like growth factor I receptors in the human brain: Quantitative autoradiographic localization. Brain Res 1989;503:299–303.

11 Smith A, Wang J, Cheng CM, Zhou J, Weickert CS, Bondy CA: High-level expression of Dok-1 in neurons of the primate prefrontal cortex and hippocampus. J Neurosci Res 2004;75:218–224.

12 Rollero A, Murialdo G, Fonzi S, Garrone S, Gianelli MV, Gazzerro E, Barreca A, Polleri A: Relationship between cognitive function, growth hormone and insulin-like growth factor I plasma levels in aged subjects. Neuropsychobiology 1998;38:73–79.

13 Paolisso G, Ammendola S, Del Buono A, Gambardella A, Riondino M, Tagliamonte MR, Rizzo MR, Carella C, Varricchio M: Serum levels of insulin-like growth factor I and IGF-binding protein-3 in healthy centenarians: Relationship with plasma leptin and lipid concentrations, insulin action and cognitive function. J Clin Endocrinol Metab 1997;82:2204–2209.

14 Kalmijn S, Janssen JA, Pols HA, Lamberts SW, Breteler MM: A prospective study on circulating insulin-like growth factor I, IGF-binding proteins and cognitive function in the elderly. J Clin Endocrinol Metab 2000;85:4551–4555.

15 Aleman A, Verhaar HJJ, de Haan EHF, De Vries WR, Samson MM, Drent ML, van der Veen EA, Koppeschaar HPF: Insulin-like growth factor-I and cognitive function in healthy older men. J Clin Endocrinol Metab 1999;84:471–475.

16 Dik MG, Pluijm SM, Jonker C, Deeg DJ, Lomeckey MZ, Lips P: Insulin-like growth factor I and cognitive decline in older persons. Neurobiol Aging 2003;24:573–581.

17 Papadakis MA, Grady D, Black D, Tierney MJ, Gooding GAW, Schambelan M, Grunfeld C: Growth hormone replacement in healthy older men improves body composition but not functional ability. Ann Intern Med 1996;124: 708–716.

18 Deijen JB, de Boer H, Blok GJ, van der Veen EA: Cognitive impairment and mood disturbances in growth hormone deficient men. Psychoneuroendocrinology 1996;21:313–322.

19 Deijen JB, de Boer H, van der Veen EA: Cognitive changes during growth hormone replacement in adult men. Psychoneuroendocrinology 1998;23:45–55.

20 Sonntag WE, Lynch C, Thornton P, Khan A, Bennett S, Ingram R: The effects of growth hormone and IGF-I deficiency on cerebrovascular and brain aging. J Anat 2000;197(4):575–585.

21 Kowalska DM, Bachevalier J, Mishkin M: The role of the inferior prefrontal convexity in performance of delayed non-matching to sample. Neuropsychologia 1991;29:583–600.

22 Veltman DJ, Rombouts SARB, Dolan RJ: Maintenance versus manipulation in verbal working memory revisited: An fMRI study. Neuroimage 2003;18:247–256.

23 Jafferali S, Dumont Y, Sotty F, Robitaille Y, Quirion R, Kar S: Insulin-like growth factor-I and its receptor in the frontal cortex, hippocampus, and cerebellum of normal human and Alzheimer disease brains. Synapse 2000;38: 450–459.

24 Deeg DJH, Beekman ATF, Kriegsman DMW, Westendorp-de Seriere M: Autonomy and well-being in the aging population. II. Report from the Longitudinal Aging Study Amsterdam 1992–1996. Amsterdam, VU University Press, 1998.

25 Van Doorn J, Cornelissen AJFH, van Buul-Offers SC: Plasma levels of insulin-like growth factor binding protein-4 under normal and pathological conditions. Clin Endocrinol 2001; 54:655–664.

26 Shacham S: A shortened version of the Profile of Mood States. J Pers Assess 1983;47:305–306.

27 Boellaard R, van Lingen A, Lammertsma AA: Experimental and clinical evaluation of iterative reconstruction (OSEM) in dynamic PET: Quantitative characteristics and effects on kinetic modeling. J Nucl Med 2001;42:808–817.

28 Mesina CT, Boellaard R, Jongbloed G, van der Vaart AW, Lammertsma AA: Experimental evaluation of iterative reconstruction versus filtered back projection for 3D [^{15}O] water PET activation studies using statistical parametric mapping analysis. Neuroimage 2003;19:1170–1179.

29 Van den Heuvel OA, Boellaard R, Veltman DJ, Mesina C, Lammertsma AA: Attenuation correction of PET activation studies in the presence of task-related motion. Neuroimage 2003; 19:1501–1509.

30 Genovese CR, Lazar NA, Nichols T: Thresholding of statistical maps in functional neuroimaging using the false discovery rate. Neuroimage 2002;15:870–878.

31 Cowan N: The magical number 4 in short-term memory: A reconsideration of mental storage capacity. Behav Brain Sci 2001;24:87–114.

32 Stebbins GT, Carrillo MC, Dorfman J, Dirksen C, Desmond JE, Turner DA, Bennett DA, Wilson RS, Glover G, Gabrieli JD: Aging effects on memory encoding in the frontal lobes. Psychol Aging 2002;17:44–55.

33 Cabeza R, Anderson ND, Locantore JK, McIntosh AR: Aging gracefully: Compensatory activity in high-performing older adults. Neuroimage 2002;17:1394–1402.

34 Fletcher PC, Henson RN: Frontal lobes and human memory: Insights from functional neuroimaging. Brain 2001;124:849–881.

35 Baddeley A: The fractionation of working memory. Proc Natl Acad Sci USA 1996;93: 13468–13472.

36 Petrides M: Specialized systems for the processing of mnemonic information within the primate frontal cortex. Philos Trans R Soc Lond B Biol Sci 1996;351:1455–1461.

37 Weissman DH, Giesbrecht B, Song AW, Mangun GR, Woldorff MG: Conflict monitoring in the human anterior cingulated cortex during selective attention to global and local object features. Neuroimage 2003;19:1361–1368.

38 Minuto F, Underwood LE, Grimaldi P, Furlanetto RW, Van Wyk JJ, Giordano G: Decreased serum somatomedin C concentrations during sleep: Temporal relationship to the nocturnal surges of growth hormone and prolactin. J Clin Endocrinol Metab 1981;52:399–403.

39 Blum WF, Albertsson-Wikland K, Rosberg S, Ranke MB: Serum levels of insulin-like growth factor I and IGF binding protein 3 reflect spontaneous growth hormone secretion. J Clin Endocrinol Metab 1993;76:1610–1616.

40 Maki PM, Resnick SM: Effects of estrogen on patterns of brain activation at rest and during cognitive activity: A review of neuroimaging studies. Neuroimage 2001;14:789–780.

41 Azad N, Pitale S, Barnes WE, Friedman N: Testosterone treatment enhances regional brain perfusion in hypogonadal men. J Clin Endocrinol Metab 2003;88:3064–3068.