


RESEARCH

Cognitive performance during senescence in untreated congenital isolated GH deficiency

Vanderlan O Batista¹, Michael Kellner², Roberto Salvatori³, Walter Lisboa⁴, André Faro⁵, Lucas B Santos⁶, Enaldo V Melo⁷, Alécia A Oliveira-Santos⁶, Carla R P Oliveira⁶, Viviane C Campos⁶, Cynthia S Barros-Oliveira⁶, Elenilde G Santos⁶, Nathalie O Santana⁶, Keila R Villar-Gouy⁶, Ângela C Leal⁶, Rivia S Amorim⁸, Davi A Oliveira Simões⁶ and Manuel H Aguiar-Oliveira⁶

¹Division of Psychiatry, Health Sciences Graduate Program, Federal University of Sergipe, Aracaju, Sergipe, Brazil

²Department of Psychiatry and Psychotherapy, University Hospital Hamburg-Eppendorf, Hamburg, Germany and Department of Psychiatry and Psychotherapy, Technical University of Munich, Munich, Germany

³Division of Endocrinology, Diabetes and Metabolism, Department of Medicine, The Johns Hopkins University School of Medicine, Baltimore, Maryland, USA

⁴Department of Psychology, Federal University of Sergipe, São Cristóvão, Sergipe, Brazil

⁵Postgraduate Program in Psychology, Federal University of Sergipe, São Cristóvão, Sergipe, Brazil

⁶Division of Endocrinology, Health Sciences Graduate Program, Federal University of Sergipe, Aracaju, Sergipe, Brazil

⁷Statistics division, Health Sciences Graduate Program, Federal University of Sergipe, Aracaju, Sergipe, Brazil

⁸Division of Geriatrics, Health Sciences Graduate Program, Federal University of Sergipe, Aracaju, Sergipe, Brazil

Correspondence should be addressed to M Aguiar-Oliveira: herminio@infonet.com.br

Abstract

Individuals with untreated isolated GH deficiency (IGHD) due to a mutation in the GHRH receptor gene from Itabaianinha Brazil have increased insulin sensitivity, normal life expectancy, and an extended health span, i.e. the period of life free from disabilities. We hypothesize that their prolonged health span is accompanied by a delayed cognitive decline in senescence. To test this hypothesis, we have administered the Literacy-Independent Cognitive Assessment (LICA) to 15 IGHD individuals aged over 50 years and 15 controls matched by age, sex, years of education, and percentage of illiteracy. All individuals were negative for HIV and syphilis serology, and there were no differences in serum levels of folate, vitamin B12 and TSH between the two groups, while free T4 was higher in the IGHD group. IGHD subjects had a higher total LICA score than controls, 215 (22.7) vs 204.2 (18.1), without reaching statistical significance. Scores of memory, visuoconstruction, language and calculation were similar between the two groups, with better attention (9.5 (1.4) vs 8.3 (1.1), $P=0.01$) and executive function (38.3 (4.8) vs 35.1 (2.5), $P=0.03$) scores in IGHD. MANCOVA revealed that group (but no age) had a significant effect on the LICA variables (partial eta squared of 0.455, power of 0.812, $P=0.02$). This effect is verified on attention (partial eta squared 0.216, power of 0.749, $P=0.01$) and executive function (partial eta squared 0.154, power of 0.570, $P=0.03$). In conclusion, IGHD in senescence is associated with similar total cognitive performance but better attention and executive function than controls.

Keywords: GH; GHRH receptor; cognition; senescence

Introduction

Growth hormone (GH) has been reported to have a role in improving cognitive function, both by inducing cognitive behaviors related to learning and memory and by affecting excitatory circuits involved in synaptic plasticity (1). GH also has a protective effect on the central nervous system, with beneficial effects of GH therapy reported in patients with spinal cord injury. Accordingly in animal models GH appears to stimulate neurogenesis (1, 2, 3). However, brain function, like immune and visual function, seems to depend more on extrapituitary circuits, namely insulin (4, 5), IGF2 (6), brain GH (7), and brain IGF1 and IGF2 (8, 9) than on the classical somatotrophic axis (pituitary GH and circulating IGF1) (10, 11).

GH administration seems ameliorate poor cognitive performance in GH-deficient (GHD) subjects (12), and GH-releasing hormone (GHRH) modulates neuronal exosome biomarkers in mild cognitive impairments (13). While a GHRH agonist appeared to improve cognitive function in humans with mild cognitive impairment (14), a ghrelin agonist (MK-677) had no clinical effect on Alzheimer's disease progression (15).

Genetically modified mice with changes in GH signaling have been used to assess the interaction of the somatotrophic system with aging, including the brain. Most of these studies show that impairing GH signaling results in delayed aging, including of the brain, with increased life span and health span, i.e. the period of life free from important morbidities (16). Accordingly, GH deficient GHRH knockout mice show cognitive decline markedly later than control animals (17). In a similar 'nature's experiment' we have described in the Brazilian city of Itabaianinha a large cohort of individuals with severe isolated GH deficiency (IGHD) caused by a homozygous (c.57+1G→A) mutation in the GHRH receptor (GHRHR) gene (*GHRHR* OMIM n.618157) (18). Most affected adults have not received GH replacement and exhibit normal lifespan (19), with an extended health span (10), reflected by greater insulin sensitivity (20) and high levels of GLP1 (21), with a favorable profile of miRNAs (22). Our hypothesis was that their extended health span is accompanied by a delayed cognitive decline. The instruments often used to assess cognitive function are appropriate for people who know how to write and read, but their application to illiterate and low-education individuals is questionable. The influence of educational level on the assessment of cognitive impairment was confirmed in two Brazilian studies (23, 24). To overcome this problem, we used the Literacy-Independent Cognitive Assessment (LICA) (25, 26), comparing subjects with IGHD with local controls, both with little or no formal education.

Subjects and methods

Subjects

In a cross-sectional study, individuals with IGHD residing in Itabaianinha were recruited by telephone by the senior author (M.H.A.O). Inclusion criteria for IGHD were homozygosity for the c.57+1G→A *GHRHR* mutation, while homozygosity for the wild-type *GHRHR* allele was required for the control group. Exclusion criteria were age less than 50 years, previous GH replacement therapy, and history of traumatic brain injury and liver and kidney diseases, hypothyroidism or thyrotoxicosis, positivity to HIV and syphilis serology, and reduced serum levels of folate or vitamin B12. Presently there are 54 homozygous IGHD subjects in the Itabaianinha cohort (27), of which 15 individuals are aged 50 or over. All the 15 agreed to participate, without any compensation. From our database of genotyped homozygous normal individuals (18), we included 15 individuals, in the same age group, in the same households or neighborhood, who did not meet our exclusion criteria. Therefore, the groups included 15 IGHD subjects (6 men, range 53–84 years old) and 15 controls (6 men, range 56–78 years old). The Federal University of Sergipe Institutional Review Board approved these studies, and all subjects gave informed consent.

Study protocol

The LICA was administered in the participants' homes, and blood samples were taken in the week following the LICA, in a group of three individuals per day. Head circumference was measured with an inelastic measuring tape over the most prominent point on the back of the skull (occipital) and over the eyebrows. These steps were carried out between May and November 2022.

Laboratory analyses

Blood samples were collected between 09:00 and 10:00 h at the University Hospital. TSH, free T4, HIV, syphilis serology, and vitamin B12 were measured by standard techniques.

LICA administration

We translated the English version of the previously published and validated South Korean LICA model (25, 26) into Portuguese (28), and adapted it to the local culture to assess the cognitive function of people with little or no formal education, for the purposes of

research. The translation protocol was adapted from Beacon's guidelines (29) and carried out in six stages by a multidisciplinary team of 17 members (English teachers, nutritionist, psychologists, geriatrician, endocrinologists, and statistician). LICA was used 59 times, 5 in the pilot study, 24 in the variability studies and 30 in the experimental step. Inter- and intraobserver variability was 99 and 96%, respectively. The Cronbach's alpha was 0.76, indicating good instrument reliability.

Literacy screen

Participants were separated into illiterate or literate groups by a brief reading and writing test. The participants were asked to read aloud the following two sentences: 'Carlos was very thirsty after exercising. Carlos opened the refrigerator door'. They were then asked to write about what would happen next. The individual should write that 'Carlos drank water'. A participant who was able to read the sentences and write the appropriate response was considered literate. The same trained researcher (V.O.B.) administered the LICA to all subjects.

Literacy-Independent Cognitive Assessment

LICA is a 300-point test, consisting of 13 subtests assessing memory, visuoconstruction, executive function, attention, language, and calculation. The story recall task involves immediate and delayed recall after approximately 20 min, and recognition. The word recall task involves three learning trials of ten nouns, an immediate recall trial, a 20-min delayed recall trial, and recognition. Visuoconstruction and memory are assessed using copy construction and recognition of 10 pictures of stick formations, which were modified from the stick pattern reversal test. Executive functions are assessed using the Digit Stroop test and the animal fluency task. Attention, concentration, and working memory are evaluated using forward and backward repetition tapping tasks of nine Corsi blocks. It involves mimicking a researcher as he taps on a sequence of up to nine spatially separated identical blocks. The sequence starts using two blocks but becomes more complex until the subject's performance fails. Language is assessed using a 15-item confrontation-naming task with animals, fruits, and vegetables and the Color and Object Recognition Test (CORT). CORT was modified from semantic knowledge tests on the visual form and color of objects. Scores below 186.0 and 154.5 define dementia in literacy and illiteracy, LICA was understood easily by literate and illiterate people.

Statistical analysis

Anthropometric measures, blood pressure (BP), vitamin B12, folate, free T4, TSH, the LICA's domains and the

total LICA score were expressed as mean (standard deviation) and compared by Student's *t*-test. Sex and illiteracy were expressed as *n* (percentage) and compared by the Fisher's exact test. MANCOVA using all the LICA domains (memory, visuoconstruction, executive function, attention, language, and calculation), and the total score as dependent variables, with two factors (age and group) as independent variables, was performed to examine main and interaction effects of the factors on the defined multiple dependent variables. This is accomplished by estimating their effect size by the partial eta-square, so defined: values for partial eta squared: 0.01, small effect size; 0.06, medium effect size; 0.14 or higher, large effect size.

The statistical software SPSS/PC 20.0 (SPSS, Inc., Chicago, IL, USA) was used. Statistical significance was set at $P < 0.05$.

Results

Table 1 shows the comparison of anthropometric, biochemical, blood pressure data and LICA's domains between the two groups. As expected, height, weight, and head circumference were lower, while FT4 was higher in the IGHD group ($P=0.02$), as previously published (30). IGHD subjects had a higher total LICA score than controls, without reaching statistical significance. However, they showed better attention ($P=0.01$) and executive function ($P=0.03$).

MANCOVA revealed that group (but no age) had a significant effect on the LICA variables (partial eta squared of 0.455, power of 0.812, $P=0.02$). This effect is verified on attention (partial eta squared 0.216, power of 0.749, $P=0.01$) and executive function (partial eta squared 0.154, power of 0.570, $P=0.03$). Both partial eta-square values were greater than 0.14, showing a large effect size that translates into superior cognitive performance in the IGHD group.

In controls, the test application time was 36.2 (9.1) min vs 47.8 (4.6) min, literate vs illiterate, $P=0.02$. In IGHD, it was 41.8 (7.6) min vs 49.3 (7.2) min, literate vs illiterate, $P=0.089$.

Discussion

Population aging has become a worldwide phenomenon, with projection that the number of people aged ≥ 65 years will be approximately 1 billion by 2030 (31). The consequences of GHD on brain aging remains controversial. Patients with acquired GHD may have several confounding factors: different etiologies of GHD, pituitary surgery, irradiation, use of anticonvulsants, and deficits of other pituitary hormones with inadequacy of the respective replacement therapies (27). IGHD is rare and often

Table 1 Demographic, anthropometric, biochemical, blood pressure (BP) and Literacy Independent Cognitive Assessment (LICA) data of 15 IGHD subjects and 15 controls.

Parameters	IGHD	Controls	95% CI	P
Age (years)	66.0 (8)	66.4 (6.5)	-5.8 to 5.0	0.88
Sex, male, <i>n</i> (%)	6 (40)	6 (40)	-0.3 to 0.3	1
Weight (kg)	46.5 (15.1)	66.6 (10.3)	-29.7 to -10.4	<0.0001
Height (m)	1.2 (0.1)	1.6 (0.1)	-0.4 to -0.3	<0.0001
BMI (kg/m ²)	31.2 (8.4)	27.4 (2.9)	-0.9 to 8.6	0.11
Head circumference (cm)	50.7 (2.8)	54.7 (2.2)	-5.9 to -2.1	<0.0001
Systolic BP (mm Hg)	131 (21)	140 (13)	-23.2 to 5.3	0.21
Diastolic BP (mm Hg)	82 (15)	82 (6)	-9.4 to 9.7	0.98
Vitamin B12 (pg/mL)	500 (175)	526 (271)	-197.0 to 144.2	0.75
Folate (ng/mL)	14.7 (3.6)	12.7 (3.3)	-0.6 to 4.5	0.14
TSH (mUI/mL)	3.2 (2.7)	1.9 (1.1)	-0.3 to 2.8	0.11
Free T4 (ng/dL)	1.0 (0.2)	0.9 (0.1)	0.03 to 0.2	0.02
Illiteracy, <i>n</i> (%)	5 (33.3)	5 (33.3)	-0.3 to 0.3	1
Education years	6.4 (5.5)	4.7 (4.1)	-1.9 to 5.3	0.33
LICA ratings				
Memory	89.2 (19.3)	83.6 (11.9)	-6.5 to 17.6	0.35
Visuoconstruction	28.0 (2.8)	26.2 (3.5)	-0.6 to 4.2	0.13
Language	39.4 (3.8)	39.4 (3.9)	-2.9 to 2.9	1
Executive function	38.3 (4.8)	35.1 (2.5)	0.3 to 6.1	0.03
Attention	9.5 (1.4)	8.3 (1.1)	0.3 to 2.1	0.01
Calculation	11.5 (0.5)	11.7 (0.5)	-0.5 to 0.2	0.37
Total Score	215.8 (22.7)	204.2 (18.1)	-3.7 to 28.0	0.13

treated with GH replacement therapy during childhood. Therefore, the Itabaianinha cohort, with severe congenital and mostly untreated IGHD, constitutes a valuable opportunity to study the effect of GHD on brain aging. In this work we have found that individuals with lifetime untreated IGHD older than 50 years exhibited a trend toward a higher total LICA score than the control group, without reaching statistical significance. However, they showed better attention and executive function than controls. These data contradict the widespread, though never proven, concept that reduced function of the somatotrophic axis (i.e. GHRH, pituitary GH, and circulating IGF1) seen during aging causes the cognitive impairment often found in senescence. Accordingly, there has been no description of cases of dementia in the almost 30 years of follow-up of this cohort, nor a mention of dementia as a cause of death in the death certificates of IGHD deceased subjects born since 1892 (19).

The results of this study expand on our previous observations that in these subjects the deficiency of GH, except for severe short stature, possibly results in more benefit than harms (10, 11, 22). The harms involve unpleasant high-pitched voice, mild hearing loss, mild vestibular (10, 11, 25) and hip problems (32), increased total and LDL cholesterol, and higher systolic blood pressure (10, 11, 22). The benefits include reduced prevalence of vertebral fractures (32) with appropriate muscle function (33), increased insulin sensitivity (20), increased GLP-1 response to mixed meal (21), delayed

atherosclerosis (34), protection against common cancers (such as breast, lung, intestine, and prostate), and prolonged health span (10, 11, 22). Remarkably, we also found a significant upregulation of age-related miRNAs, miR-100-5p, miR-195-5p, miR-181b-5p, and miR-30e-5p, which are known for targeting pathways associated with longevity such as mTOR, AKT, NFκB, and IRS1s (22). As a result, despite the cardiovascular disease risk factors, their longevity is similar to that of their unaffected siblings (19). The excellent quality of life of these IGHD individuals is also notable (35).

These IGHD individuals, despite visceral obesity (36), do not exhibit insulin resistance (on the contrary they have increased insulin sensitivity), as shown by lower homeostasis model assessment index of insulin resistance (HOMA-IR) ($P=0.04$), and trend to elevation of quantitative insulin sensitivity check index and oral glucose insulin sensitivity in 2 hours ($P=0.06$ and 0.09 , respectively) (20). Insulin resistance is a crossroads of metabolic and cognitive disorders in humans (37). The hypothalamus, frontal and striatal regions are particularly sensitive to insulin. Memory, hippocampal, and visual brain regions are also modulated by insulin. Healthy insulin signaling modulates brain networks involved in homeostatic control, human eating behavior reward processing, and cognitive function. Thus, insulin might benefit memory function (37), and support neuronal survival by triggering the release of glial cell neurotrophic factors (38).

In addition, insulin input controls neurotransmitter systems (norepinephrine and acetylcholine) with relevance to memory function (36). Conversely, brain insulin resistance can be considered a potential link between obesity, type 2 diabetes, and dementia (39) or major depressive disorder (40), and peripheral insulin resistance assessed by HOMA-IR is negatively associated with cognitive function (5). Compared with normal subjects, insulin-resistant subjects have reduced glucose metabolism in middle temporal gyrus, middle frontal gyrus, right precentral gyrus, right inferior frontal gyrus, right cuneiform lobe, and bilateral cerebellar regions as assessed by PET imaging (4). These findings substantiate the insulin resistance-executive function hypothesis, demonstrated by the differential impact of insulin resistance on processing speed and specific aspects of executive function (4). Based on all these studies in the literature, we hypothesize that the increased insulin sensitivity of these IGHD individuals (20) may contribute to their better attention and executive function.

Another possible explanation of our findings is the increased GLP-1 secretion (21). Modulation of GLP-1 activity may influence amyloid β peptide aggregation in Alzheimer's disease and dopamine levels in Parkinson's disease. GLP-1 receptor agonists might also exert a beneficial effect on the cognitive impairment induced by diabetes or obesity, improving learning and memory by modulating synaptic plasticity (41).

While IGHD subjects have a mildly increased FT4, likely due to reduced function of the deiodinase system (30), their TSH is not different from that of controls. Therefore, we do not think that the FT4 difference is involved in the difference in brain function findings.

There are some reports of cognitive impairments in adult-onset GHD (AO-GHD), with somewhat positive (42), though inconsistent, effects of GH replacement therapy, particularly on attention (43) and self-reported memory (44), but no effects in patients with normal cognitive performance at baseline (45, 46). In this setting, GH therapy seems improve intellectual tasks, but it is not able to reduce the subjects' distorted perception of body image (47). In addition, one placebo-controlled study found improved memory and attentional performance in 48 patients with CO-GHD (17 with IGHD) (48). However, AO-GHD is a different condition from childhood onset GHD. Indeed, IGHD subjects from Itabaianinha have normal quality of life (QoL), and treatment with GH depot for 6 months did not result in further total QoL improvement, except in satisfaction with physical endurance (35).

Looking at cognitive function in another model of genetic cause of GH/IGF-1 alteration (GH resistance), there appears to be some discrepancy between the Ecuadorian and Israeli Laron syndrome cohorts in terms of cognitive function, which is reported as normal or improved in the former (49) and somehow compromised in the latter (50) (despite the same

genetic mutation), which is possibly an indication of an effect of the environment. There are differences between the Laron and Itabaianinha syndromes, likely due to some residual action of GH in the latter, which is not found in the former. Finally, we cannot exclude a specific effect of lack of GHRH receptor function in other areas of the brain, as, while the expression of the full-length receptor is mostly localized in the pituitary gland, splice variants are expressed in several areas of the brain (51).

This study has several strengths, such as the application of LICA by the same experienced examiner, and the match between the IGHD and control groups. A possible limitation of this study could be an adverse influence of the coronavirus disease 2019 (COVID-19) pandemic on our results, as cognitive impairment may follow the resolution of acute COVID-19 infection (52). Such effect is unlikely, as cognitive function, assessed after the end of the pandemic in Brazil, was excellent in both groups. Furthermore, we have previously shown no difference in the prevalence of anti-SARS-CoV-2 IgM and IgG antibody positivity between IGHD subjects and matched normal stature individuals in October 2020, during the acceleration of the pandemic (53). The relatively low number of subjects is a limitation of the present study. We studied all the IGHD subjects above 50 years of age and one control for each IGHD subject, matched for gender, age, literacy, neighborhood, and socioeconomic conditions. While recognizing that increasing the proportion of controls for each case would have increased the power of the study, we think that the careful matching of the groups supports the conclusions of similar cognitive function between cases and controls. It is noteworthy that no individual presented a score compatible with dementia in either of the two groups. This work expands the description of a rare genetic syndrome in a cohort that will not last forever due to the improvement of their geographic isolation, the reduction in consanguineous marriages, and the availability of GH therapy in childhood, all resulting in the reduction in the number of GH-naive individuals throughout their lives. Considering the aforementioned facts, we believe that the approach of collecting maximum information from this cohort is highly desirable rather than applying a sample size approach (54).

Conclusion

In conclusion, congenital untreated lifelong IGHD due to a *GHRHR* mutation is associated with total cognitive performance similar to controls, and better attention and executive function.

Declaration of interest

R. Salvatori serves on Novordisk advisory board. The other authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the study reported.

Funding

This work was not supported by public or private funds.

Acknowledgements

The authors thank the *Associação do Crescimento Físico e Humano de Itabaianinha* for assistance.

References

- Nyberg F & Hallberg M. Growth hormone and cognitive function. *Nature Reviews. Endocrinology* 2013 **9** 357–365. (<https://doi.org/10.1038/nrendo.2013.78>)
- Tresguerres JÁF, Fernández-Tresguerres I, Viña J, Rancan L, Paredes SD, Linillos-Pradillo B & Vara E. Effects of GH on the aging process in several organs: mechanisms of action. *International Journal of Molecular Sciences* 2022 **23** 7848. (<https://doi.org/10.3390/ijms23147848>)
- Blackmore DG, Steyn FJ, Carlisle A, O'Keeffe I, Vien KY, Zhou X, Leiter O, Jhaveri D, Vukovic J, Waters MJ, et al. An exercise “sweet spot” reverses cognitive deficits of aging by growth-hormone-induced neurogenesis. *iScience* 2021 **0** 103275. (<https://doi.org/10.1016/j.isci.2021.103275>)
- Chen Y, Qiu C, Yu W, Shao X, Zhou M, Wang Y & Shao X. The relationship between brain glucose metabolism and insulin resistance in subjects with normal cognition: a study based on 18F-FDG PET. *Nuclear Medicine Communications* 2022 **43** 275–283. (<https://doi.org/10.1097/MNM.0000000000001511>)
- Frazier DT, Bettcher BM, Dutt S, Patel N, Mungas D, Miller J, Green R & Kramer JH. Relationship between insulin-resistance processing speed and specific executive function profiles in neurologically intact older adults. *Journal of the International Neuropsychological Society* 2015 **21** 622–628. (<https://doi.org/10.1017/S13555617715000624>)
- Pardo M, Cheng Y, Sitbon YH, Lowell JA, Grieco SF, Worthen RJ, Desse S & Barreda-Diaz A. Insulin growth factor 2 (IGF2) as an emergent target in psychiatric and neurological disorders. Review. *Neuroscience Research* 2019 **149** 1–13. (<https://doi.org/10.1016/j.neures.2018.10.012>)
- Gossard F, Dihl F, Pelletier G, Dubois PM & Morel G. In situ hybridization to rat brain and pituitary gland of growth hormone cDNA. *Neuroscience Letters* 1987 **79** 251–256. ([https://doi.org/10.1016/0304-3940\(87\)90438-1](https://doi.org/10.1016/0304-3940(87)90438-1))
- Rotwein P, Burgess SK, Milbrandt JD & Krause JE. Differential expression of insulin-like growth factor genes in rat central nervous system. *PNAS* 1988 **85** 265–269. (<https://doi.org/10.1073/pnas.85.1.265>)
- Nyberg F. Growth hormone in the brain: characteristics of specific brain targets for the hormone and their functional significance. *Frontiers in Neuroendocrinology* 2000 **21** 330–348. (<https://doi.org/10.1006/frne.2000.0200>)
- Aguiar-Oliveira MH, Souza AHO, Oliveira CRP, Campos VC, Oliveira-Neto LA & Salvatori R. Mechanisms in Endocrinology: the multiple facets of GHRH/GH/IGF-I axis: lessons from lifetime, untreated, isolated GH deficiency due to a GHRH receptor gene mutation. *European Journal of Endocrinology* 2017 **177** R85–R97. (<https://doi.org/10.1530/EJE-16-1047>)
- Aguiar-Oliveira MH & Bartke A. Growth hormone deficiency: health and longevity. *Endocrine Reviews* 2019 **40** 575–601. (<https://doi.org/10.1210/er.2018-00216>)
- Falletti MG, Maruff P, Burman P & Harris A. The effects of growth hormone (GH) deficiency and GH replacement on cognitive performance in adults: a meta-analysis of the current literature. *Psychoneuroendocrinology* 2006 **31** 681–691. (<https://doi.org/10.1016/j.psyneuen.2006.01.005>)
- Winston CN, Goetzl EJ, Baker LD, Vitiello MV & Rissman RA. Growth hormone-releasing hormone modulation of neuronal exosome biomarkers in mild cognitive impairment. *Journal of Alzheimer's Disease* 2018 **66** 971–981. (<https://doi.org/10.3233/JAD-180302>)
- Baker LD, Barsness SM, Borson S, Merriam GR, Friedman SD, Craft S & Vitiello MV. Effects of growth hormone-releasing hormone on cognitive function in adults with mild cognitive impairment and healthy older adults: results of a controlled trial. *Archives of Neurology* 2012 **69** 1420–1429. (<https://doi.org/10.1001/archneurol.2012.1970>)
- Sevigny JJ, Ryan JM, Van Dyck CH, Peng Y, Lines CR, Nessly ML & MK-677 Protocol 30 Study Group. Growth hormone secretagogue MK-677: no clinical effect on AD progression in a randomized trial. *Neurology* 2008 **71** 1702–1708. (<https://doi.org/10.1212/01.wnl.0000335163.88054.e7>)
- Bartke A. Growth hormone and aging. *Reviews in Endocrine and Metabolic Disorders* 2021 **22** 71–80. (<https://doi.org/10.1007/s11154-020-09593-2>)
- Leone S, Recinella L, Chiavaroli A, Ferrante C, Orlando G, Vacca M, Salvatori R & Brunetti L. Behavioral phenotyping, learning and memory in young and aged growth hormone-releasing hormone-knockout mice. *Endocrine Connections* 2018 **7** 924–931. (<https://doi.org/10.1530/EC-18-0165>)
- Salvatori R, Hayashida CY, Aguiar-Oliveira MH, Phillips JA, Souza AHO, Gondo RG, Toledo SPA, Conceição MM, Prince M, Maheshwari HG, et al. Familial Dwarfism due to a novel mutation of the growth hormone-releasing hormone receptor gene. *Journal of Clinical Endocrinology and Metabolism* 1999 **84** 917–923. (<https://doi.org/10.1210/jcem.84.3.5599>)
- Aguiar-Oliveira MH, Oliveira FT, Pereira RMC, Oliveira CRP, Blackford A, Valença EHO, Santos EG, Gois-Junior MB, Meneguz-Moreno RA, Araújo VP, et al. Longevity in untreated congenital growth hormone deficiency due to a homozygous mutation in the GHRH receptor gene. *Journal of Clinical Endocrinology and Metabolism* 2010 **95** 714–721. (<https://doi.org/10.1210/jc.2009-1879>)
- Oliveira CRP, Salvatori R, Barreto-Filho JAS, Rocha IES, Mari A, Pereira RMC, Campos VC, Menezes M, Gomes E, Meneguz-Moreno RA, et al. Insulin sensitivity and β -cell function in adults with lifetime, untreated isolated growth hormone deficiency. *Journal of Clinical Endocrinology and Metabolism* 2012 **97** 1013–1019. (<https://doi.org/10.1210/jc.2011-2590>)
- Oliveira-Santos AA, Salvatori R, Nogueira MC, Bueno AC, Barros-Oliveira CS, Leal ÂCGB, Marinho CG, Damascena NP, Oliveira DA, Melo MA, et al. Enteroendocrine connections in congenital isolated GH deficiency due to a GHRH receptor gene mutation. *Journal of Clinical Endocrinology and Metabolism* 2019 **104** 2777–2784. (<https://doi.org/10.1210/jc.2019-00094>)
- Saccon TD, Schneider A, Marinho CG, Nunes ADC, Noureddine S, Dhahbi J, Lopez YON, LeMunyan G, Salvatori R, Oliveira CRP, et al. Circulating microRNA profile in humans and mice with congenital GH deficiency. *Aging Cell* 2021 **20** e13420. (<https://doi.org/10.1111/ace1.13420>)
- Kochhann R, Varela JS, Lisboa CSM & Chaves MLF. The Mini Mental State Examination: review of cutoff points adjusted for schooling in a large Southern Brazilian sample. *Dementia Neuropsychology* 2010 **4** 35–41. (<https://doi.org/10.1590/S1980-57642010DN40100006>)

- 24 Baccaro A, Segre A, Wang YP, Brunoni AR, Santos IS, Lotufo PA, Benseñor IM & Goulart AC. Validation of the Brazilian-Portuguese version of the Modified Telephone Interview for cognitive status among stroke patients. *Geriatrics and Gerontology International* 2015 **15** 1118–1126. (<https://doi.org/10.1111/ggi.12409>)
- 25 Choi SH, Shim YS, Ryu SH, Ryu HJ, Lee DW, Lee JY, Jeong JH & Han SH. Validation of the literacy independent cognitive assessment. *International Psychogeriatrics* 2011 **23** 593–601. (<https://doi.org/10.1017/S1041610210001626>)
- 26 Shim Y, Ryu HJ, Lee DW, Lee JY, Jeong JH, Choi SH, Han SH & Ryu SH. Literacy independent cognitive assessment: assessing mild cognitive impairment in older adults with low literacy skills. *Psychiatry Investigation* 2015 **12** 341–348. (<https://doi.org/10.4306/pi.2015.12.3.341>)
- 27 Aguiar-Oliveira MH & Salvatori R. Disruption of the GHRH receptor and its impact on children and adults: the Itabaianinha syndrome. *Reviews in Endocrine and Metabolic Disorders* 2021 **22** 81–89. (<https://doi.org/10.1007/s11154-020-09591-4>)
- 28 Santos LB, Kellner M, Oliveira W, Faro A, Oliveira CRP, Batista VO, Oliveira-Santos AA, Brito IVA, Almeida V, Villar-Gouy KR, et al. Translation into Portuguese and cultural adaptation of the Literacy Independent Cognitive Assessment. *Endocrine Abstracts 25th European Congress of Endocrinology* 2023 **90** EP706. (<https://doi.org/10.1530/endoabs.90.EP706>)
- 29 Beaton DE, Bombardier C, Guillemin F & Ferraz MB. Guidelines for the process of cross-cultural adaptation of self-report measures. *Spine* 2000 **25** 3186–3191. (<https://doi.org/10.1097/00007632-200012150-00014>)
- 30 Alcântara MRS, Salvatori R, Alcântara PRS, Nóbrega LMA, Campos VS, Oliveira ECO, Oliveira MHS, Souza AHO & Aguiar-Oliveira MH. Thyroid morphology and function in adults with untreated isolated growth hormone deficiency. *Journal of Clinical Endocrinology and Metabolism* 2006 **91** 860–864. (<https://doi.org/10.1210/jc.2005-2555>)
- 31 The Centers for Disease Control and Prevention. Public health and aging: trends in aging – United States and worldwide. *JAMA* 2003 **289** 1371–1373. (<https://doi.org/10.1001/jama.289.11.1371>)
- 32 Epitácio-Pereira CC, Silva GMF, Salvatori R, Santana JAM, Pereira FA, Gois-Junior MB, Britto AVO, Oliveira CRP, Souza AHO, Santos EG, et al. Isolated GH deficiency due to a GHRH receptor mutation causes hip joint problems and genu valgum and reduces size but not density of trabecular and mixed bone. *Journal of Clinical Endocrinology and Metabolism* 2013 **98** E1710–E1715. (<https://doi.org/10.1210/jc.2013-2349>)
- 33 Andrade-Guimarães AL, Aguiar-Oliveira MH, Salvatori R, Carvalho VO, Alvim-Pereira F, Daniel CRA, Brasileiro GAM, Santana-Ribeiro AA, Santos-Carvalho HA, Oliveira CRP, et al. Adult individuals with congenital, untreated, severe isolated growth hormone deficiency have satisfactory muscular function. *Endocrine* 2019 **63** 112–119. (<https://doi.org/10.1007/s12020-018-1763-5>)
- 34 Costa UMM, Oliveira CRP, Salvatori R, Barreto-Filho JAS, Campos VC, Oliveira FT, Rocha IES, Oliveira JLM, Silva WA & Aguiar-Oliveira MH. Brazilian adult individuals with untreated isolated GH deficiency do not have accelerated subclinical atherosclerosis. *Endocrine Connections* 2016 **5** 41–46. (<https://doi.org/10.1530/EC-15-0118>)
- 35 Barbosa JAR, Salvatori R, Oliveira CRP, Pereira RMC, Farias CT, Britto AVO, Farias NT, Blackford A & Aguiar-Oliveira MH. Quality of life in congenital, untreated, lifetime isolated growth hormone deficiency. *Psychoneuroendocrinology* 2009 **34** 894–900. (<https://doi.org/10.1016/j.psyneuen.2009.01.001>)
- 36 Gomes-Santos E, Salvatori R, Ferrão TO, Oliveira CRP, Diniz RDCA, Santana JAM, Pereira FA, Barbosa RAA, Souza AHO, Melo EV, et al. Increased visceral adiposity and cortisol to cortisone ratio in adults with congenital lifetime isolated GH deficiency. *Journal of Clinical Endocrinology and Metabolism* 2014 **99** 3285–3289. (<https://doi.org/10.1210/jc.2014-2132>)
- 37 Kullmann S, Heni M, Hallschmid M, Fritsche A, Preissl H & Häring HU. Brain insulin resistance at the crossroads of metabolic and cognitive disorders in humans. *Physiological Reviews* 2016 **96** 1169–1209. (<https://doi.org/10.1152/physrev.00032.2015>)
- 38 Li L & Holscher C. Common pathological processes in Alzheimer disease and type 2 diabetes: a review. *Brain Research Reviews* 2007 **56** 384–402. (<https://doi.org/10.1016/j.brainresrev.2007.09.001>)
- 39 Gerozissis K. Brain insulin: regulation, mechanisms of action and functions. *Cellular and Molecular Neurobiology* 2003 **23** 1–25. (<https://doi.org/10.1023/A:1022598900246>)
- 40 Hamer JA, Testani D, Mansur RB, Lee Y, Subramaniapillai M & McIntyre RS. Brain insulin resistance: a treatment target for cognitive impairment and anhedonia in depression. *Experimental Neurology* 2019 **315** 1–8. (<https://doi.org/10.1016/j.expneurol.2019.01.016>)
- 41 Grieco M, Giorgi A, Gentile MC, d'Erme M, Morano S, Maras B & Filardi T. Glucagon-like Peptide-1: a focus on neurodegenerative diseases. *Frontiers in Neuroscience* 2019 **13** 1112. (<https://doi.org/10.3389/fnins.2019.01112>)
- 42 Golgeli A, Tanriverdi F, Suer C, Gokce C, Ozesmi C, Bayram F & Kelestimur F. Utility of P300 auditory event related potential latency in detecting cognitive dysfunction in growth hormone (GH) deficient patients with Sheehan's syndrome and effects of GH replacement therapy. *European Journal of Endocrinology* 2004 **150** 153–159. (<https://doi.org/10.1530/eje.0.1500153>)
- 43 Oertel H, Schneider HJ, Stalla GK, Holsboer F & Zihl J. The effect of growth hormone substitution on cognitive performance in adult patients with hypopituitarism. *Psychoneuroendocrinology* 2004 **29** 839–850. ([https://doi.org/10.1016/S0306-4530\(03\)00151-3](https://doi.org/10.1016/S0306-4530(03)00151-3))
- 44 Burman P, Broman JE, Hetta J, Wiklund I, Erfurth EM, Hagg E & Karlsson FA. Quality of life in adults with growth hormone (GH) deficiency: response to treatment with recombinant human GH in a placebo-controlled 21-month trial. *Journal of Clinical Endocrinology and Metabolism* 1995 **80** 3585–3590. (<https://doi.org/10.1210/jcem.80.12.8530603>)
- 45 Baum HBA, Katznelson L, Sherman JC, Biller BMK, Hayden DL, Schoenfeld DA, Cannistraro KE & Klibanski A. Effects of physiological growth hormone (GH) therapy on cognition and quality of life in patients with adult-onset GH deficiency. *Journal of Clinical Endocrinology and Metabolism* 1998 **83** 3184–3189. (<https://doi.org/10.1210/jcem.83.9.5112>)
- 46 Bollerslev J, Hallén J, Fougner KJ, Jørgensen AP, Kristo C, Fagertun H, Gudmundsen O, Burman P & Schreiner T. Low-dose GH improves exercise capacity in adults with GH deficiency: effects of a 22-month placebo-controlled, crossover trial. *European Journal of Endocrinology* 2005 **153** 379–387. (<https://doi.org/10.1530/eje.1.01971>)
- 47 Sartorio A, Conti A, Molinari E, Riva G, Morabito F & Faglia G. Growth, growth hormone and cognitive functions. *Hormone Research* 1996 **45** 23–29. (<https://doi.org/10.1159/000184754>)
- 48 Deijen JB, de Boer H & van der Veen EA. Cognitive changes during growth hormone replacement in adult men. *Psychoneuroendocrinology* 1998 **23** 45–55. ([https://doi.org/10.1016/S0306-4530\(97\)00092-9](https://doi.org/10.1016/S0306-4530(97)00092-9))
- 49 Nashiro K, Guevara-Aguirre J, Braskie MN, Hafzalla GW, Velasco R, Balasubramanian P, Wei M, Thompson PM, Mather M, Nelson MD,

- et al.* Brain structure and function associated with younger adults in growth hormone receptor-deficient humans. *Journal of Neuroscience* 2017 **37** 1696–1707. (<https://doi.org/10.1523/JNEUROSCI.1929-16.2016>)
- 50 Laron Z. Psychological aspects on patients in patients with Laron syndrome. In *Laron Syndrome From Man to Mouse* **38**, pp. 325–333. Eds Z Laron & JJ Kopchik. Berlin Heidelberg: Springer-Verlag 2011.
- 51 Halmos G, Szabo Z, Juhasz E & Schally AV. Signaling mechanism of growth hormone-releasing hormone receptor. *Vitamins and Hormones* 2023 **123** 1–26. (<https://doi.org/10.1016/bs.vh.2023.06.004>)
- 52 Ceban F, Ling S, Lui LMW, Lee Y, Gill H, Teopiz KM, Rodrigues NB, Subramaniapillai M, Di Vincenzo JD, Cao B, *et al.* Fatigue and cognitive impairment in post-COVID-19 Syndrome: a systematic review and meta-analysis. *Brain, Behavior, and Immunity* 2022 **101** 93–135. (<https://doi.org/10.1016/j.bbi.2021.12.020>)
- 53 Melo MA, Borges LP, Salvatori R, Souza DRV, Santos-Júnior HT, de R Neto JM, Campos VC, Santos AA, Oliveira CRP, da Invenção GB, *et al.* Individuals with isolated congenital GH deficiency due to a GHRH receptor gene mutation appear to cope better with SARS-CoV-2 infection than controls. *Endocrine* 2021 **72** 349–355. (<https://doi.org/10.1007/s12020-021-02728-8>)
- 54 Miller F, Zohar S, Stallard N, Madan J, Posch M, Hee SW, Pearce M, Vågerö M & Day S. Approaches to sample size calculation for clinical trials in rare diseases. *Pharmaceutical Statistics* 2018 **17** 214–230. (<https://doi.org/10.1002/pst.1848>)