MedCrave
Step into the World of Research

Journal of Dental Health, Oral Disorders & Therapy

Research Article

Open Access

CrossMark

Application of growth hormone to reduce osseointegration time in dental implants

Abstract

This studypretends to evaluate the beneficial effects of the treatmentwith growth hormone(compassive off-label use)on bone regeneration and osseointegration of dental implant surgeriesin elderly people.A total of 140 patients, between 35 and 82 years of age, undergoing dental surgery, receiving a total of 402 dentalimplants have been investigated. Informed consent was signed by all of them. From this group, 58 patients(31 malesand 27 females) received a total of 209 implants and weretreated with local growth hormone in the surgical bed during the procedure, and in 29 implants of this group,when surgical beds were more extensive also with daily systemic application for one month.

Another 82 patients between 35 and 82 years of age (39 males and 43 female) werealso submitted to implant surgery and received a total of 193 implants without growthhormone treatment. For the evaluation of results, a simple apical radiography was used. Osseointegration was determined by bone neoformation and density between and around theimplant coils. Study showed that growth hormone treatment was able to induce a statisticallysignificant decrease in the average time between the surgical approach and osseointegration of theimplant. The median value for treated patients was 82 days whereas the untreated needed amedian of 140 days. No differences were observed either regarding gender or groups ofage.

It was concluded that in all age and gender groups the reduction of theosseointegration time was very significant when growth hormone treatment was used.

Keywords: growth hormone, implants, osseointegration

Volume 11 Issue 4 - 2020

Ana Rego,[1] Jesus A F Tresguerres,[2] Jose M Tallon,[3,4] Paulo Gomes[5]

[1]Clínica do Mediterrâneo, Lisboa
[2]Department of Physiology, Medical School,University Complutense,Spain
[3]Medical Clinic, Doctor, Tallon
[4]Sports Sciences Department, Exercise and Health, Universidade de Trás-os-Montes e Alto Douro,Vila Real, Portugal
[5]NOVA IMS Information Management School, Lisbon, Portugal

Correspondence: Ana Rego, Clínica do Mediterrâneo, Lisboa, Portugal, Tel 00351917469494Email ana@clmediterraneo.com

Received: August 02, 2020 | **Published:** August 24, 2020

Abbreviations: GH, Growth hormone; PTH, parathyroid hormone; OPG, osteoprotegerin; PRGF, plasma rich in growth factors

Introduction

The great desire of nearly all people is to have a long and healthy life, which does not meanto add more years to the lifespan, but also to increase the quality of life in said years. Starting from the age of 40 a decrease in several hormones can be observed that seems to be related to the aging process. The replacement therapy with these hormones at physiological doses and after an individual evaluation to prove that the beneficial effects[1] are always greater than the possible adverse effects could be a strategy to delay the consequences of aging. Although no hormone can be recognized undoubtedly as a "rejuvenating" agent or that it may prolong life, some of its actions may be beneficial.[2] The possible treatment of elderly people usingreplacement therapy with growth hormone (GH) was first demonstrated in the paper of Rudman et al.[3] Actuallythe possibilities of GH replacement therapy for the aging process is showing a steady increase.

GH promotes body growth during infancy through puberty, inducing fundamentally an increase inthe length of long bones acting on the chondrocytes of the conjugation cartilage (or growth plate), although it also stimulates the growth of soft tissues. In childhood, a deficit in the secretion of GH leads to pituitary dwarfism, whereas its hypersecretion causes gigantism. In adults, excessive production of GH leads to acromegaly, whereas its deficit leads to a syndrome characterized by a set of factors leading to early atherosclerosis and an increased risk of cardiovascular diseases.[4]

Pulsatilesecretion of GH reaches a peak during puberty, a period in which its pulse amplitude is greatest during slow-wave sleep.[3] This phase is the most important of all stages of sleep due to its influence in the GH increase. With aging, sleep fragmentation occurs and changes in circadian rhythms appear, which leads to a decrease in slow-wave sleep. This leads to a decrease in melatonin, GH andInsulin-Like Growth Factor1 (IGF-1) levels.[5] From the fourth decade of life onwards a decrease of GH secretion levels can be observed, converting the majority of elderly people into GH deficient individuals (SDGA).

One of the actions of GH is the stimulation of the formation and/or remodelling of bone tissue, and the increase of bone density. Recent studies justifies that GH can be used in the treatment of osteoporosis in adults with GH deficiency or also during a period of healthy aging.[6] In addition, it has been shown that continuous treatment over a period of time in patients with GH deficiency is also able to increase bone mineral density and is therefore recommended as a treatment to obtain bone maturation in children in need of it.[7] Another possibility of GH administration is to contribute to accelerate the process of osseointegration in fractures.[8]

Among the actions performed by GH are the formation and remodelling of the bone tissue. The overproduction of GH (achromegaly) is accompanied by an increase in bone mass[9] whereas in adults with GH deficiency the bone mineral density is decreased,[10] and these patients develop a higher risk of osteoporosis.[11] In fact, both local[12]and systemic[8] GH administration is able to accelerate the bone remodelling process.

Several studies have demonstrated the possible utility of GH as a coadjuvant in the consolidation of fractures. Thus, the administration of exogenous GH to rats in which an experimental tibial fracture had

occurred was capable of increasing the rigidity and load capacity of the fractured bone.[13,14]These positive effects are also observed in older animals.[15,16]All these data allow hope for its potential role in the consolidation of complicated fractures, either by nature of injury or because the individual responds poorly to conventional treatment when having advanced age or osteoporosis.[17,18,19]

There are many studies that have proposed the administration of GH for the treatment of osteoporosis, since it has been proven that it is able to accelerate bone remodelling both in patients with adult GH deficiency and in healthy elderly.[20,21]Still with better results given in combination with Parathyroid Hormone (PTH).[22] The administration of GH alone or combined with estrogens, is also able to reverse vertebral osteopenia in ovariectomized rats.[23] Also, in women in which osteoporosis is a serious problem, this beneficial effect has been observed, since the administration of GH in postmenopausal osteopenic women increases bone mineral density and markers of bone formation within 7 days of treatment. Similarly, replacement therapy for 12 months[24] or 24 months[25] in adults with GH deficiency also increases bone mineral density and remodelling markers. There is a recent and very important paper which followed menopausal women with osteoporosis for ten years, in which a very important effect has been observed with the treatment with GH.[26]All this suggests that, probably in the near future, GH will be used alone or in combination with other drugs forthe treatment of osteoporosis.

Similarly, several studies have shown that local administration of GH at the time of surgery favours the transcortical osseointegration of titanium implants in rabbits, both osteoporotic and non-osteoporotic,[27] allowing a glimpse of its use in the field of dentistry.

Materials and methods

209 implants were placed using local GH in the surgical wound in 58 patients (31 men and 27 women) and 193 implants without GH in 82control patients, (39 men and 43 women)for comparison, in people between 35 and 82 years of age.

Threaded implants of various diameters and lengths were used, both in external and internal connection, a single dose in local application in the alveolus of GH 2mg implantation (Somatropin - 10mg/1.5ml). In 29 implants with more extensive surgeries, systemic GH of 1mg (Somatropin 10mg/1.5mg) per day was administered 10 days before surgery and for 20 days afterwards.

When there was a need for bone growth or filling of empty spaces, agglomerated regeneration biomaterials were applied in a Plasma Rich in Growth Factors (PRGF) autologous fibrin clot. All the implants were placed with an open flap technique on the first time and on the second time, with an exposure of the implant, with the punch technique.

All patients signed an informed consent, both in the placement of simple implants (without GH) and in those in which GH was administered.They were all informed about the benefits and the possible risks of the compassionate off label use of GH.

For the evaluation of the results, the simple apical radiography was used, always with the same apparatus, film positioner, the same intensity to be as accured and standardized as possible, assessing the osseointegration by the neoformation and bone density around and between the turns of the implant's threading. Osseointegration occurs when the image shows a similar density to normal bone, with absence of hypodense zones; some hyperdense areas due to ankylosing might be present.

Statistical analysis

A statistical analysis was carried out using the "t-student modified" adapted to situations of non-equality of underline sub population variances. The statistical analysis of the comparison of the three averages of the groups, by resorting to a model of analysis of variance to a factor (age) is not revealed feasible due to the small number of observations in the group over 70 years old.

Results

When GHadministration in single or multiple implant placement surgeries is used, the healing and osseointegration process is faster in time, (median time, 82 days vs 140 days) as compared to controls in which GHwas not used, as shown in Table 1.

In each of the Box Plot, Figure 1, three cases of "outliers" patients are identified in the right parts of the data distribution. The clinical study of the patients that generate these observed cases, revealed that there were other factors that could explain the relatively high time margin between placement and osseointegration.

For the other patients, no deterministic factors were found to explain the remaining extreme values of time, observed between placement and osteointegration.

Consequently, robust estimates of average time and variance of the sample were obtained, between the various times of osseointegration, recalculating mean time and variance "truncated to the right" by excluding the three outlier cases, in each of the samples under study.

The exploratory analysis of Table 2 indicates that

1. The use of GH decreases the average time between implant placement and osseointegration.
2. There is a visible discrepancy in the evaluation of the absolute dispersion of the data in each of the situations
3. In each case the coefficient of asymmetry is less than one, which means, the data has a high degree of symmetry.

It can be seen that there is a very significant influence of the use of GH in the reduction of the osseointegration time of the implant ($P< 10^{-5}$),Table 3.

Comparative statistical study of ages with application of GH

The previous analysis of these data seems to reveal a small but not significant difference in the effect of age on the mean times between placement and osseointegration when using GH, Figure 2. And the inter-quartile range is quite similarly for the tow first age group.

From Table 4, it can be concluded that there are nodifferences in the degree of reduction of the osseointegration time by GH in the different age groups, population of up to 50 years μ_1 and μ_2 in the group of 51 to 70 years.

Additionally, Table 5shows the significant reduction of osteointegration times inall ages groups patients treated with GH, as compared whit those without GH, around 60 days.

Citation: Rego A, Tresguerres JAF, Tallon JM, et al. Application of growth hormone to reduce osseointegration time in dental implants. *J Dent Health Oral Disord Ther.* 2020;11(4):116–124. DOI: 10.15406/jdhodt.2020.11.00529

Table 1 Statistics order for the two osseointegration times

	Nr.	Min. days	Max. days	1st Quartile	Median	3rdQuartile	Interquartile range
With GH	209	43	147	66	82	92	26
Without GH	193	66	271	126	140	1705	445

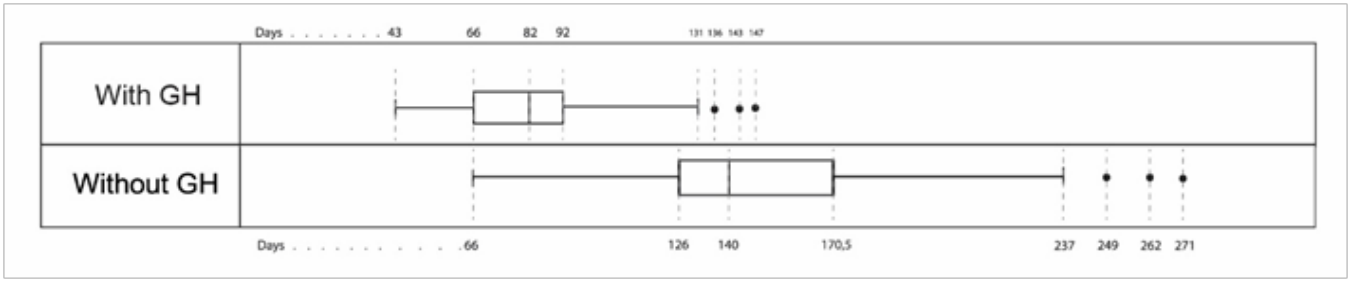

Figure 1 Box plot of the two situations, with or without GH-comparative analysis.

Table 2 Robust estimates mean time, variaance and asymmetry coefficient

	n	Truncated average time	Truncated variance	Coefficient of skewness
With GH	197	806	3103	4
Without GH	185	1456	11048	6

Table 3 Robust estimate of mean time, standard errors and p value comparative statistaical study of mean time with and without GH

	n	mean TSE	p-value
With GH	197	80.6±1.3	
Without GH	185	145.6±2.4	
Difference		65±2.7	$p<10^{-5}$

Table 4 Statistics of osseointegration times

Age	Nr. obser	Min. Time	Max. Time	1st Quartile	Median	3rdQuartil	Mean±Se
<=50	59	55	120	64	75	91	77.9±1.9
From 51 to 70 years	120	43	128	66	84	93	81.8±1.7
>70 years	18	64	121	68	69	93	78.3±4.3
Total	197						

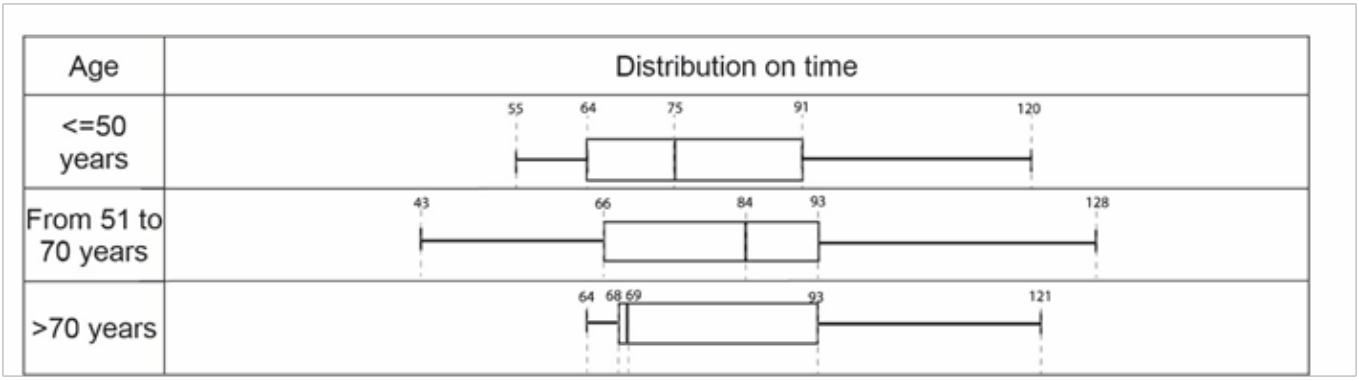

Figure 2 Box-plot of the three age groups, with GH-comparative analysis.

Citation: Rego A, Tresguerres JAF, Tallon JM, et al. Application of growth hormone to reduce osseointegration time in dental implants. *J Dent Health Oral Disord Ther.* 2020;11(4):116–124. DOI: 10.15406/jdhodt.2020.11.00529

Table 5 Comparison of age with or without GH

	Age					
	<=50 years		From 51 to 71 years		> 70 years	
Treatment	n1	Mean±Se	n2	Mean±Se	n3	Mean±Se
with GH	59	77.9±1.9	120	81.8±1.7	18	78.3±4.3
Without GH	81	147.6±4.6	86	144.9±3.4	18	134.3±2.3
Difference		69.7±2.9 ($P10^{-5}$)		63.1±3.7 ($p10^{-5}$)		56±4.8 ($p<5X10^{-4}$)

Statistical comparative study of gender with application of GH

When we compare women with less than or equal to 50 years of ageand women over 50 years of age on the action of GH taking into consideration that it is generally in this age group that hormonal alterations are likely to influence osseointegration, the data points to a non-significantdifference in the time of osseointegration in these age groups when subjected to treatment with GH, (p-value >0,10), Table 6.

Placement of implants with not enough bone

Placing the implant in the bone cortex, but having aminimum amount of marrow, even if only on one side, the bone growth was verified both on the side that had marrow and on the other side, having bone growth both in thickness as in height (Figure 3). Where was placed 4 implants.

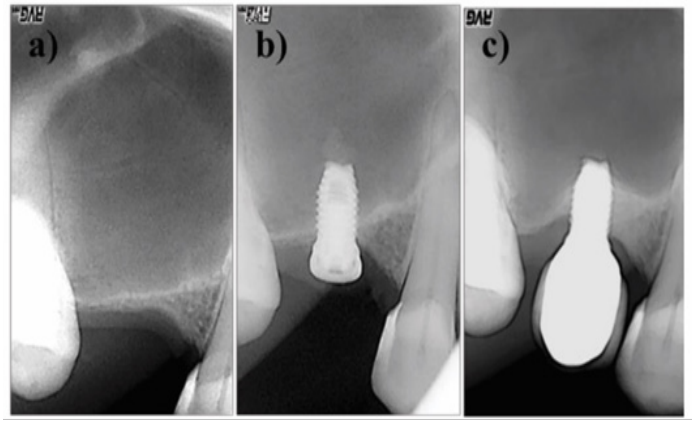

Figure 3 Bone growth around the implant with the use of GH.

Recovery of implants with Peri Implantitis

In conjunction with biomaterials and PRGF, bone neoformation was verified, whether in growth or in vertical filling (Figure 4) around an implant with Peri Implantitis. Surgeries were performed on 6 implants.

Treatments with several stages

In the 1st stage GH was not used and external scarring occurred in about 1 month and complete osseointegration at the end of 6 months, in the 2nd stage local GH was used and external scarring occurred in close to one week and complete osseointegration at the end of 2 months, in the 3rd stage local GH was used in the surgery and systemic GH (10 days before the surgery and 20 days after it , (in the case of very extensive surgery of the upper hemiarcade with extractions and immediate placement of implants) external healing occurred in two weeks and osseointegration in 3 months (Figure 5). Twenty-nine implants were placed in 5 patients.

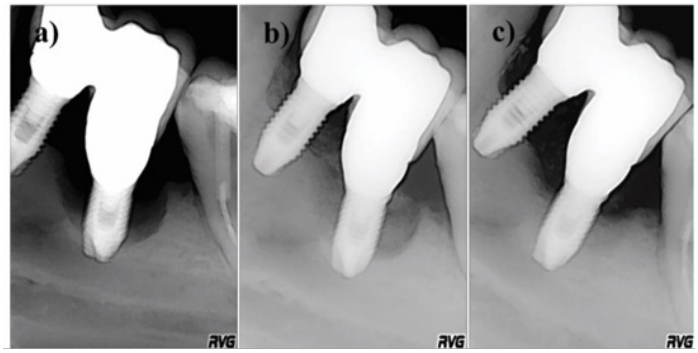

Figure 4 Recovery of implant with periimpantitis.

Dental extraction and implant placement

External scarring and complete osseointegration is faster even when there is no complete closure of the wound due to lack of gingiva. There were 13 implants placed.The scarring and osseointegration is much slower (previous statistics) and vertical bone growth is not verified in premises with less bone, sometimes causing serious problems (Figure6), and even when it has been years since the placement of the implants in areas of less bone, there is no bone neoformation or complete osseointegration (Figure 7).

Placement of implants with infectious lesions

GH treatment very quickly accelerates the growth and developmentof old lesions that were latent or hidden, generating a rapid rejection of the implant with bone loss in the corresponding space. This happened in 5 implants of 3 patients.

Discussion

It is known that from the fourth decade of life onwards there is a decrease in GH levels, making the majority of elderly people physiologically GHdeficient which is associated with muscle weakness, atherosclerosis, osteoporosis, etc.[28]

GH and mainly IGF-I promotes bone growth, in a dose-dependent manner, by direct stimulation of metaphyseal chondrocytes.[29,30]When GH is administered locally, it takes action on the chondroprogenitor cells, promoting their differentiation and proliferation, whereas IGF-I acts on already mature chondrocytes, stimulating both their

Citation: Rego A, Tresguerres JAF, Tallon JM, et al. Application of growth hormone to reduce osseointegration time in dental implants. *J Dent Health Oral Disord Ther.* 2020;11(4):116–124. DOI: 10.15406/jdhodt.2020.11.00529

proliferation and matrix synthesis.[31] IGF-I and II are the most abundant growth factors in bone and are able to stimulate the proliferation and differentiation of osteoblasts,[32]At the same time as they enhance collagen synthesis and inhibit its degradation. The actions of these IGFs are regulated to a certain extend by the Insulin-Like Growth Factor-Binding Protein 3 (IGFBP-3), whose in vivo production is under the direct control of GH.[31]

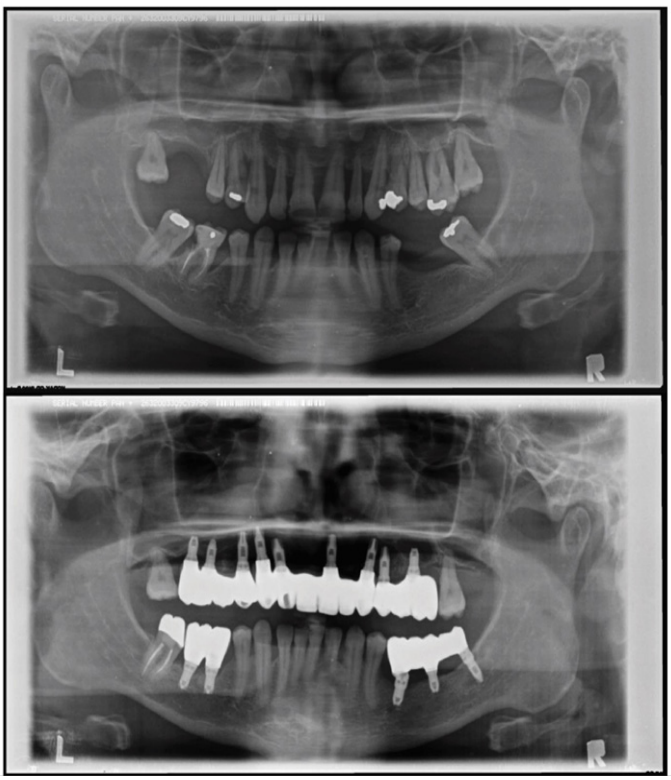

Figure 5 Use of GH at various stages of treatment.

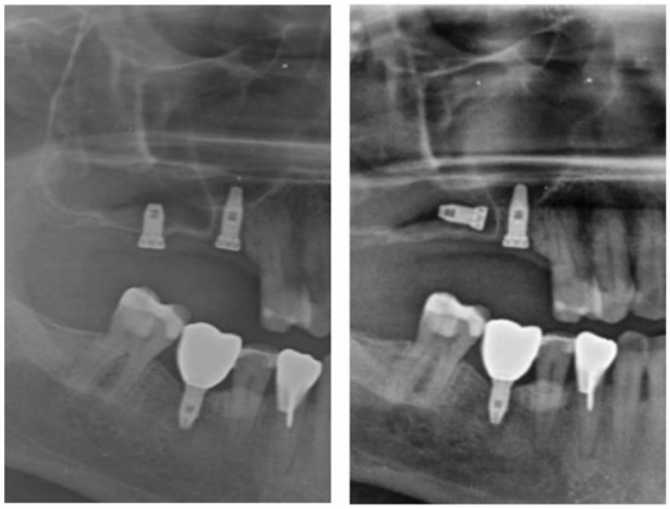

Figure 6 Problems when implants are placed with lack of bone and without GH.

It has been often observed that both resorption and bone formation show high values in young subjects with high levels of GH,[8,21]concluding that GH activates bone remodelling, by increasing the differentiation of osteoclasts and osteoblasts, and the same can be obtained with the treatment with GH.

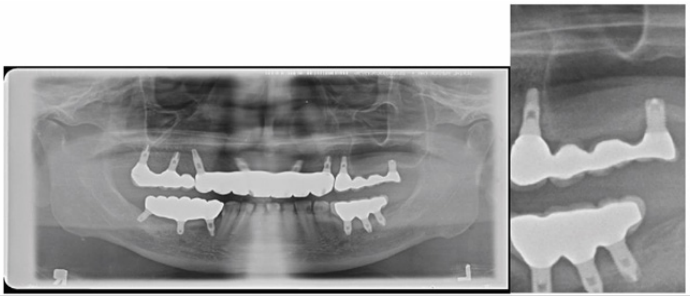

Figure 7 There is no new bone formation without GH.

GH is very important in the longitudinal growth of bone acting on the growth plates and stimulating the differentiation and proliferation of osteoblasts. However, in the osseointegrationprocess of titaniumimplants placed in situations of osteopenia/osteoporosis, its effects haven't been studied much. The systemic application of the hormone on bone physiology has been widely evaluated in animals[33–39]with satisfactory results in fracture healing, bone distraction, situations of osteopenia and osteoporosis, and in GH deficiencies.

GH is an anabolic hormone that stimulates not only osteoblasts proliferation and differentiation,[40] but also osteoclasts,[41] thus, GH is capable of stimulating bone turnover.[8] GH is also able to enhance bone fracture repair, both in young[16,42]and old animals,[13] when given systemically. Hedner et al.[43]applied GH in jaws of rats with bone defects, demonstrating that there was an increase in bone formation with application of local GH for 4 weeks. In case of fractures or osteotomy, GH has shown also positive effects on the repair, as proven by Cacciafesta et al.,[44]which have shown that in the animals treated with GH there is a significant increase almost doubling the volume of newformed bone as compared with the placebo group,Actually GH administration could be effective in the consolidation of hip fractures in humans.[45]

Several authors have found an increase in cortical thickness after treatment with GH not only in normal rats,[46] but also in aged rats,[15,16]in ovariectomized rats,[23] and also in old ovariectomized rats[35,47]Accordingly, with Andreassen and Oxlund,[18] GH treatment does not seem to have effects on trabecular bone in absence of linear growth.

In addition, when GH administration has been associated with other hormones, such as PTH, additive effects on vertebrae have been observed in old ovariectomized rats, in which an increase in both cortical and trabecular mass was also seen. GH seems to induce a subperiosteal apposition whereas PTH induces an endosteal and cancellous deposition.[17]

Elena Martin Monge in her study in 2009[48] valued the local application of growth hormone on the process of osseointegration of a titanium implant of threaded surface in the tibia of ovariectomized rabbits submitted toa hypo-calcic diet (experimental osteoporosis) and in the tibia of healthy rabbits.The "impulse effect" of the application of local GH would produce an initial acceleration of intracortical remodelling, starting with resorption and continuing with bone formation. Following the model proposed by Tresguerres et al (In which 4 U.I. of rhGH were applied in the surgical process of inserting titanium plates, and in other casesbicortical screw implants).Local

application of GH increases osteoid tissue formation and bone growth in animal models.[49–54]

Oxlund & Andreassen[55]injected for 14 days, 2 or 20mg/Kg/day of rhGH on the surface of the diaphysis of the intact tibia of ten month old rats and ina fracture healing process.After 21 days an increase in bone load capacity and strength was found, as well as in the size and volume of the fracture callus; after 98 days, the ability of bone loading, versus a control group, remained increased.

Table 6 Statistical comparative of two female age groups with GH

	Age	N. of observations	Mean±Se	Mean difference±Se
Female with GH	<=50 years	39	80.1±2.0	2.8±2.9 (p-value >0,10)
	From 51 to 70 years	49	77.3±2.1	

In humans, the application of GH activates bone remodelling units, increasing the differentiation of osteoclasts and osteoblasts, and once activated, they go through a "complete" remodelling cycle[56]. It is known that the effect of treatment with GH in adults, not only markedly accelerates bone turnover by increasing the activation of new bone metabolic units, but also stimulates periosteal bone apposition, which entails the enlargement of the bone width.

The combination of these two different effects can, simultaneously, increase both the bone mineral content and the bone projection area.

The systemic application of the hormone has been studied in humans[56–60] with satisfactory results in fracture healing, osteodistraction, situations of osteopenia and osteoporosis, and GH deficiencies.Already in 1998, Wüster et al.[56]carried out a review on the application of GH in healthy individuals; in patients with osteoporosis, in elderly people and in cases of GH deficiency (GHD) (in which the bone mass is decreased). They observed that after administering GH to the patients with GHD there was an increase in bone turnover (remodulation), mineral density initially decreased, during the first year of treatment, due to the increase in bone remodelling.

In the same way as other authors[52–54]the local application of lyophilized GH was carried out directly on the implant bed, soaking with the blood clot. In addition, since administration is in a single dose, side effects would not occur.

Data obtained in the present study are consistent with previous ones showing improvements achieved by GH administration regarding biomechanical properties of the bone.[18,35,46]

If we try to investigate the pathways by which GH is able to stimulate bone formation, it has been demonstrated that it is able to stimulate the differentiation from mesenchymal stem cells towards the osteoblast lineage, even in aged animals.[61]

In addition, GH seems to modulate the activity of Runx2, a transcription factor that is needed for chondrocyte maturation and osteoblasts differentiation.[62]

GH is able to enhance osteoclast activity,[63]but it is also capable to increase osteoprotegerin (OPG), an endogenous inhibitor of osteoclast differentiation and activation. The effect is exerted through its binding to RANKL, preventing its union to RANK and leading to a decrease of osteoclastogenesis.[64]

The age related decline in GH levels observed with aging, could lead to reduced plasma calcium levels, resulting in a compensatory increase of PTH, leading to an increase in bone resorption. Administration of GH could reduce the secondary hyperparathyroidism, since it can increase the intestinal calcium reabsorption,[65] increasing calcium availability by the bone. Furthermore, GH administration could also restore PTH levels, as demonstrated by Joseph et al.[66] in postmenopausal women with osteoporosis.

GH is able to stimulate angiogenesis,[67,68]which is the first step in the osteogenic process. In another study from our group, GH was able to reduce in the aorta of old animal's medium layer cross-sectional area that showed an increase, and to increase its relaxing responses which showed a reduction.[69]

Also, Landin-Wilhelmsen et al.,[57] did find significant differences in BMD or BMC at the end of the study with 80 osteoporotic women who had previously received Hormone Replacement Therapy, and who were given for 18 months 1 u/day of GH, or 2.5U/day. They concluded that GH produced a delayed, prolonged and dose-dependent effect in postmenopausal osteoporosis, and that it could be used as an anabolic agent in the treatment of this disease.More recently Krantz et al.[26]demonstrated also very positive effects of GH treatment for 10 years in osteoporosis of menopausal women.

In the reviewed papers, we have not found any study of the use of GH in osteoporotic subjects in which implants were placed with any type of surface covered.

In the scant literature found on local application of GH, this is done in the form of an osmotic pump or local subcutaneous injection[43,55,70]and when used with implants, the authors consulted impregnated the surface of the implant, either titanium or ceramic, with the GH[49,51,71]Downes et al.[50]used a polymethylmethacrylate cement for local application, obtaining good results, but in their case, titanium implants were not used.

The safety of potential GH administration in aged people is controversial. Since the very important paper of Rudman et al.[72]many elderly people have been treated with GH, mainly in the USA, for several "off label" indications including aging itself, and no report to our knowledge about any increase in cancer appearance has appeared until today.However, some side effects have been detected with GH treatment in adults as an increased risk for carpal tunnel syndrome, fluid retention or insulin resistance, but only when high doses of the hormone were used.[73,74]

Conclusion

In this work carried out in dental implantology, it can undoubtedly be concluded that the use of GH is beneficial in healing and osseointegration, both locally and systemically, as demonstrated in the statistical study presented.In addition, the analysis by age groups showed a small difference, statistically significant, with slightly longer average times between implantation and osseointegration in the age group of 50 to 70 years.

In the comparative analysis of implant placement with or without GH, there is great evidence for the three age groups regarding the reduction of osseointegration times in patients treated with GH (p-value close to zero).In addition, a very similarly central spread of osseointegration time is verified for the first two age groups (patients aged <50 years and between 50 and 70 years). Which means, its application would be more advisable in the age group over 50 years.

When specifically studying women of over 50 years old, we observed that without GH there is a significant difference between the times of osseointegration and when GH is used, that difference does not exist, because the use of GH will compensate for its deficit that is verified from the fourth decade of life making its use more beneficial in this group of people.

However, it was also observed in this study for Clinical practice that with previous injuries the use of GH also exacerbated them more quickly. In the remaining situations there are indications of the beneficial influence of the GH in decreasing the average time of osseointegration, but the sample is very small in statistical terms, to do it with reliability, lacking studies that can be more statistically concluding.

Funding

None.

Acknowledgments

None.

Conflicts of interest

None.

References

1. Sattler FR. Growth Hormone in the aging male. *Best Pract Res Clin Endocrinol Metab*. 2013;27(4):541–555.
2. Heutling D, Lehnert H. Hormone therapy and anti-aging: is there an indication? Internist(Berl). 2008;49(5):570–579.
3. Tresguerres JAF, Kireev R, Tresguerres AF, et al. The endocrine system during aging. In: Soler PA, and Mañas LR. Treatise on geriatric medicine: fundamentals of health care for the elderly. 1st ed. Spain: S.L.U; 2014. pp. 125–133.
4. Ariznavarreta C, Garcia AP, Salazar V, et al. Growth Horm one and Aging. *Treballs de la SCB*. 2005;56:225–235.
5. de la Calzada-Álvarez MD. Sleep modifications in aging. *Rev Neural*. 2000;30(6):577–580.
6. Tresguerres JAF. Anti-aging medicine in the 21st century: role of the endocrine system; 2008.
7. Díez JJ, Cordido F. Benefits and risks of growth hormone in adults with growth hormone deficiency. *Medicina Clínica*. 2014;143(8):354-359.
8. Brixen K, Nielsen HK, Mosekilde L, et al. A short course of recombinant human growth hormone treatment stimulates osteoblasts and activates bone remodelling in normal human volunteers. *J Bone Miner Res*. 1990;5:609–618.
9. Riggs BL, Randall RV, Wahner HW, et al. The nature of the metabolic bone disorder in acromegaly. *J Clin Endocrinol Metab*. 1972;34(6):911–918.
10. Degerblad M, Bengstsson BA, Bramnert M, et al. Reduced bone mineral density in adults with GH deficiency: Increase bone turnover during 12 months of GH substitution therapy. *Eur J Endocrinol*. 1995;133(2):180–188.
11. Rosen T, Hansson T, Granhed H, et al. Reduced bone mineral content in adult patients with growth hormone deficiency. *Acta Endocrinol (Copenh)*. 1993;129(3):201c206.
12. Guicheux J, Gauthier O, Aguado E, et al. Human growth hormone locally released in bone sites by calcium-phosphate biomaterial stimulates ceramic bone substitution without systemic effects: a rabbit study. *J Bone Miner Res*. 1998;13(4):739–748.
13. Bak B, Andreasen TT. The effect of growth hormone on fracture healing in old rats. *Bone*. 1991;12(3):151–154.
14. Nielsen HM., Bak B, Jorgensen PH, et al. Growth hormone promotes healing of tibial fractures in the rat. *Acta Orthop Scand*. 1991;3:244–247.
15. Andreassen TT, Jorgensen PH, Flyvbjerg A, et al. Growth hormone stimulates bone formation and strength of cortical bone in aged rats. *J Bone Miner Res*. 1995;10(7):1057–1067.
16. Bak B, Jorgensen PH, Andreasen TT. Increased mechanical strength of healing rat tibial fractures treated with biosynthetic human growth hormone. *Bone*. 1990;11(4):233–239.
17. Andreassen TT, Oxlund H. Additive anabolic effects of growth hormone and parathyroid hormone on vertebral body cortical and cancellous bone in old ovariectomized rats. *J Bone Miner Res*. 1996;11:S457–S460.
18. Andreassen TT, Oxlund H. The effects of growth hormone on cortical and cancellous bone. *J Musculoskel Neuron Interact*. 2001;2(1):49–58.
19. Agnus Dei D, Gentilletta R. GH and IGF1 as therapeutical agents for osteoporosis. *J Endocrinol Invest*. 2005;28:32–36.
20. Brixen K, Kassem M, Eriksen EF, et al. Growth hormone and adult bone remodelling: The potential use of GH in the treatment of osteoporosis. *J Pediat. Endocrinol*. 1993;6:65–71.
21. Marcus R., Butterfield G, Holloway L, et al. Effects of short term administration of recombinant growth hormone to elderly people. *J Clin Endocrinol Metab*. 1990;70:9–527.
22. Mosekilde L, Tornving L, Thomsen JS, et al. Parathyroid hormone and growth hormone have additive or synergetic effect when used as intervention treatment in ovariectomized rats with established osteopenia. *Bone*. 2000;26(6):643–651.
23. Eschen C, Andreasen TT. Growth hormone normalizes vertebral strength on ovariectomized rats. *Calciffied Tissue Int*. 1995;57:392–396.
24. Brixen K, Kassem M, Nielsen HK., et al. Short-term treatment with growth hormone stimulates osteoblastic and osteoclastic activity in osteopenic postmenopausal women: A dose response study. *J Bone Miner Res*. 1995;10(12):1865–1874.
25. Johannsson G, Bengtsson BA. Growth hormone and the acquisition of bone mass. *Hormone Research*. 1997;48(suppl 5):72–77.
26. Krantz E, TrimpouP, Landin-Wilhelmsen K. Effect of GH treatment on fractures and quality of life in postmenopausal osteoporosis: A 10 year follow up study. *J Clin Endocrinol.Metab*. 2015;100(9):3251–3259.
27. Fernández-Tresguerres I. Influence of growth hormone on osseointegration. Complutense University of Madrid; 1999.
28. Toogood AA, O´Neil PA, Shalet SM. Beyond the somatopause: growth hormone deficiency in adults over the age of 60 years. *J Endocrinol Metab*. 1996;81:460–465.

29. Isaksson OGP, Janson JO, Gausse IAM. Growth hormone stimulates longitudinal bone growth directly. *Science*. 1982;216(4551):1237–1239.

30. Russell SM, Spencer EM. Local injections of human or rat growth hormone or of purified human somatomedin-C stimulate unilateral tibial epiphyseal growth in hypophysectomized rats. *Endocrinology*. 1985;116(6):2563–2568.

31. Carrascosa A, Audi L. The growth plate. Hormonal regulation of their differentiation. In: Moreno Esteban B, Tresguerres JAF, eds. Growth retardation. 2nd ed. Madrid: Díaz de Santos SA; 1996. pp. 119–126.

32. Emesto Canalis, Michael Centrella, Warner Burch, et al. Insulin-like growth factor I mediates selective anabolic effects of parathyroid hormone in bone cultures.*The Journal of clinical investigation*. 1989;83(1):60–65.

33. Bail HJ, Kolbeck S, Krummrey G, et al. Systemic application of growth hormone for enhancement of secondary and intramembranous fracture healing. *Horm Res*. 2002;58 Suppl 3:39–42.

34. Andreassen TT, Oxlund H. The influence of combined parathyroid hormone and growth hormone treatment on cortical bone in aged ovariectomized rats. *J Bone Miner Res*. 2000;15(11):2266–2275.

35. Mosekilde L, Thomsen JS, Orhii PB, et al. Growth hormone increases vertebral and femoral bone strength in osteopenic, ovariectomized, aged rats in a dose-dependent and site-specific manner. *Bone*. 1998;23(4):343–352.

36. Kidder LS, Schmidt IU, Evans GL, et al. Effects of growth hormone and low dose estrogen on bone growth and turnover in long bones of hypophysectomized rats. *Calc Tissue Int*. 1997; 61:327–335.

37. Verhaeghe J, van Bree R, Van Herck E, et al. Effects of recombinant human growth hormone and insulin-like growth factor-I, with or without 17 beta-estradiol, on bone and mineral homeostasis of aged ovariectomized rats. *J Bone Miner Res*. 1996;11(11):1723–1735.

38. Ortoft G, Oxlund H. Qualitative alterations of cortical bone in female rats after long-term administration of growth hormone and glucocorticoid. *Bone*. 1996;18(6):581–590.

39. Mann DR, Rudman CG, Akinbami MA, et al. Preservation of bone mass in hypogonadal female monkeys with recombinant human growth hormone administration. *J Clin Endocrinol Metab*. 1992;74:1263–1269.

40. Ohlsson C, Bengtsson B-A, Isaksson OGP, et al. Growth hormone and bone. *Endocr Rev*. 1998;19:55–79.

41. Nishiyama K, Sugimoto T, Kaji H, et al. Stimulatory effect of growth hormone on bone resorption and osteoclast differentiation. *Endocrinology*. 1996;137(1):35–41.

42. Nielsen HM, Bak B, Jorgensen PH, et al. Growth hormone promotes healing of tibial fractures in the rat. *Acta Orthop Scand*. 1991;62(3):244–247.

43. Hedner E, Linde A, Nilsson A. Systemically and locally administered growth hormone stimulates bone healing in combination with osteopromotive membranes: an experimental study in rats. *J Bone Miner Res*. 1996;11(12):1952–960.

44. Cacciafesta V, Dalstra M, Bosch C, et al. Growth hormone treatment promotes guided bone regeneration in rat calvarial defects. *Eur J Orthod*. 2001;23(6):733–740.

45. Yang S, Cao L, Cai S, et al. A systematic review of growth hormone for hip fractures. *Growth Hormone & IGF Research*. 2012;22(3-4):97–101.

46. Jorgensen PH, Bak B, Andreassen TT. Mechanical properties and biochemical composition of rat cortical femur and tibia after long-term treatment with biosynthetic human growth hormone. *Bone*. 1991;12(5):353–359.

47. Andreassen TT, Melsen F, Oxlund H. The influence of growth hormone on cancellous and cortical bone of the vertebral body in aged rats. *J Bone Miner Res*. 1996;11(8):1094–1102.

48. Martín Monge, Elena María. Experimental osteoporosis: effect of local administration of growth hormone on the peri-implant tissue. 2009.

49. Guicheux J, Gauthier O, Aguado E, et al. Growth hormone-loaded macroporous calcium phosphate ceramic: in vitro biopharmaceutical characterization and preliminary in vivo study. *J Biomed Mater Res*. 1998;40:560–566.

50. Downes S, Wood DJ, Malcolm AJ, et al. Growth hormone in polymethylmethacrylate cement. *Clin Orthop Relat Res*. 1990;252:294–298.

51. Blom EJ, Verheij JG, de Blieck-Hogervorst JM, et al. Cortical bone ingrowth in growth hormone-loaded grooved implants with calcium phosphate coatings in goat femurs. *Biomaterials*. 1998;19(1–3):263–270.

52. Tresguerres IF, Clemente C, Donado M, et al. Local administration of growth hormone enhances periimplant bone reaction in an osteoporotic rabbit model. *Clin Oral Impl Res*. 2002:13(6);623–630.

53. Tresguerres IF, Blanco L, Clemente C, et al. Effects of local administration of growth hormone in peri-implant bone: an experimental study with implants in rabbit tibiae. *Int J Oral Maxillofac Implants*. 2003;18:807–11.

54. Tresguerres IF, Alobera MA, Baca R, et al. Histologic, morphometric, and densitometric study of peri-implant bone in rabbits with local administration of growth hormone. *Int J Oral Maxillofac Implants*. 2005;20:193–202.

55. Andreassen TT, Oxlund H. Local anabolic effects of growth hormone on intact bone and healing fractures in rats. *Calcif Tissue Int*. 2003;73(3):258–264.

56. Wüster C, Harle U, Rehn U, et al. Benefits of growth hormone treatment on bone metabolism, bone density and bone strength in growth hormone deficiency and osteoporosis. *Growth Horm IGF Res*. 1998;8 Suppl A:87–94.

57. Landin-Wilhelmsen K, Nilsson A, Bosaeus I, et al. Growth hormone increases bone mineral content in postmenopausal osteoporosis: a randomized placebo-controlled trial. *J Bone Miner Res*. 2003;18(3):393–405.

58. Nilsson AG. Effects of growth hormone replacement therapy on bone markers and bone mineral density in growth hormonedeficient adults. *Horm Res*. 2000;54 Suppl 1:52–57.

59. Baum HB, Biller BM, Finkelstein JS, et al. Effects of physiologic growth hormone therapy on bone density and body composition in patients with adult-onset growth hormone deficiency. A randomized, placebo-controlled trial. *Ann Intern Med*. 1996;125(11):883–890.

60. Thoren M, Soop M, Degerblad M, et al. Preliminary study of the effects of growth hormone substitution therapy on bone mineral density and serum osteocalcin levels in adults with growth hormone deficiency. *Acta Endocrinol (Copenh)*. 1993;123 Suppl 2:41–43.

61. Bolamperti S, Guidobono F, Rubinacci A, Villa I. The Role of Growth Hormone in Mesenchymal Stem Cell Commitment. *Int J Mol Sci*. 2019; 20(7):5264–5277.

62. Komori T. Regulation of proliferation, differentiation and functions of osteoblasts by Runx2. *Int J Mol Sci*. 2019;20:1694–1705.

63. Nishiyama K, Sugimoto T, Kaji H, et al. Stimulatory effect of growth hormone on bone resorption and osteoclasts differentiation. *Endocrinology*. 1996;137(1):35–41.

64. Mrak E, Villa I, Lanzi R, et al. Growth hormone stimulates osteoprotegerin expression and secretion in human osteoblast-like cells. Journal of Endocrinology. 2007;192:639–645.

65. Chipman JJ, Zerwekh J, Nicar M, et al. Effect of growth hormone administration: reciprocal changes in serum 1 alpha,25-dihydroxyvitamin D and intestinal calcium absorption. *J Clin Endocrinol Metab*. 1980;51(2):321–324.

66. Joseph F, Ahmad AM, Ul-Haq M, et al. Effects of Growth Hormone Administration on Bone Mineral Metabolism, PTH Sensitivity and PTH Secretory Rhythm in Postmenopausal Women with Established Osteoporosis. *J Bone Miner Res*. 2008;23(5):721–729.

67. Castillo C, Salazar V, Ariznavarreta C, et al. Effect of melatonin administration on parameters related to oxidative damage in hepatocytes isolated from old Wistar rats. J Pineal Res 2005;38(4):240–246.

68. Ramirez-Fernandez MP, Calvo-Guirado JL, de-Val JE, et al. Melatonin promotes angiogenesis during repair of bone defects: a radiological and histomorphometric study in rabbit tibiae. *Clin Oral Invest*. 2013;17(1):147–158.

69. Castillo C, Cruzado M, Ariznavarreta C, et al. Effect of recombinant human growth hormone administration on body composition and vascular function and structure in old male Wistar rats. *Biogerontology*. 2005;6(5):303–312.

70. Theyse LF, Oosterlaken-Dijksterhuis MA, van Doorn J, et al. Expression of osteotropic growth factors and growth hormone receptor in a canine distraction osteogenesis model. *J Bone Miner Metab*. 2006;24(4):266–273.

71. Downes S, Clifford CJ, Scotchford C, et al. Comparison of the release of growth hormone from hydroxyapatite, heat-treated hydroxyapatite, and fluoroapatite coatings on titanium. *J Biomed Mater Res*. 1995;29:1053–1060.

72. Rudman D, Feller AG, Nagraj HS, et al. Effects of human growth hormone in men over 60 years old. *N Engl J Med*. 1990;323(22):1–6.

73. Liu H, Bravata DM, Olkin I, et al. Systematic review: the safety and efficacy of growth hormone in the healthy elderly. *Ann Intern Med*. 2007;146:104–115.

74. Locatelli V, Bianchi VE. Effect of GH/IGF-1 on bone metabolism and osteoporosis. *Int J Endocrinol*. 2014;23:235060.