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A Prospective Study on Circulating Insulin-Like Growth Factor I (IGF-I), IGF-Binding Proteins, and Cognitive Function in the Elderly

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ABSTRACT

The objective of this study was to investigate the longitudinal relation between the insulin-like growth factor I (IGF-I)/IGF-binding protein (IGFBP) system and cognitive function. The study population consisted of a sample of 186 healthy participants from the population-based Rotterdam Study, aged 55–80 yr. At baseline, we determined fasting blood levels of free and total IGF-I, IGFBP-1, and IGFBP-3. The 30-point Mini-Mental State Examination (MMSE) was used to assess cognitive impairment at baseline (MMSE score of <26; 6% of the sample) and cognitive decline after, on the average, 1.9 yr of follow-up (drop in MMSE score of >1 point/year; 22% of the sample). Odds ratios (OR) and 95% confidence intervals (95% CI) were estimated using logistic regression, with adjustment for age, sex, education, body mass index, and fasting insulin levels. Total IGF-I appeared to be inversely related to cognitive impairment, although not significantly. Higher total IGF-I and the total IGF-I/IGFBP-3 ratio were associated with less cognitive decline (OR per SD increase = 0.65; 95% CI = 0.44–0.95 and OR = 0.59; 95% CI = 0.39–0.87, respectively). No relation was observed between free IGF-I and cognitive decline (OR = 0.99; 95% CI = 0.68–1.44). In conclusion, in this prospective study higher serum total IGF-I levels and higher total IGF-I/IGFBP-3 ratios, but not higher free IGF-I levels, were associated with less cognitive decline over the following 2 yr. Circulating total IGF-I levels may reflect an underlying biological process that influences cognitive decline. (*J Clin Endocrinol Metab* **85:** 4551–4555, 2000)

THERE IS A physiological decline of the GH/insulin-like growth factor I (IGF-I) axis with aging (1).The possibility that the GH/IGF-I axis is involved in cognitive deficits has been recognized for several years. Animal and *in vitro* studies showed that IGF-I enhances neuronal survival and inhibits apoptosis (1a). It exerts neurotrophic activities in the hippocampus, which is involved in learning and memory (1b). Finally, IGF-I may result in a reduction of the formation of tangles, which is one of the hallmarks of Alzheimer's disease (1c). Few cross-sectional epidemiological studies have found a relationship between IGF-I levels and cognitive function. In childhood GH deficiency, a state characterized by low total IGF-I levels, cognitive impairment has been reported (2–4). Healthy centenarians with a higher serum total IGF-I and total IGF-I over IGF-binding protein-3 (IGFBP-3) ratio had less cognitive impairment (5). In two other small studies of older subjects, high serum total IGF-I levels were associated with better cognitive performance as well (6, 7).

Until now, only the link between total IGF-I levels or the ratio of total IGF-I to IGFBP-3 and cognitive function has been investigated. However, the amount of total IGF-I in blood is a reflection of the sum of IGF-I bound to specific IGFBPs and free IGF-I. The bioavailability of IGF-I to the tissues is modulated by at least six IGFBPs and several IGFBP-proteases (8, 9). IGFBP-3 is quantitatively the most important binding protein and is thought to function as an intravascular reservoir for IGF-I (10). IGFBP-1 has been proposed as a regulator of IGF-I bioactivity and might simultaneously both inhibit and potentiate IGF-I action at different sites (11). Until recently, when IGF-I was assayed in serum, the total extractable IGF-I was measured, which offers only a crude estimate of biologically active IGF-I due to the wide interindividual variation in circulating IGFBP (12). Serum free IGF-I, analogous to sex and thyroid hormones, is likely to be more biologically active than bound IGF-I (13). Moreover, it has been observed that normal or even increased levels of free IGF-I may occur with subnormal circulating total IGF-I levels (14, 15). Consequently, it seems desirable to distinguish between free and total IGF-I levels. Recently, a well validated method has been developed to measure free IGF-I levels (16–18).

To our knowledge, no previous study has prospectively investigated the association between the IGF-I/IGFBP system and cognitive function in healthy subjects. Therefore, the current prospective population-based study was designed to investigate whether in healthy elderly men and women circulating serum levels of total and free IGF-I as well as the ratio of total IGF-I to IGFBP-3, as this ratio has been found to be related to cognitive impairment in a previous study (5), are associated with cognitive decline.

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Subjects and Methods

Study population

The Rotterdam Study is a single center, prospective, population-based study (19) designed to investigate determinants of chronic dis-

abling diseases in the elderly. The conduct of the study was approved by the medical ethics committee of Erasmus University, and written consent was obtained from all participants. All residents of Ommoord, a suburb in Rotterdam, aged 55 yr or over, including those living in homes for the elderly, were invited to participate. The baseline examinations started in May 1990 and continued until June 1993. Of the 10,275 eligible subjects, 7,983 (78%) agreed to participate. During a home visit, trained interviewers administered a questionnaire, covering, among other areas, socio-demographic background, medical history, and medication use. This was followed by 2 clinical examinations at the research center, including neuropsychological testing. The follow-up examination started in September 1993 and lasted until December 1994. Of the 7,215 subjects who were still alive, 6,315 (88%) agreed to participate.

Data for the present study come from an additional examination of endocrine factors. For this examination, a random sample (n = 219) was taken of subjects, aged 55–80 yr, who had completed the baseline examination of the Rotterdam Study not more than 6 months previously. Persons with a history of psychiatric or endocrine diseases, including diabetes mellitus treated with medication, were not invited.

There were no differences in age, sex, or education between our sample and other participants of the Rotterdam Study in the same age range and without dementia or known diabetes mellitus. The mean baseline Mini-Mental State Examination (MMSE) score was higher in our sample [28.1 (SD = 1.6) *vs.* 27.6 (SD = 1.9); $P = 0.002$], which was probably the result of the selection criteria.

Blood measurements

Blood was obtained after an overnight fast at the research center between 0800–0900 h and was allowed to coagulate for 30 min. Serum was separated by centrifugation and was quickly frozen in liquid nitrogen. Free IGF-I was measured with a commercially available two-site immunoradiometric assay (Diagnostics Systems Laboratories, Inc., Webster, TX; intra- and interassay coefficients of variation (CV), 10.3% and 10.7%, respectively) (16, 17). Total IGF-I was determined by a commercially available RIA (Medgenix Diagnostics, Brussels, Belgium; intra- and interassay CV, 6.1% and 9.9%, respectively). Commercially available immunoradiometric assays were also used for measurement of IGFBP-1 and IGFBP-3 (Diagnostics Systems Laboratories, Inc.; intra- and interassay CV for IGFBP-1, 4.0% and 6.7%, respectively; for IGFBP-3, 1.8% and 1.9%, respectively). Insulin was determined by a commercially available RIA (Medgenix; intra- and interassay CV, 8.0% and 13.7%, respectively). Dehydroepiandrosterone sulfate (DHEAS) was assayed by RIA (Diagnostic Products, Los Angeles, CA; intra- and interassay CV, 5.3% and 7.0%, respectively). Serum glucose levels were determined using a standard glucose hexokinase method.

Cognitive function

Global cognitive function was tested at both baseline and follow-up with the Dutch version of the 30-point MMSE during the (first) visit to the research center (20). It was administered by specially trained research assistants. The MMSE includes questions on orientation to time and place, registration, attention and calculation, recall, language, and visual construction. This screening test was originally created for a clinical setting (21) and is extensively used in epidemiological studies (21). Although it tests mainly cortical functions, these are important to daily functioning and are severely affected in dementia. If fewer than 4 individual items (of 20) were not answered by the subject, these were rated as error (22). If a subject did not answer 4 or more individual items, the total MMSE score was considered missing. Cognitive impairment was defined as a score below 26 (23), and cognitive decline was considered a drop in the MMSE score of more than 1 point/yr (approximately >1 SD). The mean follow-up time between the first and second MMSEs was 1.9 yr (SD = 0.23).

Other measurements

Height and weight were measured wearing indoor clothes and without shoes. Body mass index was defined as weight (kilograms) divided by the square of height (meters).

Blood pressure was measured twice in the sitting position with a random zero sphygmomanometer, and the average was used for further analyses. Hypertension was defined as a systolic blood pressure more than 160 mm Hg, a diastolic blood pressure more than 90 mm Hg, or the use of antihypertensive medication. Food intake was assessed with a semiquantitative food frequency questionnaire at baseline, which aimed to assess habitual food intakes during the past year and included 170 food items and questions about dietary habits, supplementation, and prescribed diets (24). This questionnaire was found to have good reliability and validity (24). Education was classified into four levels: completed primary education, lower vocational or general education, intermediate vocational or general education, and higher vocational training, college, or university (UNESCO, Paris, 1976). Self-reported health status was assessed by asking participants whether they judged the quality of their health status as better, the same, or worse than that of their peers.

Statistical analysis

Complete information on cognitive function at baseline was available for 186 subjects, because, due to logistic reasons and refusal, not everyone was given the MMSE. Follow-up data on cognitive decline were available for 166 subjects. Differences in baseline characteristics according to IGF-I levels above and below the median and according to cognitive decline were tested after adjustment for age by analyses of covariance. Logistic regression was used to estimate odds ratio (OR) and 95% confidence intervals (CI) for the risk of cognitive impairment and decline. The independent variables of interest were total IGF-I, free IGF-I, IGFBP-1, IGFBP-3, and the ratio of total IGF-I to IGFBP-3. To maximize the power, we entered the variables of interest continuously (per SD) in the logistic model. Because they were not normally distributed, we first performed a log transformation. The choice of confounding variables included in the models was based either on a statistically significant association with the determinant and the outcome, on a previously observed association between them, or on a theoretical relationship. Confounding variables included in the main model were age, sex, education, body mass index, and fasting insulin levels. Additionally, we adjusted for glucose levels, hypertension, DHEAS, cigarette smoking (former, current, or never), total energy or protein intake, and self-reported health perception. We also investigated whether there was effect modification by gender by including the product term as covariate in the model. All tests were two-sided, and $P < 0.05$ was considered statistically significant. Data analyses were performed using BMDP statistical software (BMDP Statistical Software, Inc.).

Results

The mean age of the participants at baseline was 67.4 yr (SD = 5.6). Fifty percent of the sample was female. The median baseline MMSE score was 28 (range, 20–30), and 5.9% of the subjects were cognitively impaired. The mean drop in the MMSE per yr was 0.21 (SD = 0.92); 22% showed a drop in the MMSE score of more than 1 point/yr. There were no differences in age, sex, serum free or total IGF-I, IGFBP-1, or IGF-I/IGFBP-3 ratio between subjects with and those without a baseline MMSE score. Compared with subjects who attended the follow-up examination (n = 166), those who did not (n = 20) were older (71.1 *vs.* 66.9 yr; $P = 0.002$) and had a lower median MMSE score at baseline (27 *vs.* 28; $P = 0.001$). There were no differences in IGF-I, IGFBP-1, or IGFBP-3 levels between these groups.

Subjects with a total IGF-I level above the median (17.8 nmol/L) were younger than those with a total IGF-I level below the median (Table 1). Mean DHEAS, free IGF-I, and IGFBP-3 levels were higher, whereas IGFBP-1 was lower among participants with a high total IGF-I, after adjustment for age. There were no significant age-adjusted differences in the proportion of subjects with cognitive impairment or cognitive decline, or in sex, total energy intake, total protein intake, or fasting insulin levels between subjects with a total IGF-I level above the median and those with a total IGF-I

TABLE 1. Mean characteristics according to IGF-I above and below the median

	Total population	Total IGF-I		Age-adjusted *P* value[a]
		<17.8 nmol/L (n = 94)[b]	≥17.8 nmol/L (n = 92)[b]	
Cognitive impairment (%)	5.8 (n = 11)	8.6	3.1	0.87
Cognitive decline (%)	22.5 (n = 38)	27.2	18.2	0.10
Age (yr)	67.4 (5.6)	68.2 (5.4)	66.5 (5.7)	0.05
Sex (% female)	50 (n = 94)	55	45	0.08
Only primary education (%)	32 (n = 60)	30	33	0.67
Body mass index (kg/m^2)	26.3 (3.6)	26.3 (3.8)	26.4 (3.4)	0.72
Total energy intake (kJ/day)	8313 (2184)	8243 (2044)	8385 (2326)	0.73
Total protein intake (g/day)	82.0 (20.9)	79.9 (19.9)	84.0 (21.7)	0.32
Fasting insulin level (mU/L)	13.5 (8.0)	13.8 (8.9)	13.2 (7.1)	0.99
Random glucose level (mmol/L)	6.67 (2.15)	6.56 (2.40)	6.78 (1.89)	0.25
DHEAS (μmol/L)	3.36 (2.25)	2.89 (1.91)	3.83 (2.46)	0.002
Free IGF-I (nmol/L)	0.09 (0.05)	0.08 (0.05)	0.11 (0.05)	<0.001
IGFBP-1 (nmol/L)	0.76 (0.78)	1.01 (0.96)	0.52 (0.45)	<0.001
IGFBP-3 (nmol/L)	109.8 (26.3)	104.1 (24.1)	115.3 (27.3)	0.03

[a] Adjusted for age by analyses of covariance.
[b] SD in *parentheses*.

TABLE 2. Mean baseline characteristics according to cognitive decline

	Cognitive decline[a]		Age-adjusted *P* value[b]
	Absent (n = 129)[b]	Present (n = 37)[b]	
Age (yr)	66.8 (5.5)	7.3 (6.1)[c]	0.77
Sex (% female)	48	53	0.41
Only primary education (%)	31	32	0.72
Body mass index (kg/m^2)	26.6 (3.6)	25.7 (3.9)	0.25
Total energy intake (kJ/day)	8242 (2063)	8583 (2493)	0.33
Total protein intake (g/day)	80.8 (19.1)	84.4 (22.5)	0.25
Fasting insulin level (mU/L)	14.1 (8.5)	12.3 (5.8)	0.33
Random glucose level (mmol/L)	6.9 (2.4)	6.1 (1.1)	0.06
DHEAS (μmol/l)	3.58 (2.38)	2.68 (1.65)	0.02
Total IGF-I (nmol/L)	19.6 (7.7)	16.2 (6.8)	0.006
Free IGF-I (nmol/L)	0.093 (0.06)	0.087 (0.04)	0.50
IGFBP-1 (nmol/L)	0.74 (0.83)	0.79 (0.69)	0.62
IGFBP-3 (nmol/L)	109.4 (25.8)	113.3 (26.3)	0.39
Ratio total IGF-1/IGFBP-3	0.19 (0.08)	0.15 (0.06)	0.003

[a] Defined as a drop in the Mini-Mental State Examination score of more than one point per yr.
[b] SD in *parentheses*.
[c] Adjusted for age by analyses of covariance.

TABLE 3. Risk of cognitive decline from baseline to follow-up (n = 37/166) per SD increase in log-transformed selected metabolic factors

	SD of the natural logarithm	Adjusted relative risk (95% confidence interval)		
		Basic model[a]	+ Adjustment for DHEAS	+ Adjustment for self-reported health
Free IGF-I	0.595	0.99 (0.68–1.44)	1.02 (0.70–1.50)	1.01 (0.69–1.48)
Total IGF-I	0.464	0.65 (0.44–0.95)	0.67 (0.45–0.99)	0.66 (0.45–0.97)
IGFBP-1	1.02	0.92 (0.62–1.37)	0.88 (0.59–1.31)	0.92 (0.61–1.37)
IGFBP-3	0.244	1.26 (0.84–1.89)	1.25 (0.83–1.88)	1.31 (0.87–1.99)
Total IGF-I/IGFBP-3	0.097	0.59 (0.39–0.87)	0.60 (0.40–0.91)	0.59 (0.39–0.88)

Cognitive decline was defined as a drop in the MMSE score of more than one point per yr.
[a] Adjusted for age, sex, education, body mass index, and fasting insulin levels.

below the median (Table 1). Persons with cognitive decline, *i.e.* a drop in the MMSE score of more than 1 point/yr from baseline to follow-up, had a significantly lower DHEAS, total IGF-I, and total IGF-I/IGFBP-3 level at baseline than those without cognitive decline, after adjustment for age (Table 2).

After adjustment for age, gender, educational level, body mass index, and fasting insulin levels, logistic regression analyses suggested an inverse association between total IGF-I and cognitive impairment, although this was not significant (OR per SD increase = 0.76; 95% CI = 0.41–1.41), but not between free IGF-I and cognitive impairment (OR per SD increase = 0.94; 95% CI = 0.47–1.88). In the longitudinal analyses, total IGF-I and, not surprisingly, the total IGF-I to IGFBP-3 ratio, but not free IGF-I, were associated with a significantly reduced risk of cognitive decline (Table 3). Per SD increase in the natural logarithm of IGF-I, the risk of cognitive decline was reduced by approximately 35%. Additional adjustment for DHEAS or self-reported health status

did not essentially alter the results (Table 3), nor did adjustment for hypertension, smoking, total energy intake, total protein intake, or baseline MMSE score (data not shown). There was no significant interaction between IGF-I and sex. To examine whether the relationship was nonlinear or influenced by outliers, we redid the analyses according to tertiles of IGF-I and the total IGF-I to IGFBP-3 ratio, which showed essentially the same results.

Discussion

In this population-based sample of elderly subjects, serum total IGF-I and the total IGF-I to IGFBP-3 ratio were both inversely related to cognitive decline in the next 2 yr. These results were independent of differences in age, sex, insulin levels, body mass index, or other major confounders. However, we found no association between free IGF-I and cognitive decline.

Before interpreting our findings, some methodological issues should be taken into account. The MMSE score was used in our study to assess cognitive decline. It was not originally created for this purpose and it may be less sensitive to small changes in cognitive function (25). The reliability of a change in the MMSE has been examined in patients with dementia, and it was found that for a time interval between the MMSEs of 1 yr or more the reliability was approximately 0.74, which is reasonable (26). We chose a cut-off point for cognitive decline of a drop in the MMSE score of more than 1 point/yr, *i.e.* more than 1 SD (SD = 0.92), which may not be pathologically significant on an individual level, but can be of major importance on a population level. Participants in our sample were healthier because of the exclusion criteria, and the follow-up duration was short, leading, on the average, to only a small drop in the MMSE score. However, combined with the small sample size, this would only impede the detection of a significant modest association.

It could be argued that selection bias might have affected the validity of our results. The 20 subjects in our sample without a MMSE score at follow-up (due to death or nonresponse) had a significantly lower baseline MMSE score and were older than subjects who were not lost to follow-up, but there were no differences in IGF-I and IGFBP levels, making selection bias less likely.

In accordance with our findings, in two previous small studies of elderly subjects, high circulating total IGF-I levels were associated with better cognitive performance (6, 7). Another study also found that a high total IGF-I to IGFBP-3 ratio was related to less cognitive impairment (5). As GH secretion is one of the main regulators of circulating total IGF-I and IGFBP-3 (27), our findings may suggest at first glance that GH secretion plays an important role in age-related cognitive decline. However, free IGF-I levels were not associated with cognitive decline in our study. Free IGF-I levels are probably a better indicator of GH secretion than total IGF-I (28–31). In addition, Rollero *et al.* observed that MMSE scores in elderly subjects were not related to basal GH or GH peaks after GHRH stimulation, whereas they were positively associated with total IGF-I levels (7). In accordance with this, GH treatment of healthy older men (in physiological doses, which will have elevated free IGF-I levels) did not improve cognitive function (32). Moreover, GH replacement of subjects with adult-onset GH deficiency was not associated with significant alterations in cognitive function (33). Our study suggests that factors other than GH secretion are involved in the relationship of total IGF-I and the total IGF-I/IGFBP-3 ratio to cognitive decline.

What could then be the explanation for the observed relationships of total IGF-I and the total IGF-I/IGFBP-3 ratio to cognitive decline? There are many different conditions that might have altered the balance between bound and free IGF-I (8, 34). Could DHEAS be the missing link? We previously observed in this study population that higher DHEAS levels were associated with better cognitive function (35), whereas Morales *et al.* found that DHEA administration increases IGF-I concentrations in middle-aged and elderly (36). However, the observed associations between IGF-I and cognition did not change after adjustment for DHEAS.

Nutrition is considered to be another major regulator of circulating total IGF-I levels (37). Protein repletion of elderly subjects increases serum levels of total IGF-I (38). In contrast, protein deficiency in the diet causes suppression of circulating total IGF-I (27). Elderly persons are often undernourished, particularly with respect to protein (39). It has also been found that daily dietary protein intake in elderly correlates positively to cognitive performance in old age (40). In the study by Rollero *et al.* (7), nutritional indexes, such as mid-arm circumference, were positively correlated with the MMSE scores and total IGF-I. We therefore hypothesized that daily protein intake might be responsible for the observed relationship between total IGF-I and cognitive decline in our study. However, adjustment for daily dietary protein intake did not alter the results.

Finally, total IGF-I and the ratio of total IGF-I over IGFBP-3 may be indicators of general health status, and thereby predict cognitive decline, as well as other disorders of old age. This is in accordance with the hypothesis suggested by Blum that the total IGF-I level signals the cell about the well-being of the organism (27). When we adjusted for self-reported health status, the results did not change. Self-reported health status is, however, only a rough indicator of general health status.

In conclusion, in this population-based study, higher serum total IGF-I levels and a higher total IGF-I over IGFBP-3 ratio, but not higher free IGF-I levels, were associated with less cognitive decline over the next 2 yr. Circulating total IGF-I levels may reflect an unknown underlying biological process that influences cognitive decline.

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