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Original Article

Correlation between Serum FGF21 and Cognition in Men and Women Over 60 Years of Age

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SUMMARY

Background: Previous studies have shown that mitochondrial dysfunction (MtD) could be responsible for various age-related disorders, including cognitive decline. The main aim of the present study was to investigate possible link between serum levels of fibroblast growth factor 21 (FGF21, surrogate biomarker of MtD) and biomarkers of cognition in people over 60 years of age.
Methods: Eighty-seven adults aged 61 to 93 years (56 women) were enrolled in this cross-sectional study. Cognitive assessment included tests of global cognition, memory, and category fluency. Spearman's rank correlation was used to test an association between serum FGF21 and cognitive outcomes.
Results: Serum FGF21 levels are significantly higher in women compared to men (431.5 ± 268.8 vs. 274.0 ± 218.7 pg/ml; $p < 0.01$), with FGF21 positively correlated with global cognition ($\rho = 0.35$, $p < 0.05$), learning ability ($\rho = 0.56$, $p < 0.01$), immediate ($\rho = 0.41$, $p < 0.01$) and delayed memory ($\rho = 0.34$, $p < 0.05$) in oldest participants (> 70 years).
Conclusion: A positive correlation between serum FGF21 and cognition are found in women and the oldest participants, suggesting modulation of mitochondrial function with age. Possible gender- and age-driven MtD in the elderly should be corroborated in further longitudinal studies.

1. Introduction

Mitochondrial dysfunction (MtD) occupies scientific attention as one of the possible mechanisms that influence age-related disorders.[1] Several studies suggest that fibroblast growth factor (FGF21) could be a novel marker of impaired mitochondrial function.[2,3] FGF21 is a hormone-like cytokine involved in the regulation of carbohydrate and lipid metabolism. Changes in FGF21 dynamics were initially investigated in metabolic disorders, and its mediating role in improving glucose tolerance, reducing fatty acids and weight loss in obese mice has been established.[4] After it had been discovered that FGF21 could pass the blood-brain barrier,[5] researchers' interest was extended to its possible role in brain metabolism and MtD. The results suggest that expression of FGF21 could be triggered by brain-specific MtD in neurons and glial cells of the hippocampus and cortex,[6] two main sites involved in cognitive impairment.

Cognitive decline is one of the prominent features of aging. FGF21 link to cognition has been explored in mice, where the improvement in cognition was found after FGF21 modulation.[7,8] There is growing evidence that FGF21 has the potential to empower mitochondrial integrity by reducing reactive oxygen species (ROS) production and enhanced FGF21-mediated signaling in the brain, ultimately leading to preventing, improving, and even restoring impaired cognitive functions.[9,10] Since the existing literature provides very limited empirical data about the relation between FGF21 and cognition in humans,[11] the aim of this explorative study was to investigate possible association between these two parameters.

2. Materials and methods

2.1. Participants

The study sample consisted of 87 elderly people, aged 61–93 years (70.7 ± 5.8 years; 56 women). The participants over 60 years of age, who did not change dietary regime during the last month, and who gave written consent to participate were included in the study. Exclusion criteria for study enrollment were the history of mental illness, brain traumatic injury and acute infection, and surgery during the last six months. The research was conducted from October 2018 to January 2019 in FSPE Applied Bioenergetics Lab at the University of Novi Sad. The study was approved by the EC Faculty of Sport and Physical Education at the University of Novi Sad (# 114-451-710) in accordance with the Declaration of Helsinki.

2.2. Measurements

FGF21 was sampled from the venous blood, with the sample immediately centrifuged and serum analyzed afterwards using commercial ELISA kit (EDI™ Human Intact FGF21 ELISA KT-879, Epitope

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Diagnostics Inc., San Diego, CA). Anthropometric measurements included weight, height, body mass index (BMI), and handgrip strength (HG). BMI was calculated by dividing weight in kilograms by squared height in meters. Handgrip strength was assessed with a handgrip dynamometer (Baseline Model 12-0241; Fabrication Enterprises, Inc., White Plains, NY, USA) according to ACSM protocol.[12] Cognitive testing included Montreal Cognitive Assessment Test (MoCA), Rey Auditory Verbal Learning Test (RAVLT), and Category Fluency Test (CAT). MoCA is a screening test of global cognitive functioning which comprises 12 tasks of various cognitive domains.[13] Maximal score is 30 with score ≥ 26 is considered normal. RAVLT measures learning ability and verbal episodic memory using two different lists (A and B), both containing 15 unrelated words that should be memorized.[14] List A is read five consecutive times allowing the learning process to take place by repeating each trial. After 5 trials, interference list B is read-only once and asked to be repeated immediately (RAVLT trial B), after which follows a recall of list A. Half an hour later, participants should recall words from list A, and then to recognize words from both lists among 50 semantically and phonemically similar words. Measurements are learning ability presented by the total number of words memorized during 5 consecutive trials from list A (RAVLT trials 1–5), immediate and delayed recall of list A (RAVLT trial 6 and 7) and recognition of words from A and B list (recognition of list "A" and "B"). CAT was assessed through the enumeration of animals in one minute. All participants underwent the same testing protocol after an overnight fast, with blood sampling collected between 07:00–08.00, followed by anthropometric measurements and cognitive tests. The overall examination lasted for approximately one hour.

2.3. Statistical analysis

Due to the non-normal distribution of FGF21, non-parametric statistics were applied. Mann-Whitney U test was applied to test differences between FGF21 serum levels and gender, age education, BMI, and HG. Spearman's rank correlation was employed to establish an association between FGF21 and cognitive variables, both in the entire and stratified sample by each of the four categorical variables: age (61–69 yrs vs. > 70 yrs), years of education (< 12 yrs and $\geq$ 12 yrs), BMI (normal weight $\leq$ 24.9 and overweight $\geq$ 25), and HG. HG strength was first divided into gender subsamples and then by age groups into two categories – average and strong (man: 61–69 yrs < 44 kg and $\geq$ 44.1 kg, +70 yrs < 35.1 kg and $\geq$ 35.2 kg; women: 61–69 yrs < 27.2 kg and $\geq$ 27.3 kg, +70 yrs < 24.5 kg and $\geq$ 24.6 kg). Data were analyzed in statistical package SPSS 20.0 (SPSS Inc., Chicago, IL, USA), with the significance level set at $p \leq 0.05$.

3. Results

All participants completed the study. Serum FGF21 levels ranged from 70 to 1182 pg/ml, with average levels at 383.0 $\pm$ 260.5 pg/ml. The study outcomes are presented in Table 1. Results indicated that women had significantly higher serum FGF21 than men (431.5 $\pm$ 268.8 vs. 274.0 $\pm$ 218.7; $p < 0.01$), while levels were similar among different age-, education- and BMI-specific groups. No significant correlations were found between serum FGF21 and cognitive outcomes after accounting for gender, age, education, and BMI differences in the study sample, although correlation with MoCA test, as an indicator of global cognition, shows a tendency toward statistical significance ($p = 0.06$).

After the sample has been stratified, few significant correlations emerged between FGF21 and cognition indices (Table 2). Significant correlations were found between serum FGF21 and learning ability (RAVLT trials A1–A5, $p < 0.05$) and MoCA test ($p < 0.05$) among women. In the subsample of older participants (70–93 yrs), serum FGF21 levels significantly correlated with RAVLT trials A1–A5 ($p < 0.01$) (Figure 1), immediate recall (RAVLT trial A6, $p < 0.01$), delayed recall (RAVLT trial A7, $p < 0.05$), and MoCA ($p < 0.05$), while no significant correlations were found in younger counterparts. Among those who have normal BMI and in women with average handgrip strength, significant correlation was established with MoCA test ($p < 0.01$) and RAVLT A1–A5 ($p < 0.05$), respectively.

4. Discussion

The findings of this study indicate that serum FGF21 tended to be associated with global cognition ($p = 0.06$) yet the correlation has not reached statistical significance. Contrary to previous studies,[2,3] we did not find a rise in FGF21 levels with age, while a positive correlation was demonstrated between serum FGF21 and global cognition and memory in participants over 70 years as compared to younger counterparts. In addition, gender appears to play an important role in explaining the relationship between FGF21 and cognition since women had higher levels of FGF21 then men, with levels were positively correlated with both memory and global cognition.

The positive association between sFGF21 and cognition could

Table 1
Sample characteristics (mean, SD) on entire and gender-divided sample.

Variables	Total	Male	Female	p [a]
Age	70.74 $\pm$ 5.76	73.19 $\pm$ 7.48	69.39 $\pm$ 4.02	0.003
Education	13.20 $\pm$ 3.24	13.66 $\pm$ 3.66	12.93 $\pm$ 2.99	0.316
Serum FGF21 (pg/ml; median)	383 $\pm$ 260.45	274 $\pm$ 218.65	431.5 $\pm$ 268.82	0.011
Body mass index (kg/m^2)	28.37 $\pm$ 4.34	26.85 $\pm$ 3.25	29.21 $\pm$ 4.65	0.015
Handgrip strength (kg)	33.59 $\pm$ 9.52	44.08 $\pm$ 7.80	27.78 $\pm$ 4.62	0.000
RAVLT trials A1–A5	37.59 $\pm$ 8.91	34.26 $\pm$ 8.169	39.43 $\pm$ 8.83	0.009
RAVLT trial B	4.83 $\pm$ 1.81	4.19 $\pm$ 1.54	5.18 $\pm$ 1.86	0.014
RAVLT trial A6	6.87 $\pm$ 2.97	5.71 $\pm$ 2.96	7.52 $\pm$ 2.80	0.006
RAVLT trial A7	5.33 $\pm$ 3.25	3.97 $\pm$ 3.42	6.09 $\pm$ 2.91	0.003
RAVLT recognition of list A	10.30 $\pm$ 2.79	8.90 $\pm$ 2.81	11.05 $\pm$ 2.49	0.000
RAVLT recognition of list B	4.89 $\pm$ 3.24	5.17 $\pm$ 3.69	4.75 $\pm$ 3	0.573
MoCA[c]	22.11 $\pm$ 3.52	21.32 $\pm$ 3.18	22.55 $\pm$ 3.65	0.119
CAT[d]	17.94 $\pm$ 4.15	16.84 $\pm$ 4.08	18.55 $\pm$ 4.10	0.065

Abbreviations: FGF21, fibroblast growth factor; RAVLT, Rey Auditory Verbal Learning test; MoCA, Montreal Cognitive Assessment test; CAT, category fluency test.
[a] t-test was applied to test differences between genders, except for FGF21 where Mann-Whitney test was applied. [b] RAVLT measurements are expressed as number of words. [c]MoCA indicates total scores from 12 tasks (maximal score 30). [d] CAT indicates total number of words.

Table 2
Spearman's rank correlation between serum FGF21 and cognitive outcomes.

	RAVLT A1–A5	RAVLT A6	RAVLT A7	RAVLT B	RAVLT REC. A	RAVLT REC. B	MoCA	CAT
Gender								
Male (N = 31)	-0.220	-0.232	-0.155	-0.171	-0.118	-0.054	0.077	-0.120
Female (N = 56)	0.284*	0.098	0.030	0.035	0.198	0.254	0.263*	-0.040
Age								
Younger (N = 43)	-0.114	-0.162	-0.153	0.037	0.206	0.191	0.203	-0.245
Older (N = 44)	0.555**	0.405**	0.340*	0.155	0.206	0.111	0.351*	0.221
Education								
Less (N = 41)	0.084	0.037	0.048	0.045	0.228	0.069	0.132	-0.047
More (N = 46)	0.187	0.054	0.036	0.011	0.029	0.271	0.255	-0.056
BMI								
Normal (N = 21)	0.313	0.089	-0.015	0.209	0.170	0.381	0.561**	0.139
Overweight (N = 66)	0.114	0.046	0.099	-0.013	0.221	0.085	0.114	-0.033
HG strength man								
Normal (N = 9)	0.176	0.043	-0.230	-0.308	-0.134	-0.361	0.325	0.184
Strong (N = 22)	-0.278	-0.251	-0.170	-0.078	-0.127	-0.052	0.081	-0.168
HG strength women								
Normal (N = 25)	0.454*	0.164	0.130	0.233	0.199	0.359	0.257	-0.035
Strong (N = 31)	0.193	0.049	-0.022	-0.053	0.238	0.206	0.283	-0.068

Abbreviations: RAVLT, Rey's Auditory Verbal Learning Test; RAVLT A1–A5, total number of words during 5 consecutive trials (max. 75); RAVLT A6, number of words from list A reproduced immediately after list B (max. 15); RAVLT A7, number of words from list A reproduced 30 min later; RAVLT B, number of words reproduced from list B; RAVLT Rec A, number of words accurately recognized from list A; RAVLT Rec B, number of words accurately recognized from list B; MoCA, Montreal Cognitive Assessment; CAT, category fluency test; BMI, body mass index; HG, handgrip.
* Indicates $p < .01$ and ** Indicates $p < .05$.

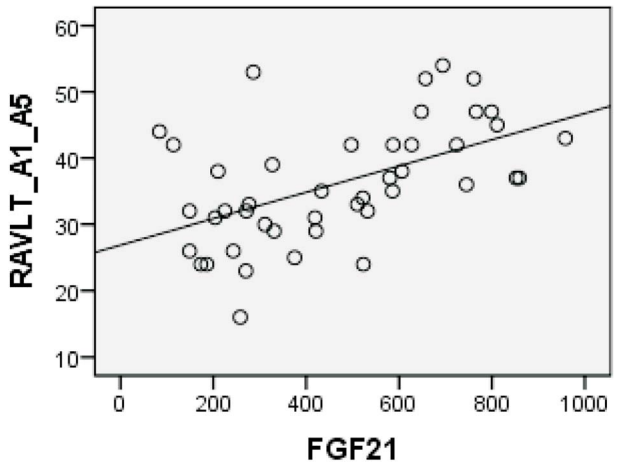

Figure 1. A correlation between serum FGF21 and RAVLT learning ability score in participants over 70 years of age (n = 44).

be due to two possible reasons. First, an FGF1-related pathway could act as a compensatory mechanism that enables cognitive functions to be preserved in old age. A substantial amount of evidence suggests the neuroprotective role of elevated FGF21 levels.[7,9] This effect of FGF21 is mainly achieved by decreasing oxidative stress in MtD.[8,9] A compensatory function of FGF21 has been suggested in healthy elderly who demonstrated elevated FGF21 levels, despite energy disturbances and enhanced inflammation found in this population.[3,18,21] Higher levels of FGF21 could be due to augmented mitochondrial unfolded protein response (UPR^{mt}) and uncoupling protein 1 (UCP1) expression, both related to enhanced longevity.[22,23] Increased lifespan is observed in mild-to-moderate MtD conditions[15,16] which is consistent with mitochondrial threshold theory. It proposes that mitokines are able to cope with mitochondrial stress up to a certain threshold after which its secretion became harmful.[16] This imposes a problem of establishing a cut-off point for the FGF21 level since the extensive range in the healthy population is detected (5 pg/ml to 5 ng/ml).[20] Without reference values, it remains difficult to interpret the changes in the levels of this metabolic cytokine as either beneficial or detrimental to senescence.[2]

On the other hand, it is plausible that FGF21 biodynamics could change independently from cognition, although both could vary in the same direction leading to positive interrelation. The only study that addresses the link between FGF21 and cognition in humans with metabolic syndrome did not find a significant correlation in adults over 65 years while negative correlation has been found in people below 65 years.[11] It is also interesting that research on centenarians, successfully aged people, found both decreased[17] and increased[3] FGF21 levels. One possible explanation of inconsistent results suggests that aging might be an FGF21 resistant condition.[18] The 'resistant' state possible appears with normal levels of endogenous FGF21 while high pharmacologic doses might induce a favorable effects.[19] Experiments with mice demonstrated beneficial effects of exogenously administered FGF21 in relation to cognitive functioning.[7,8,10]

Obesity is another FGF21 resistant state, and old age could diminish the responsiveness of tissues (including adipose tissue) to FGF21. Reduced sensitivity could be due to impaired FGF21 signalling owing to inhibition of β-klotho expression, an obligatory co-receptor of FGF21 secretion.[18] Briefly, if aging is an FGF21 resistant condition, then the age-associated impairments (including cognitive worsening) might occur whatever high FGF21 levels are, implicating their independence.

Nevertheless, the regulation of FGF21 appears to involve gender-driven mechanism(s). The higher FGF21 serum levels in women corroborates previous findings in mice[24] and humans.[25] For instance, transgenic female mice with the high circulating concentrations of FGF21 lived longer than male counterparts, and female and male transgenic mice outlived their wild-type littermates, indicating that FGF21 might be a pro-longevity factor.[26] A more pronounced effect in females is in line with results obtained in our study where women had significantly higher scores on almost all cognitive variables.

Our study has several limitations, including a cross-sectional design with a small sample size, and gender disbalance in favor of women. It should be noted that the youngest subject recruited in

this study is 61 years old, and expanding study subjects to a younger and more gender-balanced population may give us a more accurate trend of serum FGF21 level with age. In addition, a lack of control group of younger people precludes any conclusions about a causal relationship between age and FGF21, which warrants further research. Future studies should also include more biomarkers of MtD, especially in the brain, which is associated with age-dependent cognitive decline.[27,28] Neural and biochemical mechanisms that underlie age-related changes in the FGF21 level should be addressed as well. This study was explanatory and aimed to get the initial information about whether FGF21 is related to human cognition; well-controlled, robust and longitudinal studies should be done to evaluate a link between cognitive aging and mitochondrial dysfunction.

In conclusion, significantly higher serum FGF21 levels in women implicate the involvement of gender-based mechanisms in the regulation of FGF21. A positive correlation between FGF21 and cognition, especially in the participants aged > 70 years, suggests the potential benefit of FGF21 towards cognitive functioning in senescence. It seems that cognition and FGF21 perhaps tackle the common underlying mechanism responsible for age vitality in humans. At this time, it is still not clear how various FGF21 pathways are interconnected with mitochondrial function and how this link could contribute to cognition, which should be addressed in further studies.

Conflict of interest

The authors declare that they have no conflict of interest.

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