ORIGINAL ARTICLE

PTH improves titanium implant fixation more than pamidronate or renutrition in osteopenic rats chronically fed a low protein diet

R. Dayer · T. C. Brennan · R. Rizzoli · P. Ammann

Abstract

Summary We evaluated the effects of parathyroid hormone (PTH), pamidronate, or renutrition on osseointegration of titanium implants in the proximal tibia of rats subject to prolonged low-protein diets. PTH improved mechanical fixation, microarchitecture, and increased pull-out strength. Pamidronate or renutrition had lesser effects. PTH can thus improve implant osseointegration in protein-malnourished rats.

Introduction Protein malnutrition impairs implant osseointegration in rats. PTH and pamidronate prevent deleterious effects of protein restriction introduced just prior to implantation. Whether these treatments improve osseointegration after chronic protein deprivation, i.e., in osteopenic bone at time of implantation, is unknown. We evaluated effects of PTH, pamidronate, or renutrition on resistance to pull-out of titanium rods implanted into the rat tibiae following isocaloric low-protein intake.

Methods Forty-one adult female rats received normal or isocaloric low-protein diets. Six weeks later, implants were surgically inserted into proximal tibiae. Following implantation, rats on low-protein diets were treated with PTH (1-34), pamidronate, saline vehicle, or normal protein diets, for another 8 weeks. Tibiae were removed for micro-computerised tomographic morphometry and evaluation of pull-out strength.

Results Pull-out strength decreased in rats on isocaloric low-protein diets compared with normal protein group (−33.4%). PTH increased pull-out strength in low-protein group, even compared to controls from the normal protein group. PTH and pamidronate increased bone volume/tissue volume, bone-to-implant contact, and trabecular thickness, whilst trabecular separation was reduced, with a shift to more plate-like bone surrounding the implants.

Conclusions PTH reversed the deleterious effects of long-term protein undernutrition on mechanical fixation and bone microarchitecture and improved implant osseointegration more than pamidronate or renutrition, likely through changes to structure model index.

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Introduction

As the population continues to age, undernutrition and protein deficiency persist amongst elderly patients living in nursing homes and in community-dwelling subjects [1–6]. This problem is widely acknowledged and has even driven the need for assessment tools to diagnose under- or malnutrition [5, 7–9]. Decreases in dietary protein can be caused by age-related physiological changes, diseases of the gastrointestinal tract or of an inflammatory nature, physical impairment, and psychological as well as psycho-social issues. It is widely acknowledged that protein

undernutrition leads to prolonged illness periods [10], reduced immunity [11–13], and severely negative effects on the musculoskeletal system [14–16]. It is not surprising then that a high prevalence of undernutrition is observed in elderly patients undergoing total hip or total knee arthroplasty [17–20] and that the prognosis for these patients is rather poor as it is difficult to stimulate food intake in these patients.

In a previous report, we demonstrated that 26 weeks of an isocaloric low protein diet induces a trabecular bone volume (BV) loss of around 75% in female rats and 70% in male rats. This was associated with a diminution of ultimate strength (or maximal load) in both females and males. Stiffness and energy were also negatively affected by the low protein diet in these animals. More recently, we reported that isocaloric low protein intake, introduced around the time of surgical implantation, impairs titanium implant osseointegration in adult female rats. This was associated with poor bone microarchitecture surrounding the implant [21].

Both bisphosphonates and parathyroid hormone (PTH) treatment have been reported to improve implant fixation, allowing for more durable joint replacements [22–32]. Indeed, in a recent study, we showed that both PTH and pamidronate were able to prevent the deleterious effects of an isocaloric protein restricted diet on bone microarchitecture close to the implant and on mechanical fixation in adult female rats [33]. In these experiments, rats were subject to low protein for only 2 weeks before the implants were inserted. Immediately following the implant surgery, PTH or pamidronate was administered for 8 weeks. Both PTH and pamidronate improved the mechanical fixation, even when compared to animals with normal protein intake.

It is more likely that a longer duration of protein malnourishment prior to implantation would more closely resemble what is often observed in the clinical setting, with many elderly patients having osteopenia caused by a long-term lack of sufficient dietary protein. No data has been reported on whether systemic treatment with bisphosphonates or PTH (1-34) improve the altered titanium implant osseointegration observed in prolonged low protein-induced osteopenic models, or modify the tridimensional trabecular microarchitecture in the vicinity of an implant. The effects of renutrition following implantation in such models have also never been tested. The aim of the current study was, therefore, to test the hypothesis that systemic treatments of PTH, bisphosphonates, or renutrition are able to augment titanium implant osseointegration in a rat model of protein-malnourished-induced osteopenia, a model which closely resembles what is frequently observed in the clinical setting of orthopaedic wards.

Materials and methods

Animals and diet

All experimental designs and procedures have received approval of the Animal Ethics Committee of the Geneva University Faculty of Medicine. Forty-one 10-month-old Sprague-Dawley female rats (Charles River Laboratories, L'Arbresle, France), housed individually at 25°C with a 12:12-h light–dark cycle, were employed. Rats were strictly pair-fed a laboratory diet provided by Provimi Kliba AG (Kaiseraugst, Switzerland) containing either 15% or 2.5% casein, 0.8% phosphorus, 1% calcium, 70–80% carbohydrates, and 5% fat throughout the experimental period. Isocaloric intake by all rats was ensured by the addition of corn carbohydrate to the low protein diet, thus providing the same energy intake across all groups. Demineralised water was available ad libitum. Since the minimal protein intake in rats necessary to maintain normal bone homeostasis is 5% [34, 35] and it is known that casein is not wholly utilisable by rats, a restriction to 2.5% casein corresponds to a reduction of more than 50% of the minimum recommended intake, and a reduction of 50% is often observed in patients who sustain a hip fracture [36, 37].

Experimental design

One week after an initial adaptation period to a 15% casein-containing diet, rats were divided into five groups. One group received a diet containing 15% casein ($n=8$, 15% control group), and three groups were placed on a diet containing 2.5% casein until the end of the study. The final group received the 2.5% casein diet until the implantation, after which, they were commenced on 15% casein ($n=8$, renutrition group). Six weeks following the beginning of isocaloric protein restriction (i.e., at week 7), a titanium implant was inserted in each tibia of the animals under general anaesthesia. We inserted implants into both tibias to avoid the possibility of disuse or overuse often observed when only one limb is operated on. Following implantation, rats from one of the groups receiving 2.5% casein were injected subcutaneously five times a week with human PTH (1-34; Eli Lilly and Company, Indianapolis, IN, USA) at a dose of 40 µg/kg body weight per injection, until the end of the study ($n=8$, 2.5% PTH group). This dose has previously been demonstrated to effectively correct the altered bone mechanical properties associated with protein deprivation in rats [33, 38]. It is higher than that used in humans. Similarly, another of the groups receiving 2.5% casein-containing diet was treated five times a week during the first and the fifth weeks post-implantation surgery with subcutaneous injection of 3-amino-1hydroxypropylidene-1,1-

bisphosphonate (Pamidronate, Novartis International AG, Basel, Switzerland) at a dose of 0.6 mg/kg (n=9, 2.5% pamidronate group). This dose has been shown to prevent the deleterious effect of isocaloric protein deprivation on bone strength [39]. Rats in the pamidronate group also received a subcutaneous injection of vehicle (sodium chloride, 0.9%, 0.5 ml/kg) five times a week in those weeks they did not receive pamidronate. All treatments and the vehicle were injected at the same volume per kilogramme body weight. The renutrition group was fed with a 15% casein-containing diet after the implantation as described above. Finally, the group fed a diet containing 15% casein (n=8, 15% control group), the renutrition group, and the remaining group on 2.5% casein diet (n=8, 2.5% control group) were injected with vehicle (sodium chloride, 0.9%, 0.5 ml/kg) five times a week during the 8 weeks following implantation. At 8 weeks post-implantation (i.e., at week 15), blood was obtained from all rats by aortic puncture under general anaesthesia, and animals were killed by an overdose of ketamine hydrochloride. The tibias were removed for micro-computerised tomographic (μCT) morphometry and mechanical testing.

Implants design and preparation

The implants were made from commercially pure titanium (Institut Straumann AG, Waldenburg, Switzerland), were 1.0 mm in diameter and 4.1 mm long, and were sandblasted and acid etched on the non-threaded part. The threaded part was 1.25 mm long (type M1) and remained outside of the bone, permitting prehension for the pull-out test. This type of implant was selected as titanium is well tolerated by bone tissue. The rough surface, fashioned by sandblasting and acid-etching, ensures good osseointegration. The implants were cleaned in phosphate-free Deconex® (Borer Chemie AG, Zuchwil, Switzerland), rinsed in pure water in an ultrasonic bath, and sterilised with ethylene oxide.

Implantation technique

Animals were anaesthetised by abdominal injections of ketamine (100 mg/kg) and xylazine (10 mg/kg). The skin of the bilateral tibial region was shaved and cleaned with 70% ethanol. We have previously provided a pictorial description of the implanted region [21]. Under aseptic conditions, a 10-mm incision was made anteriorly to gain access to the proximal medial section of the tibia metaphysis. The periosteum was reflected medially. The periosteum in adult rats is a relatively thin and fragile tissue, which cannot be replaced around the implant. It was thus left reclined. A 1.0-mm diameter hole was drilled through one of the cortices with a hand-held drill into which the implant was inserted. Rotatory speed did not exceed 2,000 rpm, and drilling was

accompanied by profuse saline irrigation to avoid thermal bone necrosis. The proximal limit of the entry point was delimited by a virtual line perpendicular to the long axis of the tibia and crossing the anterior edge of the growth plate centrally, which is curved in this central region anteriorly and inferiorly. The second anatomical landmark utilised was a virtual line passing from the inferior border of the tendinous insertion on the proximal anterior tibial crest to a medial tendinous insertion, which most likely corresponds to the pes anserinus in humans. The point of insertion was halfway between these two tendinous insertions. We have previously demonstrated by X-ray examination that employing such landmarks leads to the implant being in contact with the trabecular bone of the proximal tibial metaphysis [21], at a distance of 2 mm distal to the growth plate along the great axis of the tibia. The implant was inserted perpendicularly to the surface of the cortical bone on the medial part of the proximal tibia. The depth of the inserted implant section into the bone corresponded to the length of the non-threaded section of the implant (2.85 mm). Only the threaded part remained outside the bone. The implants were specifically designed taking into account the X-ray determined dimensions of the proximal tibia in adult female Sprague-Dawley rats. After insertion of the implant, the skin was sutured using a 3-0 resorbable polyglactic suture (Vycril®; Ethicon, Spreintenbach, Switzerland). Postoperative analgesia was maintained by subcutaneous injection of buprenorphine (0.06 mg/kg) twice a day for 3 days.

Micro-computerised tomographic (μCT) morphometry

The tibias were carefully excised immediately after death and frozen at −20°C in plastic bags. The night before μCT analysis, the bones were thawed slowly at 7° and maintained at room temperature. During the whole analysis, they were immersed in saline solution. Parameters of volume, architecture, and percentage of implant surface in direct contact with trabecular bone (percentage of attachment, bone-to-implant contact) were studied using μCT morphometry (μCT 40; Scanco Medical AG, Bassersdorf, Switzerland). The voxel was 20 μm in all spatial directions. For penetrating radio-opaque titanium and improving signal-to-noise ratio, the system was set to 70 kEV with a 350-ms integration time. The number of tomographic projections was 1,000 projections per 360°. Images were filtered with a 3-D Gauss philtre with Sigma 1.5 voxels and then segmented with threshold 1.4 cm^{-1} for bone (25% of maximal grey scale value, visually chosen) and threshold 5.8 cm^{-1} for titanium (attenuation coefficient). Trabecular bone was analysed on a circular band of 0.5 mm around the implant (Fig. 2). Relative BV/tissue volume (TV), trabecular number, trabecular thickness, and trabecular separation were calculated by directly measuring the 3-D distances in

the trabecular network. Connectivity density based on Euler number (Conn.D) and the structure model index (SMI) were calculated. When a bone voxel was in the vicinity (60 µm boundary) of a titanium voxel, this titanium voxel was classified as "attached surface". With a distance of 3 voxels (60 µm) from the detected titanium surface, the presence or absence of bone can be accurately determined (IPL, image processing language software, available with the µCT 40, Scanco Medical AG, Bassersdorf, Switzerland). The artefacts of the titanium in the CT image prevented us from analysing the presence of bone at a range closer to the implant [40, 41]. The number of attached surface voxels divided by the total number of titanium surface voxels yielded the percentage bone-to-implant contact. The tridimensional reconstructions obtained were always verified against the original grey scale images.

Pull-out test

After µCT analysis, implanted tibias were subjected to a pull-out test. The tibias were held in place on a metal device by two flat supports 5.6 mm apart. Another metal piece was screwed onto the threaded, i.e., external part of the implant and was used to grip the implant during the test. Pull-out strength was determined as the peak force, i.e., the maximum failure force, applied to completely loosen the implant from the bone, and was measured with a servo-controlled electromechanical system (Instron 1114; Instron Corp., High Wycombe, UK) with the actuator displacement speed set at 2 mm/min. Reproducibility was 13.4% (3.3–22), as evaluated by the mean of the absolute differences between paired-sample measurements (left/right). A preliminary study showed that freezing the tibias did not alter pull-out strength values. The mean of the absolute differences between values obtained before or after freezing procedure was 12.5% (0.9–35.5). Regression between values obtained before or after freezing procedure was characterised by an r^2 coefficient of 0.96 [21].

Biochemical assays

Plasma insulin-like growth factor I (IGF-I) was measured by immunoenzymometric assay, with a kit from Nichols Institute (San Juan Capistrano, CA, USA) following the manufacturer's instructions, and osteocalcin employing a kit from Biomedical Technologies Inc. (Stoughton, MA, USA).

Statistical analysis

All results are expressed as mean ±SEM. Significance of difference was evaluated by analysis of variance (ANOVA) and post-analysis by Tukey's honestly significant difference test (Statistical Package for the Social Sciences (SPSS) 14.0; SPSS Inc., Chicago, IL, USA).

Results

Effect of PTH, pamidronate, or renutrition on pull-out strength

As previously shown [21, 33], mean pull-out strength values were decreased in rats fed an isocaloric low protein diet (−33.4%) compared with animals in the normal protein group (Fig. 1). Neither pamidronate nor renutrition were able to increase mechanical fixation above the low protein control group. PTH treatment, on the other hand, markedly increased the pull-out strength compared to the control animals receiving the same diet (2.5%). Pull-out strength values in the 2.5% PTH group were also higher than in animals never subject to a low protein diet, i.e., the 15% control group, as well as compared to the renutrition and pamidronate-treated groups.

Effect of PTH, pamidronate, or renutrition on µCT morphometry of the trabecular bone around the implant

To evaluate the effects of PTH, pamidronate, and renutrition on pull-out strength, we analysed the trabecular bone microarchitecture around the implant and bone-to-implant contact. BV/TV values and bone-to-implant contact were lower in the low protein group compared to the normal protein group (Figs. 2, 3, and 4). Both PTH and pamidronate treatments increased BV/TV values and bone-to-implant contact compared with the low-protein intake control group and the renutrition group. Systemic administration of PTH or pamidronate under protein restriction increased BV/TV and bone-to-implant contact to values higher than those observed in control animals with normal protein intake. Finally, renutrition was unable to increase BV/TV or bone-to-implant contact compared to the low-protein control group.

Protein restriction, PTH, and pamidronate affected other parameters of the trabecular bone architecture in the vicinity of the implant (Table 1). A significant increase of trabecular number was noted in the 2.5% pamidronate group compared with the 15% and the 2.5% controls. Isocaloric protein restriction was associated with lower values of trabecular thickness when compared to the normal protein intake control group. PTH and pamidronate treatments increased trabecular thickness when compared to the low-protein control animals, the normal protein control animals, or the animals in the renutrition group. Accordingly, similar changes were noted for trabecular separation when compared to 2.5% and 15% control groups. This was associated with
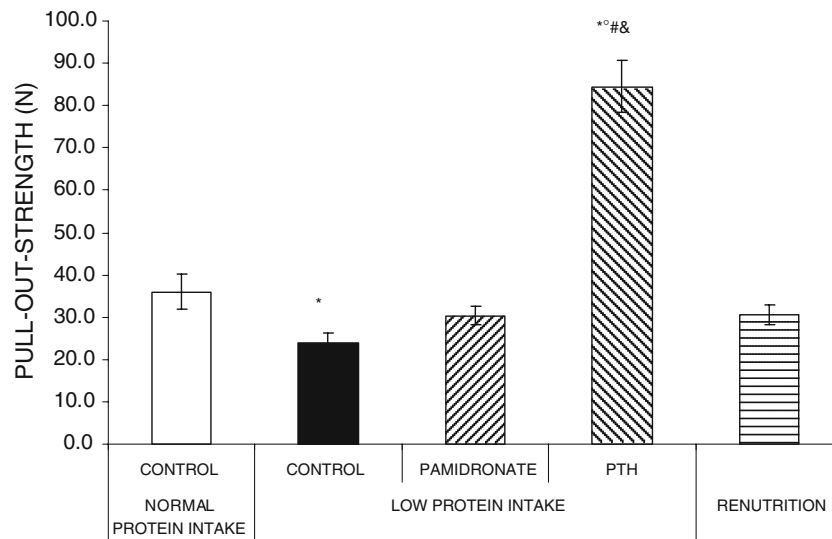
alterations of the trabecular structure, as illustrated by changes in the SMI. Protein restriction induced a shift toward more rod-like bone microstructures, although more plate-shaped bone was observed in the animals treated with PTH or pamidronate compared with 2.5% controls and the renutrition group. This effect was stronger under PTH treatment, with a decrease of SMI even when compared to the values obtained with pamidronate or in the normal protein control group. A non-significant decrease of connectivity density was noted in the low-protein-fed animals. This value was higher with pamidronate treatment, when compared to animals in the renutrition group.

Fig. 2 Microtomographic morphometry by microcomputed tomography. Microtomographic tridimensional reconstruction of the trabecular bone around titanium implants at 8 weeks after implantation. The *two upper panels* are from samples in the control groups (15% control and 2.5% control), and the *three lower ones* are from samples in the pamidronate and parathyroid hormone (PTH) low protein intake groups (2.5% pamidronate and 2.5% PTH) and renutrition group. Relative bone volume (BV/TV) around titanium implants and bone-to-implant contact (BIC) of the corresponding samples are indicated
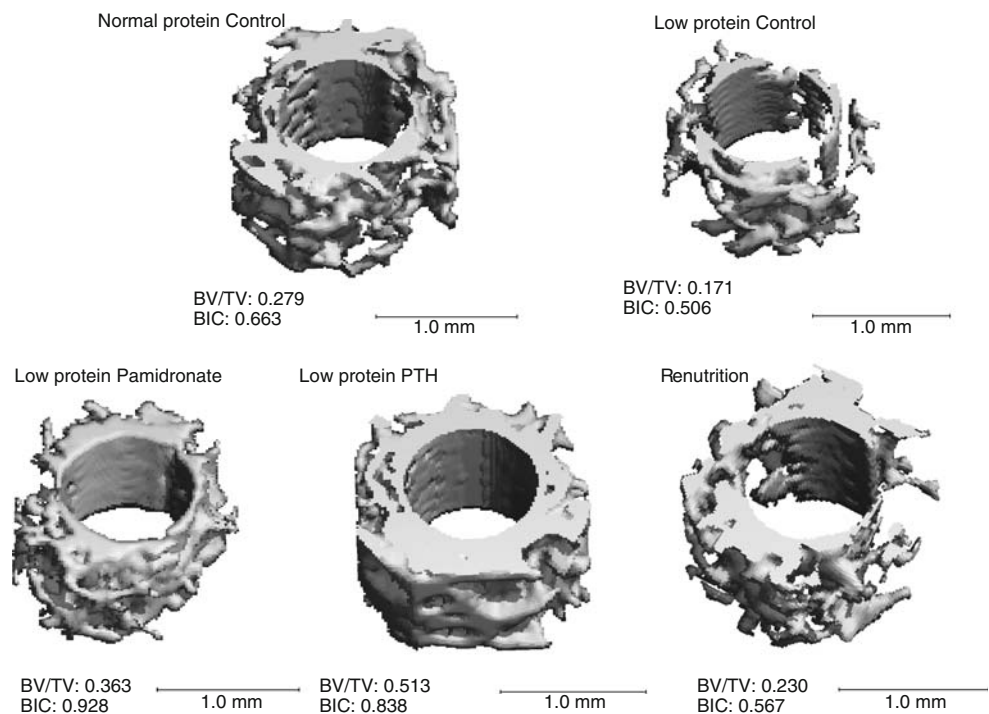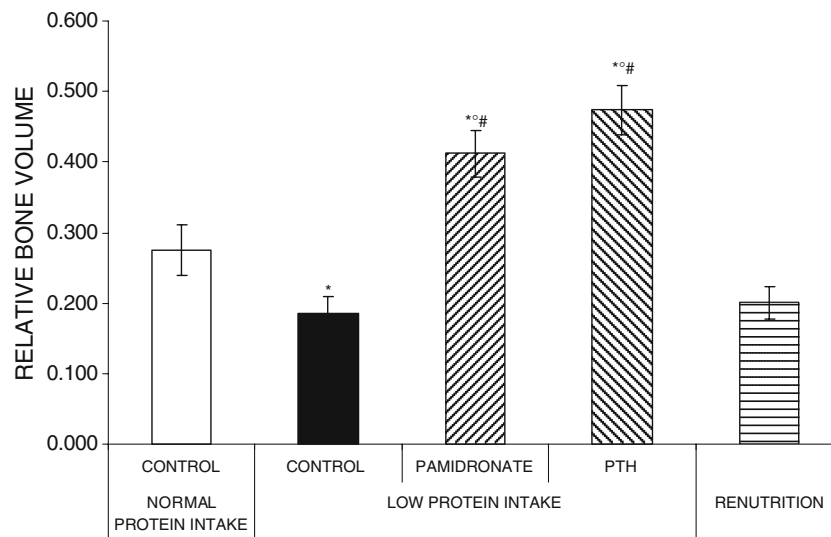
Fig. 3 Effects of systemic pamidronate or parathyroid hormone treatment under isocaloric low protein intake, or renutrition on relative bone volume (BV/TV) around titanium implants in the proximal tibial metaphysis of adult female rats 8 weeks after surgery. Values are means±SEM, *asterisks* corresponds to $p<0.05$ vs the control normal protein intake group, *degrees* corresponds to $p<0.05\%$ vs the control low protein intake group, and *number sign* corresponds to $p<0.05\%$ vs the renutrition group, as evaluated by analysis of variance and post-analysis by Tukey's honestly significant difference test

Effect of isocaloric protein restriction and renutrition on IGF-I, osteocalcin, and on body weight

Isocaloric low protein intake decreased plasma IGF-I levels (Table 2) consistent with previously shown data [21, 34, 35, 42]. Protein restriction was associated with lower body weight, despite strict pair-feeding with isocaloric diets. Renutrition increased plasma IGF-I levels and body weight when compared to the three other groups subjected to protein restriction throughout the entire study.

Isocaloric protein restriction was not associated with significant changes of osteocalcin levels, when compared to the 15% controls and the renutrition groups (Table 2). Treatment with pamidronate was associated with a reduction of osteocalcin levels when compared with the 2.5% control group and the renutrition group. PTH induced
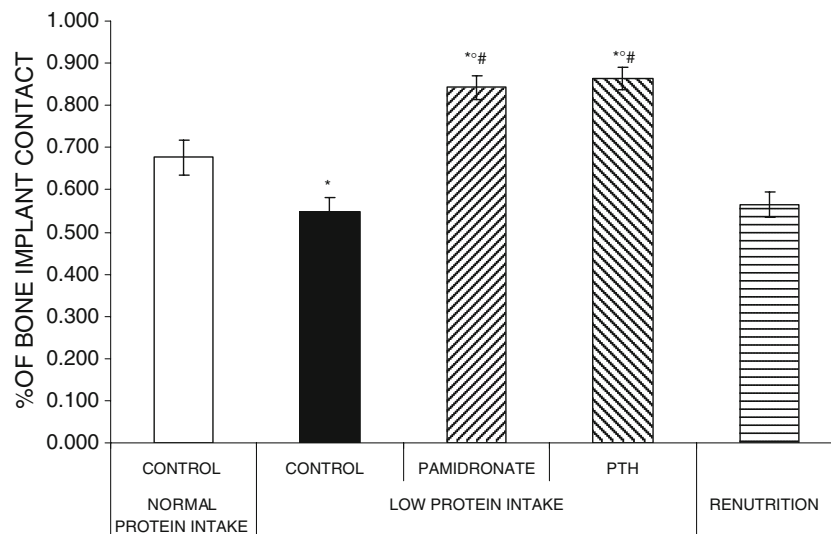

Fig. 4 Effects of systemic pamidronate or parathyroid hormone treatment under isocaloric low protein intake or renutrition on bone-to-implant contact in the proximal tibial metaphysis of adult female rats 8 weeks after surgery. Values are means±SEM, *asterisk* corresponds to $p<0.05$ vs the control normal protein intake group, *degrees* corresponds to $p<0.05\%$ vs the control low protein intake group, and *number sign* corresponds to $p<0.05\%$ vs the renutrition group, as evaluated by analysis of variance and post-analysis by Tukey's honestly significant difference test

Table 1 Effects of pamidronate or parathyroid hormone under isocaloric dietary protein restriction or renutrition on bone architecture around the titanium implant

Casein (%)	15	2.5			
Treatment	Controls (n=8)	Controls (n=8)	Pamidronate (n=9)	PTH (n=8)	Renutrition (n=8)
Tb.N (1/mm)	4.85±0.26	4.30±0.21	5.37±0.17*°	5.09±0.19	4.51±0.18
Tb.Th (μm)	118.9±5.5	105.6±3.8*	152.5±4.4*°#	150.1±6.5*°#	114.5±3.6
Tb.Sp (mm)	0.250±0.020	0.274±0.015	0.197±0.011°#	0.203±0.013°#	0.267±0.015
SMI	0.97±0.22	1.47±0.16	0.01±0.42°#	−1.07±0.32*°#&	1.58±0.09
Conn.D.	41.22±5.28	31.23±5.29	45.96±3.51#	37.20±4.31	27.59±3.77

Values are means±SEM. Results are trabecular number (Tb.N), trabecular thickness (Tb.Th), trabecular separation (Tb.Sp), structure model index (SMI), and connectivity density based on Euler number (Conn.D.). SMI is an estimate of the rod characteristics of trabecular bone (0 value= purely plate-shaped bone, 3 value=purely rod-like bone, negative values indicate a solid mass with only few holes in it). Isocaloric protein restriction was started 6 weeks before bilateral tibial insertion of titanium implants. After surgery, rats were treated with daily subcutaneous injections of a saline vehicle (control), pamidronate, or human parathyroid hormone (PTH), as specified in the "Materials and methods". Another group received a diet containing 2.5% casein until the implantation; after the surgery, animals in this group received a diet containing 15% casein and were treated with saline vehicle (renutrition group). Animals were sacrificed at 8 weeks after implantation

asterisk corresponds to $p<0.05$ vs the control normal protein intake group; *degrees* corresponds to $p<0.05\%$ vs the control low protein intake group; *number sign* corresponds to $p<0.05\%$ vs the renutrition group; *ampersand* corresponds to $p<0.05\%$ vs the pamidronate low protein group (as evaluated by ANOVA and post-analysis by Tukey's HSD test)

significant higher levels of osteocalcin when compared to the animals treated with pamidronate.

Discussion

We have previously shown that an isocaloric low protein diet induces increased bone resorption and depressed bone formation, resulting in bone loss in rats [34]. The results of the current study indicate that a prolonged isocaloric low protein intake also results in deleterious effects on titanium implant osseointegration. Systemic treatment with PTH (1-34) markedly increased the mechanical fixation of the implant under protein restric-

tion. Interestingly, PTH treatment in addition to a low protein diet was able to increase the pull-out strength needed to completely loosen the implants, even compared to control animals with normal protein intake. We also observed that PTH treatment significantly improved bone-to-implant contact and induced a higher relative BV in the vicinity of the implants. PTH also induced an increase in trabecular thickness, a decrease in trabecular separation, and a shift to more plate-like bone structures, which have all been shown to improve some of the biomechanical properties that aid in the prevention of bone fragility and decrease fracture risk [43]. It is also likely, therefore, that such improvements in these properties contribute to the osseointegration of titanium implants. It should be noted

Table 2 Effects of isocaloric protein restriction on insulin-like growth factor I, osteocalcin plasma levels, and change in body weight

Casein (%)	15	2.5			
Treatment	Controls (n=8)	Controls (n=8)	Pamidronate (n=9)	PTH (n=8)	Renutrition (n=8)
IGF-I (ng/mL)	687.1±35.8	480.7±20.2*#	427.9±17.7*#	444.3±26.3*#	678.3±29.8
Osteocalcin (nmol/L)	10.8±0.5	11.9±0.9	6.7±0.2*°#	12.6±0.7&	10.3±0.5
Δ Weight (g)	−13.8±8.6	−70.1±8.2*#	−66.4±6.0*#	−71.1±7.4*#	−22.6±5.5

Values are means±SEM. Isocaloric protein restriction was started 6 weeks before bilateral tibial insertion of a titanium implant. After surgery, rats were treated with daily subcutaneous injections of a saline vehicle (control), pamidronate, or human parathyroid hormone (PTH), as specified in "Materials and methods". Another group received a diet containing 2.5% casein until the implantation; after the surgery, animals in this group received a diet containing 15% casein (renutrition group) and were treated with saline vehicle. Determinations were performed 8 weeks after implantation, when rats were killed. Values are mean insulin-like growth factor I plasma levels (IGF-I) and mean difference of weight from the beginning of the experiment (Δ weight)

asterisk corresponds to $p<0.05$ vs the control normal protein intake group; *degrees* corresponds to $p<0.05\%$ vs the control low protein intake group; *number sign* corresponds to $p<0.05\%$ vs the renutrition group; *ampersand* corresponds to $p<0.05\%$ vs the low protein group treated with pamidronate (as evaluated by ANOVA and post-analysis by Tukey's HSD test)

that PTH treatment may have a greater anabolic effect on bone in rats than in humans, as an increased mean wall thickness has been reported in rats given intermittent PTH treatment [44], whereas this parameter is unchanged in humans [45]. Treatment with pamidronate, an inhibitor of bone resorption, was also able to effectively prevent the negative impact of isocaloric protein restriction on bone architecture surrounding the titanium implants. Pamidronate induced an increase in relative BV, bone-to-implant contact, trabecular number, and trabecular thickness, even when compared to control animals with normal protein intake. Improved trabecular separation and a shift to more plate-like trabecular structures were also noted when compared to control animals on an isocaloric low protein diet. However, these positive morphometric effects of pamidronate were not associated with significant increases in the pull-out strength. Finally, renutrition implemented immediately following implantation was unable to improve mechanical fixation, relative BV, bone-to-implant contact, or any of the other measured parameters of trabecular bone microarchitecture around the titanium implant, despite completely correcting the low IGF-I levels induced by the low protein diet. It should also be noted that in other studies performed in our group, it has been reported that dietary protein intake in patients who sustained hip fractures averaged between 0.6 and 0.7 g/kg of body weight [46, 47], whereas the recommended daily allowance for elderly adults is 1–1.2 g/kg of body weight [48], with anything below 0.8 g/kg of body weight being considered to represent protein undernutrition [14]. Furthermore, attempts to improve protein intake have been performed with insufficient success [36, 49]. It is very difficult to change the habits of both the patients and the caretakers. In addition, following dental implant procedures, masticular function is often decreased, and maintaining food intake, let alone increasing it in order to obtain higher dietary protein, is rarely possible. In our opinion, there is no doubt that the best case scenario would be to improve protein intake post-surgery; however, it remains to be the situation, even within care-facilities, and such intervention is therefore not common practise. As such, post-surgical protein malnutrition remains a problem. This, taken with the results of the current study, highlights the need to look to treatments to improve osseointegration following implantation. It should be noted that one limitation of the current study is that while casein is a very common protein source for rats, it is not wholly digestible or utilisable by rats [50], and therefore, the rats receiving the low protein diet may actually be receiving less than 50% of the minimal requirement for protein.

The changes in bone remodelling previously observed with protein deprivation [34] were also associated with decreased IGF-I concentrations and a resistance to IGF-I at the level of the target organs. In the present study, decreased plasma IGF-I was found in all animals exposed to the isocaloric low protein diet; however, IGF-I levels returned to normal in the renutrition group only. This confirms the low IGF-I levels under a low protein diet. Such malnutrition may also interfere with the process of osseointegration, in which both bone resorption and formation are implicated [51, 52].

A recent study in animals indicated that treatments with PTH or pamidronate immediately following implantation fully prevented the deleterious effects associated with an isocaloric low protein diet commenced 2 weeks prior to implantation. Both treatments significantly increased pull-out strength in animals fed an isocaloric low protein diet, and PTH even induced a higher mechanical fixation when compared to animals with normal protein intake [33]. The current results establish that PTH treatment provides beneficial effects following long-term protein deprivation, a condition which more closely mimics the nutritional status of many elderly patients in orthopaedic wards undergoing total hip or knee arthroplasty [17–20]. The increases seen in bone-to-implant contact and relative BV following PTH treatment contribute to the positive effects of PTH on implant fixation after long-term low protein diet. We have previously shown that bone-to-implant contact is a strong predictor of pull-out strength [21]. It should be noted, however, that relative BV and bone-to-implant contact were not significantly different between PTH and pamidronate treatments, yet the pull-out strength values with PTH were higher than those obtained with pamidronate. Linear regression analysis indicates that there is a relationship between SMI and pull-out strength, characterised by an r^2 value of 65% (Fig. 5). SMI characterises the form of the trabecular structure, describing whether this
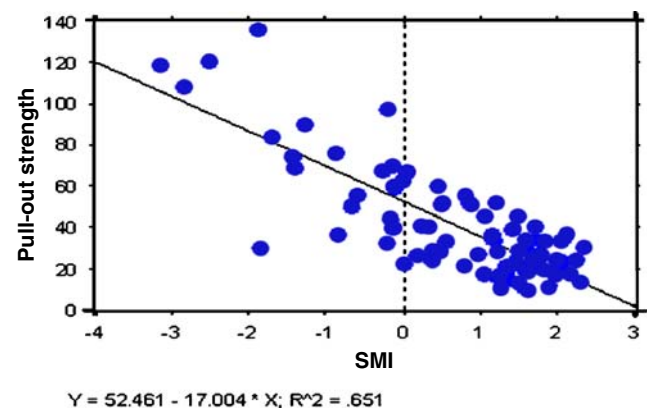

$$Y = 52.461 - 17.004 * X; \ R^{\wedge}2 = .651$$

Fig. 5 Regression analysis between structure model index (SMI) and pull-out strength. Regression analysis revealed that SMI is a good predictor of pull-out strength, with an r^2 value of 0.651: Y=2.249− 0.038×X; R^2=0.651

bone takes on rod-like shapes or more plate-like structures and was the only microstructural parameter which predicted pull-out strength. This is the first time a relationship between SMI and implant pull-out strength has been reported and may indeed underlie the interesting differences in pull-out strength observed between the PTH-treated and alendronate-treated rats. These results do not rule out the involvement of other factors, including enhanced material level properties and/or an increase in bone ingrowth into the microscopic pits of the textured titanium surface with PTH treatment. It is possible that this would generate a stronger bone interlacing and mechanical coupling at the interface. The nanometric pits on the titanium surface are largely below the resolution power of the µCT. The only technique available for such a tridimensional analysis would be a synchrotron [53], which was not available for this study.

Another possible explanation for the less potent effects of pamidronate under long-term protein deprivation is linked to the observation that isocaloric protein restriction is initially able to transiently decrease both bone resorption and bone formation [35]. Decreased bone formation may be the dominant feature of the changes in bone remodelling seen during the first weeks of protein undernutrition. This is also consistent with the strong positive effects of PTH treatment under such a diet, promoting bone formation into the microscopic pits on the titanium surface and thus inducing stronger mechanical fixation of the implant. With bone formation already depressed by protein deprivation, pamidronate, whose effects are primarily to inhibit bone resorption, is unable to reverse this deleterious effect [34]. However, our results regarding osteocalcin levels do not support this hypothesis. Osseointegration represents a complex interaction in which local bone biology plays a key role. This could contribute to the difficulty of drawing explanations based on biochemical markers reflecting general bone metabolism.

Finally, renutrition was unable to correct the deleterious effects of protein restriction on implant fixation, bone-to-implant contact, and the other tested parameters of trabecular bone microarchitecture, even when applied over a longer period (8 weeks) than protein deprivation (6 weeks). It should be noted, however, that plasma IGF-I was increased by protein renutrition, mimicking observations seen in clinical studies [54, 55]. This lack of effect of renutrition on osseointegration, even with increased IGF-I plasma levels (when compared to the other low protein groups), was unexpected. Indeed, in a previous study, essential amino acid supplements, at doses, which effectively increased plasma IGF-I, corrected bone strength via an increase in cortical thickness [42]. Taken together, these results combined with the current data support the hypothesis that essential amino acids may be more efficacious than protein repletion alone. Another possibility

is that IGF-I production stimulated by protein intake affects trabecular bone less than it does cortical bone.

In conclusion, in an experimental model of prolonged isocaloric dietary protein restriction, we have shown, for the first time, that systemic treatment with PTH is able to completely reverse the negative effects of long-term protein undernutrition on titanium implant osseointegration. In animals fed a low protein diet, mechanical fixation was significantly increased by PTH treatment, even when compared with the animals fed a normal protein diet. These positive effects were associated with increased bone-to-implant contact and with beneficial changes in the trabecular structure around the implant, including increased relative BV, thicker trabeculae, diminished trabecular separation, and a more plate-like trabecular network. Pamidronate treatment also significantly increased bone-to-implant contact and relative BV, even when compared with animals in the normal protein group. Positive changes in other parameters of trabecular bone microarchitecture around the implant were also noted; however, a significant corresponding increase in the mechanical fixation of the implant was not observed, while renutrition was unable to improve bone microarchitecture or pull-out strength values.

Conflicts of interest None.

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