Peer-reviewed version available at Int. J. Mol. Sci. 2018, 19, 2294; doi:10.3390/ijms19082294

Type of the Paper (Case Report)

Title: Growth Hormone (GH) administration increases the metabolic activity of the left hippocampus in an elder patient with cognitive disorders.

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Abstract: 1) Background: We analyzed, by PET-SCAN, how growth hormone (GH) could act on the brain of an older woman, not GH-deficient, which was beginning to show some cognitive deficiencies and presented an ApoE genotype 4/3; 2) Methods: After performing a first psychometric study (TAVEC verbal learning test), the metabolic activity of brain structures related to knowledge, memory, and behavior was analyzed using 18-F Fluorodeoxyglucose PET-SCAN. The patient was then treated with GH (0.4 mg/day) for three weeks and on the last day under this treatment, a new PET-SCAN was performed. One month after beginning treatment with GH, a new TAVEC test was performed; 3) Results: GH administration normalized the cognitive deficits observed in the first psychometric test and increased significantly ($P < 0.025$) the metabolic activity in practically all brain cortical areas, specifically in the left hippocampus and left amygdala, although not in the left parahippocampus; and 4) Conclusions: This is the first study in which the positive effects of GH on cerebral metabolism have been visualized in a human patient. Our data confirm the positive effects of this hormone on cognition, memory and behavior in patients affected by mild cognitive impairments.

Keywords: Growth Hormone; cognition; recent memory; PET-SCAN; hippocampus; amygdala; parahippocampus; ApoE genotype.

1. Introduction

In 1993 it was discovered that growth hormone (GH) receptor gene was expressed in many different areas of the CNS of rats and rabbits [1], a finding confirmed three years later when it was described that not only the GH receptor (GHR) but also that the hormone itself was present in the CNS [2]. These findings led to the assumption that GH should perform important functions at the central level, challenging the classic concept that GH is a pituitary hormone whose actions take place at the metabolic level, and on the longitudinal growth of the organism before puberty ends. In fact, the current concept is that GH is a pleiotropic hormone, which is expressed in virtually all tissues and organs in which, in addition to endocrine effects, exerts a number of auto- and paracrine actions [3, 4].

GH plays a key role in the organization of the brain during the development of the CNS [5-7], and also in its normal functioning after birth, both in animals and humans. The hormone has been found to be produced within the postnatal hippocampus in rats [8,9], where GH administration increases the proliferation of stem cells in normal adult rats [10]; this also occurs when the hormone is given after a brain injury produced by kainic acid administration, perhaps by cooperating with the

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GH endogenously expressed, as its receptor, in hippocampal progenitor cells [11]. Moreover, memory tasks induce synthesis of GH in mice hippocampus leading to the appearance of newly formed neurons [12]. Since many years ago it is well known that GH-deficient (GHD) adult patients show clear psychological improvements when being treated with the hormone [2], particularly in aspects related to memory and cognition [13]. On the contrary, adults with untreated GHD usually show significant psychological effects related to lack of energy, memory and cognitive alterations [14, 15]. These effects of GH on cognitive functions in humans have been widely reviewed recently [4, 16]. However, it is not known if they occur as a direct action of the hormone at the central level, or if they depend on a higher production of hepatic IGF-I induced by GH (or directly induced by the hormone in the CNS), or if those actions are consequence of the effects of both hormones [4, 17, 18]. In fact, GH has been demonstrated to induce the local expression of IGF-I in the human fetal cortex [19], although this has not yet been shown in the adult brain.

The pituitary secretion of GH reaches very high levels at puberty, but once it ends, a progressive decline in secretion begins, starting from 18 to 30 years of age, until the plasma levels of the hormone are virtually undetectable as we age [4, 17]. Therefore, it seems logical that aging is associated with a cognitive deterioration, produced, among other factors, by a deficit of the GH/ IGF-I axis [17, 18].

Therefore, in this study, we tried to visualize, by using PET-SCAN, how GH treatment could act in brain areas involved in cognitive aspects in an older woman who was beginning to show memory deficits and temporal and spatial disorientation. Our results clearly indicate that the treatment with low doses of GH produced a significant increase in the metabolic activity of the left hippocampus and the left amygdala, and also, although in this case not significant, in the left parahippocampus, structures that previously showed hypometabolism. Moreover, the increased metabolism was also shown in practically all the cerebral cortical areas. The improvements observed after treatment with GH were also confirmed by the TAVEC verbal learning test, and by the patient herself.

2. Results

2.1. Cognitive test: TAVEC test

Most of the values recorded as deficient in the first test changed to a mean value for a normal population during the second test performed. This indicates a positive learning curve, as well as increased attention. Likewise, the discrimination index also indicated learning on the part of the patient, because in the second test she was already able to store the information in a discriminatory way. Also, during the second test performed there was no loss of information (fading) over time.

These data indicate that the treatment with GH induced an improvement in learning, attention, and memory, although, due to the short time elapsed between the two tests, no statistical analysis of the results obtained was performed.

The scores of this test are shown in Table 1.

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ASSAY		Pre	1 Month
1. RI-A1	Immediate recall of the first learning assay	-1	0
2, RI-A5	Immediate recall of the fifth learning assay	-2	0
3. RI-AT	Total words recalled in the whole of the 5 assays	-2	0
4. RI-B	Immediate recall of the interference list	-1	1
5. RG-Pr	Percentage of words from the region of primacy, on the total number of words remembered in the whole of the 5 assays	-2	0
6. Rg-Rc	Percentage of words from the middle region, on the total number of words remembered in the whole of the 5 assays	0	0
7. Rg-Rc	Percentage of words from the region of recency, on the total number of words remembered in the whole of the 5 assays	-1	0
8. RL-CP	Short-term free memory	-1	1
9. RC-CP	Long-term free memory	-1	0
10. RL-LP	Memory with short-term keys	-1	0
11. RC-LP	Memory with long-term keys	-2	0
12. Esem-RI-A	Use of the serial strategy in the immediate recall of list A	-1	-1
13. Esem-RI-S	Use of the serial strategy in the immediate recall of list B	-1	-1
14. Esem-RL-CP	Use of serial strategy in short-term free recall	-1	0
15. Esem-RL-LP	Use of serial strategy in long-term free recall	-1	-1
16. Eser-RI-A	Use of the semantic strategy in the immediate recall of list A	-1	-1
17. Eser-RI-B	Use of semantic strategy in the immediate recall of list B	-1	0
18. Eser-RL-CP	Use of semantic strategy in short-term free recall	0	0
19. Eser-RL-LP	Use of semantic strategy in long-term free recall	1	0
20. P	Total number of Perseverations	1	1
21. I-RL	Number of Intrusions in the whole of free recall tests	0	1
22. I-RL	Number of Intrusions in the whole of memory tests with keys	-1	0
23. Recon-Ac	Number of success in the Recognition test	-2	1

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24. FP	Number of false positives in the Recognition test	1	0
25. Discriminability	Discriminability Index	-2	0
26. Bias	Response Bias Index	0	0
27. RI-S versus R1-A1	Comparison between the memory of list B and the memory of the first learning assay on list A	0	1
28. RL-CP versus RI-A5	Comparison between short-term free recall and the immediate recall of the fifth learning assay on list A	-1	0
29. RC-CP versus RO-LP	Comparison between remembering with short-term keys and remembering with long-term keys	1	0
30. RL-LP versus RL-CP	Comparison between long-term free memory and short-term free memory	1	0
31. RC-LP versus RL-LP	Comparison between memory with long-term keys and long-term free recall	0	1
32. Recon-Ac versus RL-Lp	Comparison between recognition and long-term free recall	-1	0
33. Recon-Ac versus Rcl-LP	Comparison between recognition and remembrance with long-term keys	-1	0

Table 1.- Results obtained in the TAVEC test performed in basal conditions (Pre) and 1 Month later (seven days after finishing the treatment with GH). The values in the first and second tests correspond to Z-scores (mean value for a normal population is 0). The scores in the first test were, in general, below (highlighted in blue color) the mean of the normal subjects, indicating a mild cognitive deficit. However, the second test indicates that most of the results obtained are now in the mean for normal subjects or even higher than the mean.

2.2. PET-SCAN studies

The treatment with GH led to a global increase in the metabolic activity observed in most of the cortical brain regions in both hemispheres. This was identified both qualitatively and quantitatively.

When evaluated by using the software described in Methods, the first PET-SCAN showed significant hypometabolism in the following regions of interest (ROI), as shown in Table 2: the left hippocampus (Z-score = -2.79) and the left amygdala (Z-score = -2.23), the left parahippocampus (Z-score = -2.12), the left cuneus (Z-score = -2.06), and the subgenual area of the anterior cingulate cortex (Z-score = -2.17) of the right hemisphere. These deficits, excepting the left cuneus, were 1.5 standard deviations (SD) lower than the mean value recorded in the database of the normal population, and clearly asymmetric with respect to the metabolic activity detected in the other hemisphere. These findings were corroborated by the SPM analysis (see Methods), which confirmed that these hypometabolisms had statistical significance (P <0.025).

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	First PET-SCAN					Second PET-SCAN			
ROI	L	R	Asym	Hypom	*P*<	L	R	Asym	Hypom
Hippocampus	-12.49	-6.55	-5.94		*0.025*	-6.49	-2.84	-3.65	
Amygdala	-13.38	-7.20	-6.18	Left	*0.025*	-9.47	-1.52	-7.96	
Parahippocampus	-11.93	-7.24	-4.69		*0.025*	-10.86	-8.19	-2.67	Bilat
Cuneus	-5.42	4.06	-9.48	Left	*0.025*	-0.90	6.41	-7.31	Left
AC/SGA	-2.43	-11.16	8.73	Right	*0.025*	3.08	0.91	-2.17	

Table 2.- Quantitative analysis of the metabolism (Neurocloud PET) in the regions of interest expressed as percentage of deviation with respect to the normal population of the database, in the first and second PET-SCAN performed. The values highlighted in blue indicate a standard deviation < 1.5 with respect to the mean of the normal population. L: Left side; R: Right side; Asym: Asymmetry; Hypom: Hypometabolism; Bilat: Bilateral; AC/SGA: Subgenual area of the anterior cingulate cortex. *P:* Statistical significance of each hypometabolism with respect to the normal population.

These hypometabolisms were normalized after treatment with GH, as indicated by Table 2, with the only exception of the left parahippocampus, which in the ROI-based analysis still showed a decrease in the acquisition of FDG (Z- score = -2.04).

Qualitative changes in the metabolism observed in these regions are shown in the following figures:

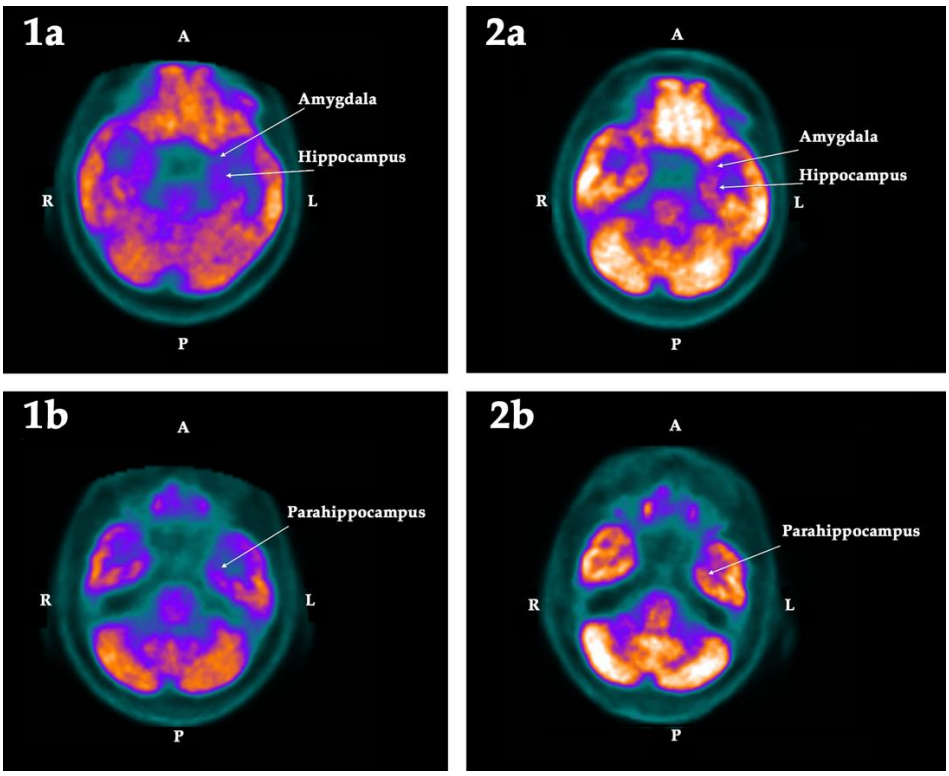

Figure 1.- Cross-section of the brain showing the metabolic activity in the left amygdala, the left hippocampus (1a) and the left parahippocampus (1b), in the first (1) and the second (2) PET-SCAN studies. Note that the low metabolic activity in those structures observed in the first PET-SCAN was normalized in the left amygdala and the left hippocampus (*P*< 0.025) after treatment with GH (2a),

but it was not statistically significant in the left parahippocampus (2b). A: Anterior. R: Right. L: Left. P: Posterior.

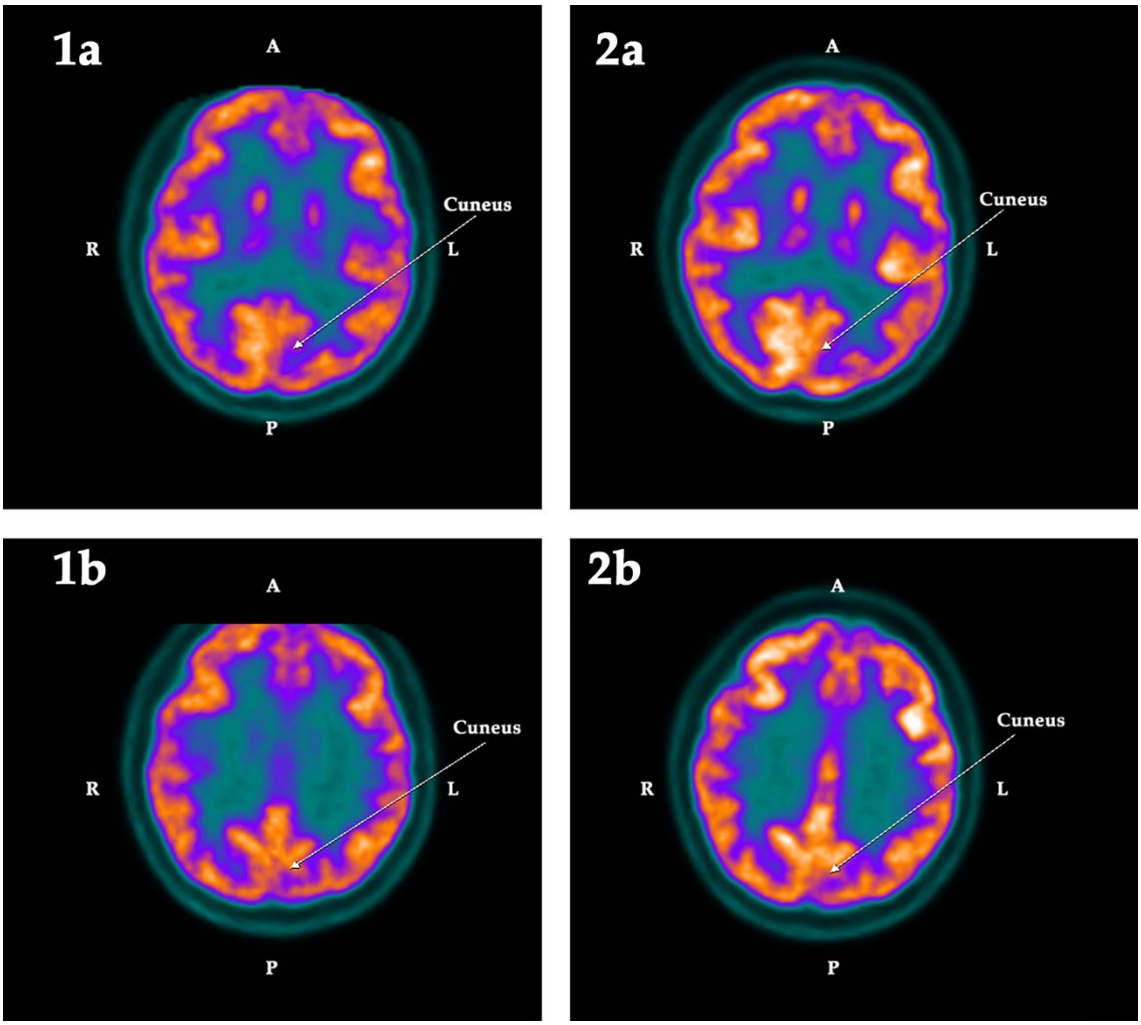

Figure 2.- Cross-section of the brain showing, in two consecutive sections, the metabolic activity in the left cuneus, in the first (1) and the second (2) PET-SCAN studies. Note that the low metabolic activity observed in the left cuneus in the first PET-SCAN (1a and 1b) was normalized ($P < 0.025$) after treatment with GH (2a and 2b). A: Anterior. R: Right. L: Left. P: Posterior.

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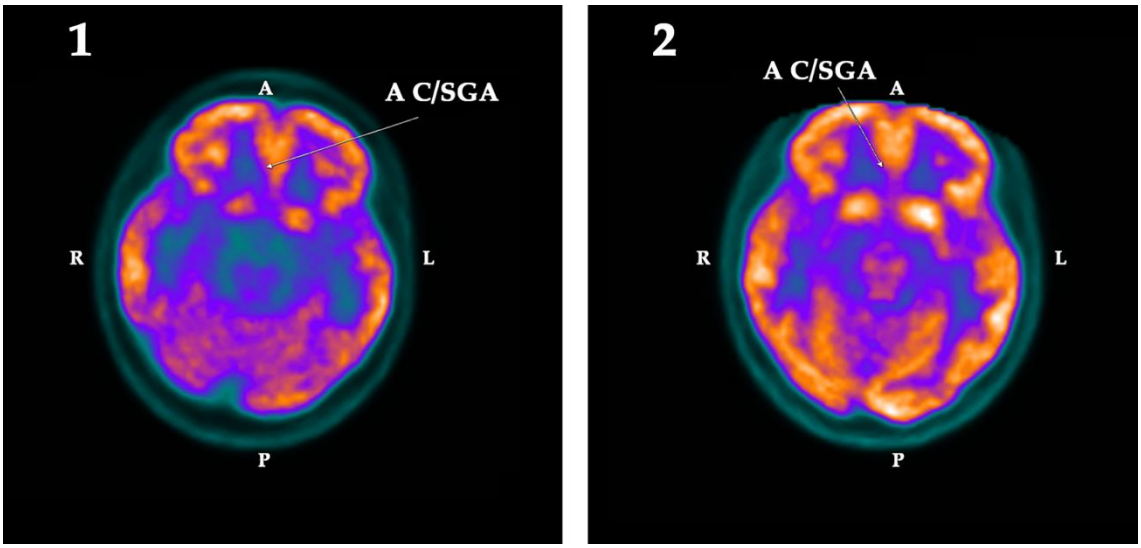

Figure 3.- Cross-section of the brain showing the metabolic activity in the subgenual area of the anterior cingulate cortex (A C/SGA), in the first (1) and the second (2) PET-SCAN studies. Note that the low metabolic activity observed in A C/SGA in the first PET-SCAN was normalized ($P< 0.025$) after treatment with GH. A: Anterior. R: Right. L: Left. P: Posterior.

2.3. Blood analysis

The blood test prior to the treatment showed that the patient had dyslipidemia (total cholesterol plasma values: 275 mg/dl (normal: 110-200 mg/dl), plasma triglycerides: 195 mg/dl (normal: 50-150 mg/dl). The erythrocytes (4.95 x 10^6 /µl) and Hb (14.2 g/dl) were in normal values, as were the values of plasma glucose (94.8 mg/dl), proteins (total proteins: 7.1 g/dl; albumin: 4.3 g/dl), liver transaminases, urea, creatinine and tumor markers (CA-125, CA15-3, CA19-9, alpha-fetoprotein and CEA). Plasma thyroid stimulating hormone (TSH) was normal (2.18 µUI / ml), as was free thyroxine (fT4, 1.1 ng/dl); Plasma cortisol at 8 am was also normal (24 µg/dl; normal values: 8-25 µg/dl). The values of plasma IGF-I (125 ng/ml) and plasma insulin-like growth factor-binding protein 3 (IGFBP3: 2.8 µg/ml) were also normal for the sex and age of the patient. A test of intravenous arginine hydrochloride showed that the patient did not have GHD (maximum GH peak: 4.6 ng/ml).

Interestingly, the Apolipoprotein E (ApoE) genotype of the patient was E4/3, presenting an ApoE4 allele (*ε4*) that is related to familial hypercholesterolemia, but also to increased risk of Alzheimer's disease (AD) or mild cognitive impairments (MCI) [20].

The blood test performed after the second PET-SCAN indicated that there were no significant changes in the parameters previously analyzed, except that the plasma IGF-I and IGFBP3 values in plasma had increased to 185 ng/ml and 3.2 µg/ ml, respectively (both in normal values). Blood glucose remained at normal values (97.3 mg/dl).

The treatment with GH did not produce any side effects.

3. Discussion

In this study, we demonstrate for the first time, with PET-SCAN brain images, the positive effect that GH exerts on the human brain in an elder patient with cognitive deficits, not GHD; particularly in areas related to learning, recent memory, behavior and visuospatial perception, although as the images indicate, the metabolic effects of the hormone took place in practically all cortical areas. Furthermore, as the patient herself reported, her quality of life and performance of daily activities improved after treatment with GH, despite the fact that the administration of the hormone lasted only a short time.

Unlike what happens in children and adults with GH deficiency, few studies show that the administration of GH increases cognition in not-GHD human patients with cognitive deficits, produced as a consequence of different pathologies [21-26]. However, these positive effects of GH have been widely demonstrated in different experimental animal models [27-31]; even in aged animals, in which the cognitive decline in hippocampal-dependent functions, such as learning and memory, is associated with a decrease in the secretion of GH and IGF-I, as in our species [4, 17, 18, 30].

In the case of the hippocampus, it has been shown to be an important neurogenic niche, where adult neurogenesis takes place in humans [32]. Functionally, the hippocampus is responsible for the acquisition of memory, learning, and recent spatial orientation and navigation (in neurons known as "place cells"). These functions are affected in personality disorders, perhaps due to a continuous excess of glucocorticoids [33], which negatively regulates the formation of new neurons in the subgranular zone (SGZ) of the dentate gyrus of this structure [34].

Recently, it was published that hippocampal neurogenesis begins to decrease abruptly from adolescence to undetectable in adults [35]. This led to great controversy and very recent publications question or contradict these postmortem findings [36-38], although there is a possibility that neurogenesis in the adult hippocampus may be deregulated by neurological diseases, such as epilepsy or behavioral disorders [39], which would explain the current divergent opinions on the persistence of adult neurogenesis in man throughout life.

In our study, the metabolic activity of the left hippocampus significantly increased after the treatment with GH. This may indicate that the treatment with the hormone induced an increase in the number of neurons in this area, as we and others have shown in rats [10, 11]. Another possibility is that GH has induced the sprout of dendritic spines and changes in the length and density of pre-existing dendrites in the hippocampus, as has been shown to occur after intracerebroventricular administration of the hormone in adult rats [40]. If we could have used 3'-deoxy-3'-[18F] fluoro-L-thymidine instead of FDG, we could have detected whether the changes observed in the PET-SCAN were or were not due to adult neurogenesis, as a study demonstrated in adult rats [41].

Interestingly, hippocampal atrophy (measured by MRI) is an early marker of AD that correlates with impairments of memory [42], and it has been found that cerebral glucose metabolism is significantly reduced in early stages of this disease [43]. In addition, it has recently been postulated that a treatment with GH could be useful in this neurodegenerative disease [44, 45]. In any case, an early stage of AD must be discarded in our patient, because the decrease in metabolism in the left hippocampus could only be detected after the quantitative analysis performed with Neurocloud, and there was no decrease in the temporoparietal acquisition. However, the ApoE genotype of the patient was ε4/3, and the presence of only one copy of the ε4 allele has been described to increase the risk of developing AD, MCI or other cerebral pathologies with cognitive affectations [20], although the major risk for AD exists in individuals homozygous showing two ε4 copies (ε4/4) [20].

We cannot discard the existence of GH-induced neurogenesis in the hippocampus of the patient described here, thus improving its recent memory; but it is very unlikely, or practically impossible, that adult neurogenesis has been the cause of the increase in glucose metabolism in virtually all cerebral cortical areas, as shown by the images of the second PET-SCAN performed after treatment with GH.

Other regions of interest in which hypometabolism was observed before treatment with GH were the left amygdala, the left parahippocampus, the left cuneus and the subgenual area of the anterior cingulate cortex.

The amygdala is involved in emotional responses (pleasure, fear, anger, anxiety), but it also determines how emotions adhere to memories, mainly forming new memories related to fear, although a recent article describes fear as the result of a very complex memory network [46]. A very recent study reports that in the basolateral amygdala of adult mice there are neurogenic precursor cells that give rise to newly formed interneurons [47]. However, adult neurogenesis in the human

amygdala has not yet been demonstrated; therefore, it is unlikely that the increased metabolism observed in this structure after the treatment with GH could be due to adult neurogenesis.

Regarding the parahippocampus, it has recently been described that, in humans, its posterior section is involved in visuospatial perception, while the previous section is related to mnemonic processes, suggesting that this structure acts as a functional interface between perception and memories [48]. GH treatment also enhanced the low metabolic activity before observed in this structure, but changes, in this case, did not reach statistical significance.

The cuneus is the cuneiform portion of the occipital lobe, located in the angle formed by separating the parietooccipital (internal perpendicular sulcus) and calcarine (calcarine sulcus) furrows. The cuneus receives information from the upper area of the contralateral retina, representing the lower visual field. Its function is basic visual processing, related with attention and work memory. In this case, the existence of left hypometabolism before the treatment with GH was detected after the Neurocloud PET analysis, showing an asymmetry between the left and the right side of this structure, although the percentage of acquisition of the left side was inside the range of values for the normal population of the database used, as shown in Table 2. Therefore, these data do not seem to have any special meaning with respect to the deficits initially observed in our patient. In any case, the treatment with GH significantly increased the metabolism of the left cuneus, but also that in the right side; therefore, the asymmetry between both sides continued to be present.

In the case of the subgenual area of the anterior cingulate cortex, there was a clear asymmetry between the left and the right side before the administration of GH, but this disappeared after the treatment with the hormone, being the metabolism already normal on both sides. This area has been related to be associated with depression [49], and its affectation is linked to mood disorders [50], which may explain some of the symptoms observed in our patient. Moreover, structural and functional abnormalities in this region had been associated to major depressive disorders, mainly when accompanied by a decreased volume of both temporal lobes and the left hippocampus and the parahippocampus [51], but this was not the case in the patient here described.

The brain uses mainly glucose to obtain the energy needed to function properly [52]. In fact, a recent study describes that there is a significant correlation between the cerebral metabolic rate of glucose, measured by FDG PET, and the level of consciousness in patients in vegetative state or minimally conscious state, being the metabolic rate of glucose capable of differentiating between both conditions [53].

An elegant study, carried out in a model of dwarf rats specifically deficient in GH and IGF-I, showed that in these animals there was a marked decrease in glucose metabolism in many areas of the brain, particularly those involved in learning and memory, dependent on the hippocampus, indicating that the decrease in GH / IGF-I production associated with aging plays an important role in the evolution towards an elderly brain [54]. Moreover, in that study it was seen that the production of ATP in the hippocampus was decreased by 15%, which contributed to the statement that GH and IGF-I play a clear role in the regulation of glucose use and cerebral energy metabolism [54]. The same happens in man. Aging leads to a series of brain deficits, such as learning and memory, neurogenesis, synaptic density and modifications in dendrite architecture [see 54, for review]. In addition, it is logical that a decrease in the production of GH/IGF-I will alter the metabolic turnover of important neurotransmitters, such as acetylcholine and noradrenaline, given the complexity of the hypothalamic regulation of the synthesis and release of pituitary GH [55].

These age-related cerebral deficits have been shown to be reversed by chronic infusion of IGF-I into the lateral ventricle of aged rats [56], or by GH treatment in very old rodents [57], and more recent studies, in man, indicate that high plasma levels of GH and IGF-I maintain the functional quality of working memory during aging [58].

In this study, we show that GH administration markedly increased the acquisition of glucose (as indicated by FDG PET-SCAN) in brain structures previously showing deficient metabolism of this key neuronal nutrient. However, we don't know how this occurred. Despite her age, the patient did not have GH deficiency and her plasma IGF-I levels were in the normal range for her age. Brain glucose hypometabolism has been related to early stages of AD [59]; in addition, the genotype of the patient presents a copy of the ε4 allele, which implies, as described [20], an increased risk of

developing AD, and also a decrease in the metabolism of glucose in the brain [60]. In addition, a recent study, in mice genetically engineered to carry one of the three human ApoE alleles instead of their normal ApoE gene, showed that the ApoE3 and ApoE4 brains of these animals exhibited a significant reduction in the expression of molecules involved in signaling by IGF-I, such as IGF-I itself, Irs 1 (insulin receptor substrates) and the glucose transporter Glut4 [61], leading to reduced glucose uptake. In the same study, it was shown that ApoE4 brains presented lower levels of Pparg, a nuclear receptor which regulates neuronal survival [62], and mitochondrial biogenesis [63]. In all, these data may explain the reduced glucose uptake and the deficient production of energy in the brain of subjects presenting an ε4 allele, and they also can explain the mild cognitive deficits observed in our patient, and also the recoveries (metabolic and cognitive) we observed after treating her with GH. As indicated above, GH induces the local expression of IGF-I in the human fetal cortex [19], and there is the possibility that this effect also takes place in the adult brain, but has not yet been seen. GH increases liver production of IGF-I and, consequently, the plasma levels of this peptide, as it happened in this study. However, differently from what happens with GH, IGF-1 plasma hardly cross the blood brain barrier. Therefore, if the effects we observed in this study were produced by IGF-1, this must have occurred due to the induction exerted by GH of the synthesis of that peptide in the brain. Moreover, IGF-I signaling implies the activation of PI3K/Akt pathways, and phosphorylated Akt induces the translocation of Glut4 vesicles to the plasma membrane for allowing the entry of glucose into the cells [64]; the activation of PI3K/Akt is also a key signaling pathway for GH actions, as our group demonstrated [65, 66]. Therefore, a direct effect of GH cannot be discarded for explaining the results obtained in this study. In addition, GH may have produced an increase in blood flow to the brain, allowing greater uptake of FDG. Recently, we described that GH induces a significant reparative effect on endothelial dysfunction that appears after atherogenic stimuli, such as hypercholesterolemia [67]; In addition, the hormone is a mitochondrial protector [24, 68], and atherogenesis is related to oxidative stress [67]. Therefore, since the patient we treated had high levels of plasma cholesterol and triglycerides it is also possible that, despite the short time of treatment, GH may have contributed to improve the blood supply to the brain, facilitating the appearance of the changes here described (both in terms of PET-SCAN images and cognitive tests).

GHRH (Growth hormone-releasing hormone) is a major inducer of the synthesis and secretion of GH in pituitary [54] and perhaps other territories [4]. A randomized, double-blind, placebo-controlled trial analyzed the effects of a synthetic analog of GHRH (Tesamorelin; Theratechnologies Inc., Canada) administered subcutaneously (1 mg/day) for 20 weeks in 61 adults with MCI and 76 healthy adults; the results obtained demonstrated that this GHRH analog had positive effects on cognition in both groups studied [69]. A further clinical trial from the same group carried out in 30 adults (age 55-87 years; 17 with MCI), utilizing the same GHRH analog at the same doses and during the same time, demonstrated that this treatment significantly increased gamma-aminobutyric acid (GABA) levels in three left side brain regions (dorsolateral frontal, posterior cingulate, and posterior parietal), increased N-acetylaspartylglutamate in the frontal cortex, and decreased myoinositol (an osmolyte linked to AD) in the posterior cingulate, inducing a positive effect on cognition in both groups of participants, without affecting the regulation of plasma glucose [70]. However, in that study, no changes were found in brain glutamate levels, unlike what has been reported in preparations of hippocampal slices from old rats treated with GH or IGF-I [30], but consistent with the effects of GH on the density and functionality of GABAB receptors, in male rats, in areas of the brain related to cognition [71].

In summary, for the first time, we demonstrated with FDG PET-SCAN the positive effects that GH exerts on the brain of an older woman with MCI. Our data do not allow us to conclude if these effects are directly produced by GH or if they depend on the brain expression of IGF-I induced by the hormone but corroborate our previous data in patients and animal models with cognitive deficits produced by acquired brain damage. Moreover, GH treatment is safe if given at low doses and during short periods of time.

4. Materials and Methods

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The patient was a 61-year-old woman, Caucasian, whose father had died of a very aggressive AD at 64 years of age, and who had also suffered from type II diabetes very poorly controlled for years. Her family history also included that an older sister had begun to present important cognitive alterations, at the age of 59, and frequent episodes of absences, for unknown reasons, that required antiepileptic treatment.

The patient has 4 children, works as a director of a company. The only clinical history of interest is a hysterectomy for a fibromyoma, and familial dyslipidemia that she never wanted to be treated with medications since she alleged that her diet was very rich in Omega-3 fatty acids. Her only medication was melatonin, before sleep.

Upon admission to our Medical Center, the patient reported that in the last two years she had suffered significant stress due to work problems, a decrease in recent memory, sporadic episodes of disorientation in time and space and some behavioral alterations. Due to the illness that her father had suffered, she feared that AD also began to develop in her.

The clinical examinations were normal. The blood pressure was 135/70 mm Hg. The body mass index (BMI) was normal: 23 kg / m2. Before starting treatment with GH and one month after starting it, a blood test was performed (hematimetry, biochemistry, thyroid hormones, cortisol, IGF-I, IGFPB3 and tumor markers). To evaluate the possibility of a GHD, a typical arginine test (30 grams of iv infusion of arginine hydrochloride between 0 and 30 min) was performed during the first blood test, and samples were taken to analyze plasma levels of GH at times 0, 30, 60, 90 and 120 min.

In a blood sample, the ApoE genotype of the patient was analyzed by molecular hybridization with amplification PCR (polymerase chain reaction).

The studies and treatment were carried out according to the protocols of the Foltra Medical Center in accordance with Spanish legislation for the use of GH off-label and the Code of Ethics of the World Medical Association (Declaration of Helsinki).

Before the GH treatment, the signed informed consent of the patient was obtained to be treated with the hormone and the results obtained could be published. The study was approved by the Ethical Committee of the Foltra Medical Center (Fol2018-002).

4.1. Cognitive tests

Initially, a TAVEC verbal learning test was performed. This test is based on other similar psychometric tests, such as the test of 15 word of Rey or the more recent CVLT-California verbal learning test. In all of them, the verbal element learning test is used. In the TAVEC test specifically, 3 lists are used for Learning, Interference and Recognition. The test allows to establish the normality of the patient (in comparison with a similar sample in age, sex and educational level), and describe the functioning of the patient's memory and determine the form and reasons for its deviation (if any).

One month after the first test, a new TAVEC test was performed.

4.2. PET-SCANS

After the first TAVEC test, the metabolic activity of the patient's brain was analyzed by PET-SCAN images in cross sections acquired 30 minutes after the administration of Fluorodeoxyglucose (FDG) 4.2 mCi 18-F. One day later, and once the signed informed consent was obtained, the patient began treatment with GH (0.4 mg/day, sc., at 10 am, Nutropín, Ipsen, Spain).

21 days later a new PET-SCAN (requested by the patient to know if GH exercised any action) was carried out in the same conditions, excepting that the dose of FDG administered was now slightly lower (3.6 mCi).

Last GH administration took place 1 hour before the second PET-SCAN was performed. Both PET-SCAN were performed at 11.00 am.

In both cases, the patient was fasting from 14 hours before PET-SCAN.

One month after the first test, a new TAVEC verbal learning test was carried out.

4.3. Statistical analysis

Due to the short time elapsed between the two TAVEC tests, no statistical analysis of the results obtained between the two was performed.

Both PET-SCAN were quantified by using Neurocloud PET software (Qubiotech Health Intelligence, A Coruña, Spain), which provides both ROI (regions of interest)-based statistical analysis using the regions on the Hammersmith Atlas [72] and voxel-based "statistical parametric mapping" (SPM), both comparing the patient images with a database of 97 healthy subjects. This allows evaluating the values obtained in any subject in terms of percentage of standard deviations (SD) with respect to the normal population, and from this the statistical significance of the data obtained. This software provides embedded spatial and intensity normalization methods. Spatial normalization is performed by a 12-parameter affine registration between the patient PET and a template on the MNI (Montreal Neurological Institute) space. Intensity normalization is performed in a ratios histogram fashion.

The software provides a proprietary database with 97 FDG-PET acquisitions performed on pre-treatment oncologic patients in which an acquisition was added using the standard brain protocol before whole body acquisition. All patients underwent a neurological examination and signed informed consent to be included in the trial. All the images in the database were visually verified and reported as normal by three independent nuclear medicine specialists. The studies carried out in these patients consisted of MRI T1 3D and -FDG-PET (brain protocol). As criteria for normality, the following were established: 1) no detection of cerebral atrophy in MRI. 2) 3 independent reports from three specialists in nuclear medicine, which affirmed the non-existence of brain anomalies in the FDG-PET. 3) An absence of neurological or psychiatric history and normal neurological examination (MMSE [Mini-mental state examination] normal scores: 25-30).

The statistical significance of the data was established for a value of $P < 0.05$.

5. Conclusions

The results of this study clearly show, for the first time with cerebral images in human patients, that GH exerts a strong and positive effect on cortical cerebral structures. Of special interest is the fact that the hormone increases metabolic activity in areas related to memory and cognition.

Although more studies are needed and with more patients, there is the possibility that treatment with GH in the early stages of Alzheimer's disease may be useful to slow the progression of this disease and/or other neurodegenerative disorders that lead to cognitive alterations.

Author Contributions: "Conceptualization, J.D. and P.D.; Methodology, J.D., I.N., C.A., A.B. and P.D.; Software, I.N. and A.B.; Validation, J.D., I.N., C.A.; Formal Analysis, I.N.; Investigation, J.D. and P.D.; Writing-Original Draft Preparation, J.D.; Writing-Review & Editing, J.D. and I.N.; Supervision, J.D.; Project Administration, J.D.; Funding Acquisition, J.D.".

Funding: "This research was funded by Foundation Foltra grant number [2018-02]."

Acknowledgments: We acknowledge the support and suggestions of Jesús Silva, PhD, Associated Researcher, Laboratory of Biomarkers of Molecular Imaging (MIBioLab), in the statistical interpretation of PET-SCAN data. Voxel-Based Morphometry was performed by Qubiotech Health Intelligence S.L. (Coruña. Spain).

Conflicts of Interest: "The authors declare no conflict of interest." "The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results".

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