The role of growth hormone in bone response for implant treatment. Experimental study using presenile animals

C. Clemente de Arriba [1], R. Rodríguez-Tor res[1], I. Fernández-T resguerres[2], J.A.F. Tresguerres[3] and L. Gómez-Pellico [1]

1- *Morphological Sciences and Surgery Department, Medical School, University of Alcalá, 28871 Alcalá de Henares, Madrid, Spain*

2- *Medicine and Bucofacial Surgery Department, Odontology School, Complutense University, Madrid, Spain*

3- *Department of Physiology, Medical School, Complutense University, Madrid*

SUMMARY

Many factors can modify osteointegration, among them the subject's age and the possibility of using hormone treatment to improve the phenomenon. In the present study we used a histological technique that permits study not only of interface, but also bone response in distant areas. Growth hormone (GH) was used topically in aged animals that had received a titanium plate as an implant in their tibiae. A similar degree of osteointegration was observed in both, the control and treated groups. Distantly located bone issue urderwent certain modifications in its osteocyte lacunae in the treated group but not in the control group.

Key Words: Growth hormone – Bone – Bone-implant interface – Osteointegration – Age

INTRODUCTION

One of the main disadvantages of uncemented titanium protheses is the difficulty in achieving an immediate fixation. This can be lead to micromovements that generate fibrous tissue around the bone-implant, thus hindering osteointegration (Lind 1996a, b). To overcome this drawback, other substances have been considered with a view to promoting rapid growth of the osseous tissue at the interface. Currently, many studies are being carried out with the aim of improving the different types of coatings used to cover the metallic implant surface. In recent years, growth factors have been incorporated into the search for improved osteointegration. Urist first introduced the concept of osseous formation stimulating proteins (BMP) in 1965 and subsequently several families of such proteins have been characterized.

Later, other agents with osteogenic capability have appeared, among them the growth factors. It has been demonstrated that the utilization of these factors improves osseous healing in several animal models, and their good performance in the osteointegration in unloaded hydroxyapatite coated implants has been reported.

This can benefit patients who show difficulty in obtaining a good osseous response, as happens in the elderly, who have been shown to have a lower osteointegration capacity than young people due to, among other factors, to senile osteoporosis (Hollister, 1996; Murai, 1996).

Many works have confirmed the improvement in osseous mass and muscle tissue in elderly people treated orally with GH for six months (Papadakis, 1996). However, no improvement in their functional capacities has been described. Recent studies have highlighted the risk of the development of tumor process in people receiving hormonal treatment and its use is therefore debatable (Rosen, 2000).

One way of improving osteointegration (Siegel, 1999) with growth hormone treatment, with no undesirable effects, is to administer it

Submitted: July 2, 2001
Accepted: August 30, 2001

Correspondence to:
Dr. C. Clemente de Arriba. Dpto. de Ciencias Morfológicas y Cirugía, Facultad de Medicina, Universidad de Alcalá, Ctra. Madrid-Barcelona Km. 33.600, 28871 Alcalá de Henares, Madrid, Spain. Phone: +34-91-8854879; Fax: +34-91-8854593.
E-mail: celia.clemente@uah.es

only at the site of implantation. In this sense, some studies have reported the use of GH adsorbed in the biomaterial with a good response, although the results are variable, depending on the dose, the animal, and the coating used (Lind, 1996a, b; Carpenter, 1992; Downes, 1990).

Despite these latter studies, however, the literature consulted by us contains few references to a direct topical treatment with growth hormone. Thus, for example, in 1989 Lynch used growth factor in gel form for the treatment of periodontitis in dogs, obtaining greater osteoblastic activity and more neoformed bone tissue than in the control group.

Topical GH treatment is less disconforting for patientes and faster than its adsortion into the coating-implant, and hence could improve the prognosis of implants prognosis in elderly people. The purpose of the present study was therefore to evaluate the effects of topical GH in the osteointegration of a titanium implant in aged rabbits.

Materials and Methods

Ten presenile New Zeland rabbits (aged two years) were used, their controlled maintenance and stabling conditions being similar. Each animal received an experimental implant, consisting of titanium layers 1 mm thick and 1 cm long and wide. They were emplaced in the middle third of their tibia, in the transversal position from the internal face without reaching the external one (Fig. 1). One half animals were operated and received lyophilised topical rhGH (4 I.U.) on emplacing the implant, which was coated with the hormone and pushed into (press-fit) the osseous bed. The rest were implanted (press-fit) without the topical hormone and used as the control group. All animals were sacrificed 15 days after the surgical treatment.

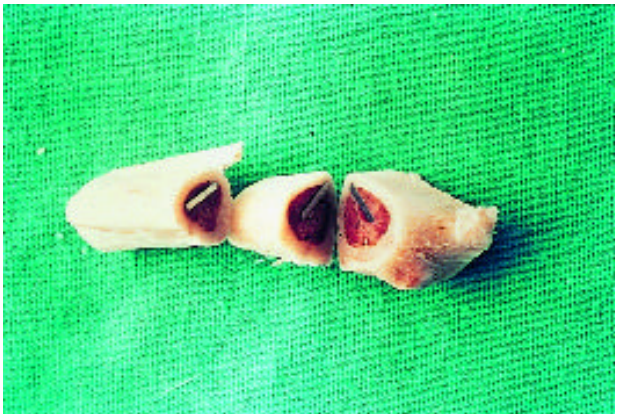


Fig. 1.- Macroscopic image of the fragment of tibia, with layers transversal to the cortical bone from the internal face.

Pieces of bone were collected to make the following studies:

- Assessment of bone mineral density (BMD) at 1 cm around the implant.
- Calculation of the bone fraction area (Aa) and affinity index with morphometric analysis.
- Histological evaluation of the bone-implant interface and its surrounding bone tissue.

To calculate the BMD, dual energy x-ray absorptiometry was used, employing a Norland XR densitometer.

Once the BMD has been obtained, the pieces of bone were processed in order to get histological samples using the technique described by Donath and Breuner (1982) which permits one to obtain preparations of bone and implant together, without decalcifying the bone previously nor separating the implant. In this way, study of the bone tissue and the bone-implant interface is not modified by the histological technique employed.

Then, a conventional histological analysis of the cuts was made, evaluating bone morphology and the interface characteristics (Fig. 2).

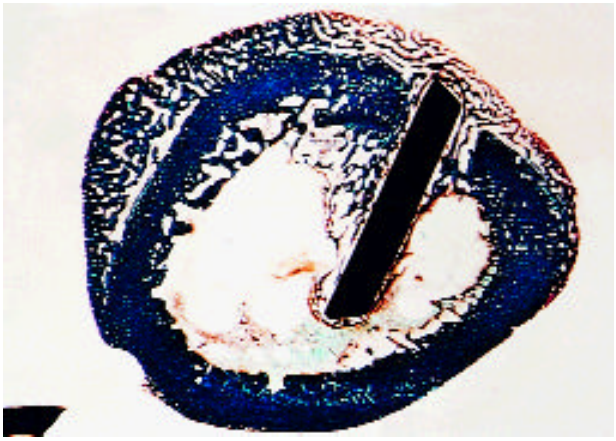


Fig. 2.- Histological image, showing the implant disposition. Transversal cut. Masson stain. x 2.

Additionally, for each histological sample two morphometric parameters were evaluated: "*bone area fraction*" and "*affinity index*" (percentage of osteointegration). In both cases, the MIP-4 image analizing system was used, connected to an light microscope and histological lens.

Area density or "bone area fraction" (Aa= B.Ar/T.Ar) (Parfitt, 1987) is defined as the ratio between the area occupied by bone (B.Ar) and the total area (T.Ar= B.Ar + Ma.Ar: Bone area, B.Ar, and marrow tissue area, Ma.Ar), as seen in Figure 3.

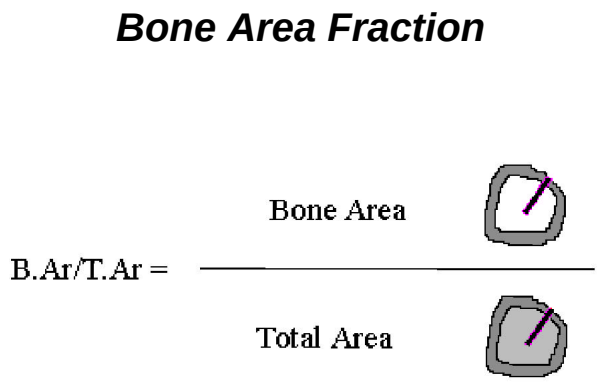


Fig. 3.- Bone area calculation.

The "*affinity index*" is calculated as the ratio between the implant perimeter and the part of implant in contact with bone tissue.

The areal bone value provides information about the quantity of neoformed bone and the affinity index affords information about the actual percentage of osteointegration (Masuda, 1998).

All data were processed with the SPSS 8.0 statistical system.

RESULTS

Our results were divided into histological, morphometric and densitometric.

Histological results:
Three assessments were made at different levels of the bone tissue.

1. Assessment of the existing interface around the implant portion that remained lodged in the medullary cavity (medullary interface).
2. Assessment of the interface around the implant which remained lodged in the cortical thickness (cortical interface).
3. Assessment of the total bone visible at the histological cut.

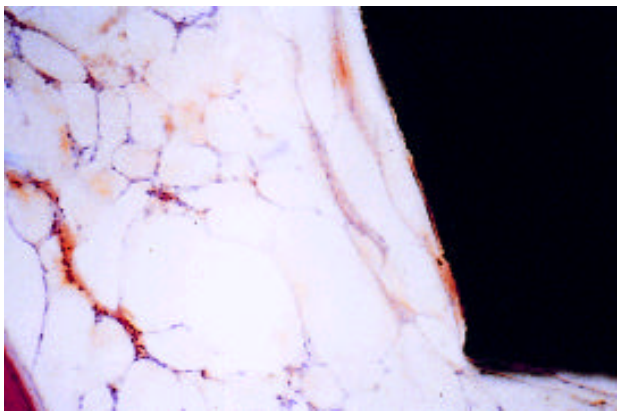


Fig. 4.- Medullary interface without bone tissue in untreated animals. Haematoxylin-Eosin stain. x 2. The presence of connective tissue and bone marrow cells can be observed.

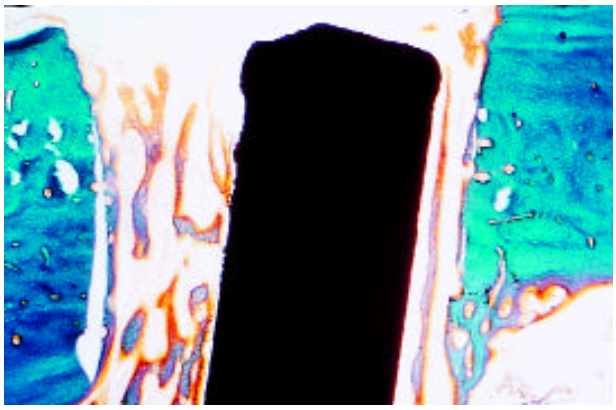


Fig. 5.- Cortical transversal interface with bone trabeculae and osteoid tissue surrounding it. Without treatment. Masson stain. x 3.

1. We did not observe any peri-implant bone tissue at the medullar interface in any of our groups. Fibres of connective tissue and cells of the bone medulla were observed in both groups at the medullar interface (Figure 4).
2. The implant portion lodged in the cortical thickness, in the group without GH, displayed thin trabeculae of peri-implant bone, of type D4 according to Misch's classification (Meffert, 1997). These trabeculae were oriented parallel to the implant in which osteoid tissue, formed from the nearby periosteum and endosteum, could be differentiated. There were also a few trabeculae formed from the edge of the bone injury, with the presence of a few osteoblasts at this level. Among the newly-formed trabeculae, connective tissue was predominant. In the group receiving hormone treatment (GH) there were fewer neoformed trabeculae at this level, although osteoid tissue could be also observed. However, most of the interface corresponded to connective tissue (Figs. 5 and 6).
   In the analysis of bone tissue we found that the cortical part remained compact,

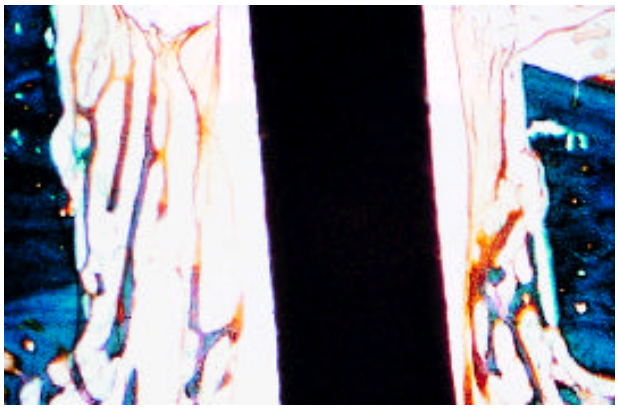


Fig. 6.- Cortical transversal interface with bone trabeculae and osteoid tissue surrounding it. With treatment. Masson stain. x 3.

with few signs of resorption; osteons were arranged in oblique lines at the external face in both the treated and the control group, while at the internal face the arrangement were less ordered (Fig. 7). It

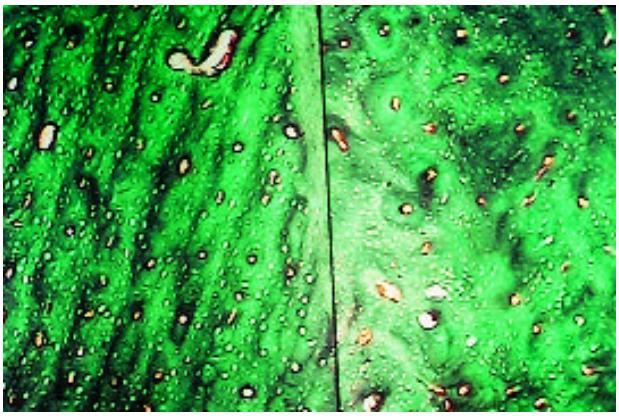


Fig. 7.- External (**left**) and internal (**right**) cortical bone of the same preparation. A different disposition of osteons can be observed. Treated animal. Masson stain. x 3.

should be noted that in the GH group there was a significant increase in osteocyte lacunae size appears, which were much more visible than in the control group. The disposition of the lacunae to form osteons was clearly visible, indicating that the bone was not neoformed but native, with a change in the aspect of its osteocyte lacunae (Fig. 8).

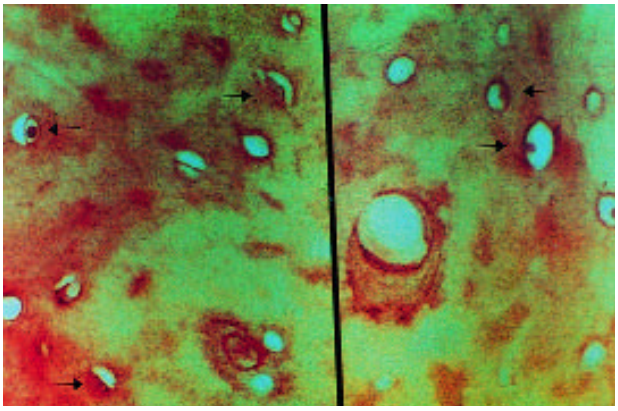


Fig. 8.- Osteocyte lacunae in untreated (**left**) and treated animals (**right**). The large size of some of them and their rounded aspect can be observed; the retracted osteocyte can be differentiated inside (arrows). Masson stain. x 20.

Morphometric and Densitometric results:

Our results were analysed with the SPSS statistical package. Levene's test for homogeneous samples and oneway analysis of variance (ANOVA) were performed. No significant differences between the two groups were found, although all the measurements performed reveled a trend towars a lower bone response in the treated group than in the control group (Tables 1, 2 and 3).

Discussion

In our study, the use of topical GH aged animals afforded results similar to those obtained in the control group. Other authors (Holloway, 1994; Marcus, 1997) have also found a similar response in aged animals using a systemic treatment. In both the control group and treated group, osteointegration was not complete, since it is considered to have been reached when 60% of the implant has been coated with osseous tissue (Masuda, 1998). In the present study, lesser values were obtained: 50% in untreated animals and 40% in the treated ones. In Sennerby´s study, carried out with screws as implanted material, the autor obtained degrees of osteointegration varying from 0 to 40%, depending on whether the analysis was performed in the cortical or in the medullary part. The same author failed to achieve good osteointegration in adult rabbits at 15 days after surgical treatment.

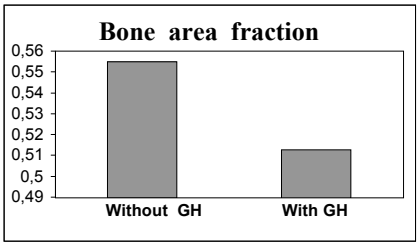
The absence of response to GH could be related to the dose of hormone since Lind (1996a, b) reported a different reactions to varying GH concentrations. It would be interesting to extend the study to different topical GH concentrations.

Assuming that in our study GH elicited different effects, opposite to those obteined with systemic GH, it might be thought that it is due to the topic treatment performed. However, there are data that confirm the limited response in aged animals treated with GH, also through the systemic route. Rudman et al. (1990), and Papadakis et al. (1996), performed density studies on bone mass in the spinal column of elderly men using photonic dual absorptiometry and the results obtained by both authors, at six months of treatment, were not significant either. Some authors believe that the lack of response would be due to the limited time of treatment in both the systemic and topical route. However, in another study by Halloway et al. (1994), after one year, not significant results were obtained. Marcus (1997) performed an analysis of the results obtained around that time and concluded that GH alone, without combining it with any other product, cannot be used as a strategy for increasing bone anabolism in elderly people.

Under normal conditions, in presenile bone a decrease occurs in the capacity for bone neoformation. However, in our study we observed that the presence of the implant elicited a response

Table 1.- Bone area fraction

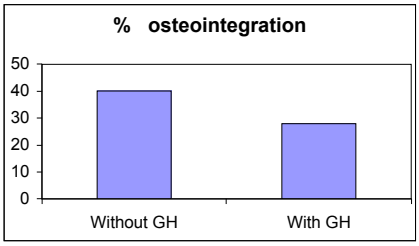
Type of treatment	Bone area fraction (n=5)	Standard deviation
Without GH	0,555	0,056
With GH	0,513	0,065



Mean values of bone area fraction evaluated with morphometrical analysis. The significance of differences between both groups was calculated using Student´s test. Non significant results.

Table 2.- Affinity Index

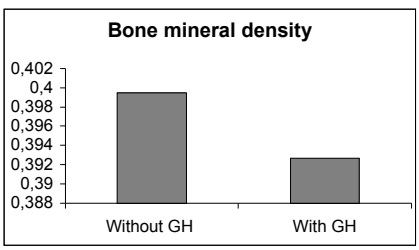
Type of treatment	Affinity index (n=5)	Standard deviation
Without GH	40%	18
With GH	28%	7



Mean values of the affinity index. The significance of differences between both groups was calculated using Student´s test. Non significant results.

Table 3.- Bone mineral density

Type of treatment	Bone mineral density (g/cm^2) (n=5)	Standard deviation
Without GH	0,3995	0,0198
With GH	0,3927	0,0179



Mean values of bone densitometry**.** The significance of differences between both groups was calculated according to the Student´s test. Non significant results.

localized in the native bone. This response consists of an enlargement of osteocytic lacunae. Both, the decrease in neoformation capacity and the enlargement of osteocytic lacunae, are stimulated by the use of topic GH. The emplacement of a biomaterial involves a change in the behaviour of the surrounding osseous tissue, wich may affect load transmittal, one of the main functions of the skeleton. It is therefore not surprising that the emplacement of an implant causes a response of the neighbouring osteocytes. Studies perfomed in experimental animals have shown that increased age is associated with a low response by the bone challenged by a mechanical stimulus, together with a small but progressive decrease in bone mass. Skerry et al. (1990) explained this as a decrease in the capacity of osteocytes, the main sensors of mechanical loads, to maintain a apprppriate porosity within the environment of their intercelular matrix. We have found something similar, since the mechanical variations caused by emplacement of the implant did not produce a intense neoformation, but instead a variation in the osteocytic lacuna, perhaps due to an inappropiate response of the osteocyte, a process which is strengthened by hormonal treatment. The size variation of the osteocytic lacuna could be due to the need to maintain the appropiate calcium levels to meet existing demands and in this case, according to Boyde 1981, we could speak in terms of "osteocytic osteolysis". A detail in favour of this, is the existence of receptors for 1,25-(OH)2D3 (Aarden, 1994) suggesting that the osteocyte participates actively in phosphocalcic metabolism, as reported previously (Boyde, 1981; Aarden, 1994). In this case, the growth hormone could elicit first the release of periosteocytic calcium, which would cause an enlargement of its lacuna. It could be speculated that age modifies the response of osteocytes when challenged with growth hormone. Other authors (László, 1991), failed to abserve lacunar reabsorption and considered that the enlargement found by Belanger could be due to the decalcification that occurs during the histological technique, which would have caused artificial retraction of the lacunae. Since we used non decalcifying techniques, the increase in the osteocytic lacunae cannot be due to an artificial retraction but rather to periosteocytic demineralization, which was more intensive in the group treated with GH, or to a deficit in the production of periosteocytic osseous matrix, which would avoid calcium deposition at that level.

Finally, some authors have distinguished four developmental stages in the life of the ostecyte. These are: formation, stabilization, reabsorption and degeneration. Other authors only distinguish three states in the development of osteocyte and their lacunae; formation, reabsortion and degeneration. In the first case, there is more fibrous collagen than in the others, and glycosaminoglycans predominate in the reabsortion phase. Our study revealed size modifications, which perhaps associated with the predominance of the reabsortion phase. It would therefore be interesting to evaluate the proteoglycan composition in the osteocytic lacunae.

Thus, although at present it cannot be confirmed that hormones control the bone matrix production by osteocytes (Belanger, 1989; Lean, 1995), a certain effect of hormone on osteocytes can definitively be considered; in the case of senile animals, this reflected in increased lacuna reabsorption.

Conclusions

The densitometric and morphological results obtained in the present study fail to reveal significant differences between the treated group and their controls. From the point of view of histological characterization a smaller "de novo" bone tissue formation was observed in the treated group.

Also, some differences were found in the aspect of the native bone between both groups in the area of the osteocytic lacunae. Topical GH increases size of osteocytic lacunae. This suggests an action of GH on osteocyte, reflected in reabsortion of their lacunae.

References

Aarden EM, Burger EH and Nijweide PJ (1994). Function of osteocytes in bone. *J Cell Biochem*, 55: 287-299.

Belanger LF (1969). *Calcif Tissue Res*, 4: 1-12. In: *Le Tissu Osseux*. Colection: Biologíe de l'appareil locomoteur. Ed. Sauramps Medical. 1989.

Boyde A (1981). Evidence against osteocytic osteolysis: In: Jee WSS, Parfitt AM (eds.). *Bone histomorphometry* Armour Montagu, Paris, pp 239-256.

Carpenter JE, Hipp JA, Gerhart TN, Rudman CG, Hayes WC and Trippel SB (1992). Failure of growth hormone to alter the biomechanics of fracture healing in a rabbit model. *J Bone Joint Surg*, 74-A, 3: 359-367.

Donath K and Breuner G (1982). A method for the study of undecalcified bones and teeth with attached soft tissues. *J Oral Pathol*, 11: 318-326.

Downes S, Wood DJ, Malcolm AJ, Path C and Ali SY (1990). Growth hormone in polymethylmethacrylate cement. *Clin Orthop rel Res*, 252: 294-298.

Hollister SJ, Guldberg RE, Kuelske CL, Caldwell NJ, Richards M and Goldstein SA (1996). Relative effects of wound healing and mechanical stimulus on early bone response to porous-coated implants. *J Orthop Res*, 14: 654-662.

Holloway L, Butterfield G, Hintz R, Gesunheit N and Marcus R (1994). Effect of recombinant human growth hormone on metabolic indices body composition, and bone turnover in healthy elderly women. *J Clin Endocrinol Metab*, 79: 470-479.

László M (1991). Organization of the extracellular matrix: a polarization microscopic approach. CRC Press, Boca Ratón.

LEAN JM, JAGGER CJ, CHAMBERS TJ and CHOW HW (1995). Increased insulin-like growth factor I mRNA expression in rat osteocytes in response to mechanical stimulation. *Am J Physiol*, 268: E318-327.

LIND M, OVERGAARD S, ONGPIPATTANAKUL B, NGUYENT, BÜNGER C and SOBALLE K (1996a). Transforming growth factor-b1 stimulates bone ongrowth to weight-loaded tricalcium phosphate coated implants. *J Bone Joint Surg*, 78-B: 377-382.

LIND M, OVERGAARD S, SOBALLE K, NGUYEN T, ONGPIPATTANAKUL B and BUNGER C (1996b). Transforming growth factor-b1 enhances bone healing to unleaded tricalcium phosphate coated implants: an experimental study in dogs. *J Orthop Res*, 14: 343-350.

LYNCH SE, WILLIAMS RC, POLSON AM, HOWELL TH, REDDY MS, ZAPPA UE and ANTONIADES HN (1989). A combination of platelet derived and insulin-like growth factors enhances periodontal regeneration. *J Clin Periodontol*, 16: 545-548.

MARCUS R (1997). Skeletal effects of growth hormone and IGF-I in adults: *Horm Res*, 48 (suppl): 60-64.

MASUDA T, YLIHEIKKILA PK, FELTON DA and COOPER LF (1998). Generalizations regarding the process and phenomenon of osseointegration. Part I. In vivo studies. *Int J Oral Maxillofac Implants* , 13. 17-29.

MEFFERT RM (1997). Do implant surfaces make a difference?. Current Opinion in Periodontology, 4: 104-108.

MURAI K, TAKESHITA F, AYUKAWA Y, KIYOSHIMA T, SUETSUGU T and TANAKA T (1996). Light and electron microscopic studies of bone titanium interface in the tibiae of young and mature rats. *J Biomed Mat Res*, 30: 523-533.

PAPADAKIS MA, GRADY D, BLACK D, TIERN MJ, GOODING GAW, SCHAMBELAM M and GRUNFE C (1996). Growth hormone replacement in healthy older men improves body composition but not functional ability. *Ann Intern Med*, 12: 708-716.

PARFITT AM, DREZNER MC, GLORIEUX FH, KANIS JA, MALLUCHE H, MEUNIER PJ, OTT SM and RECKER RR (1987). Bone histomorphometry: standardization of nomenclature, symbols and units. Report of the ASBMR histomorphometry nomenclature committee. *J Bone Mineral Res*, 2: 595-610.

ROSEN CJ (2000). Growth hormone and aging. *Endocrine*, 2: 197-201.

RUDMAN D, FELLER AG, NAGRAJ HS, GERGA GA, LALITHA PY, GOLDBERG AF, SCHLENKER R, COHN L, RUDMAN IW and MATTSON DE (1990). Effects human growth hormone in men over 60 years old. *N Eng J Med*, 323: 1-6.

SENNERBY L, THOMSEN P and ERICSON LE (1993). Early tissue response to titanium implants inserted in rabbit cortical bone. *J Mat Sci: Mat Med*, 4: 240-250.

SIEGEL MA (1999). Osseointegration?. *Oral Surgery, oral medicine, oral pathology, oral radiology and endodontics*, 88: 2-3.

SKERRY TM, SUSWILLO R, HAJ AJ, ALI NN, DODDS RA and LANYON LE (1990). Load-induced proteoglycan orientation in bone tissue. *In Vivo and In Vitro Calcif Ttissue Int*, 46: 318-326.

URIST MR (1965). Bone: formation by autoinduction. *Science*, 150: 893-899. Opus cit: Influencia de la hormona de crecimiento en la osteointegración. Isabel Fernández-Tresguerres. Tesis doctoral Facultad de Medicina. Universidad Complutense de Madrid. Spain. 1999.