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Clinical, histological, and nanomechanical parameters of implants placed in healthy and metabolically compromised patients

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ABSTRACT

Objectives: To evaluate the clinical outcomes, histological parameters, and bone nanomechanical properties around implants retrieved from healthy and metabolic syndrome (MS) patients.
Methods: Twenty-four patients with edentulous mandibles (12/condition), received four implants between the mental foramina. An additional implant prototype was placed for retrieval histology. The following clinical outcomes were evaluated: insertion torque (IT), implant stability quotient (ISQ) values at baseline and after 60 days of healing, and implant survival. The prototype was retrieved after the healing and histologically processed for bone morphometric evaluation of bone-to-implant contact (%BIC) and bone area fraction occupancy (% BAFO), and bone nanoindentation to determine the elastic modulus (Em) and hardness (H). Descriptive statistical procedures and survival tests were used to analyze the data.
Results: The final study population was comprised of 10 women and 11 men (~64 years). A total of 105 implants were placed, 21 retrieved for histology. Implant survival rates were similar between groups (> 99 %). Similarly, IT and ISQ analyses showed no significant association with systemic condition ($p > 0.216$). Histological micrographs depicted similar bone morphology, woven bone, for both conditions. While MS (33 ± 5.3 %) and healthy (39 ± 6.5 %) individuals showed no significant difference for %BIC ($p = 0.116$), significantly higher %BAFO was observed for healthy (45 ± 4.6 %) relative to MS (30 ± 3.8 %) ($p < 0.001$). No significant differences on bone nanomechanical properties was observed ($p > 0.804$).
Conclusions: Although no significant influence on clinical parameters and bone nanomechanical properties was observed, MS significantly reduced bone formation in the peri-implant area in the short-term.
Clinical Significance: A lower amount of bone formation in the peri-implant area was observed in comparison to healthy patients, although the other short-term clinical outcomes were not significantly different. Considering the escalating prevalence of MS patients in need for implant treatment, it becomes crucial to understand bone-to-implant response to determine the ideal loading time in this population.

1. Introduction

Implant-supported reconstructions are a well-established treatment option for single, partial, or full-arch dental rehabilitations [1–5]. Osseointegration is achieved by anchoring the implant through the formation of bone tissue in a dynamic modeling-remodeling process without fibrous tissue growth at the peri-implant interface [6,7]. Although implant-supported prosthesis is one of the most successful reconstructive strategies in dentistry [5,8], compromised systemic conditions, such as pro-inflammatory metabolic diseases, have shown to influence peri-implant healing process leading to increased levels of osseointegration failure and onset and progression of peri-implant

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diseases through severe tissue breakdown with time [9–14].

Nearly 50 % of the adult global population is projected to suffer from some form of a metabolic disease by 2050 [15], including metabolic syndrome (MS) and diabetes mellitus (DM), indicating a substantial increase in the number of patients that will be in need for implant-supported reconstructions in a pro-inflammatory health condition. The pathophysiological clinical consequences associated with the chronic low-grade inflammatory state on implant treatment and/or maintenance over time are still inconclusive, especially due to the high number of poorly-controlled symptoms or non-diagnosed patients under dental treatment, as well as the interrelationship between different metabolic conditions and lack of standard globally accepted definitions [14]. In fact, multiple labels and concepts based on signs and symptoms have been suggested by experts in endocrine system about metabolic deficiencies and diagnostic criteria, which led to the formalization of a differential diagnosis for MS [16,17]. Such criteria recognizes that MS consists of a spectrum of conditions in which the presence of any three of the following risk factors constitute a MS diagnosis, including elevated waist circumference that is population and country specific defined, elevated triglycerides, reduced high-density lipoprotein cholesterol (HDL-c), elevated blood pressure, and elevated fasting glucose, or drug treatment for any of the above mentioned risk factors [16,17].

There is a general consensus in the medical community that MS causes an elevation of plasma free fatty acids (FFA), meal-derived fatty acids and endogenous fatty acids from adipose tissue lipolysis, which can impair insulin sensitivity [15,16,18–20]. High blood levels of FFAs may further impair glucose metabolism due to predominant mitochondrial oxidation of lipids, which decreases glucose uptake and creates a state of chronic hyperglycemia [16,19,20]. Excess circulating glucose can trigger the pathological glycation of circulating proteins and formation of advanced glycation end products (AGEs) [15,16]. Also, excess circulating FFAs are diverted into non-oxidative pathways producing lipid metabolites, such as diacylglycerol and ceramide [15,16,20]. Such toxic metabolites can cause organ-specific oxidative damage and cellular dysfunction [15,16,18–20]. Additionally, adipocyte-derived cytokines such as tumor necrosis factor alfa (TNF-α), interleukin (IL)-1ß, IL-6, and C-reactive protein along with an increased number of pro-inflammatory macrophages and pathogenic T cells perpetuate a pro-inflammatory state [15,16].

Osseointegration is a complex phenomenon directly dependent on a healthy bone metabolism, as mentioned above [6,7]. Nonetheless, the highly integrated and sequential immune-inflammatory response is known to be adversely affected in metabolically compromised patients due to vascular supply reduction secondary to microangiopathies, dysfunctional cellular activity related to the exposure to toxic metabolites and decreased host immune resistance due to the sustained pro-inflammatory state [21,22]. Scientific findings demonstrated that toxic metabolites, such as AGEs, may alter tissue function through direct effect on the collagen structure resulting in compromised bone matrix, as well as on the mesenchymal stem cell differentiation, balance between osteoblastic and osteoclastic activity reducing osteoblast proliferation and function and increasing osteoclast-related bone resorption, features strongly indicative of delayed healing and deteriorated bone quality [23–28].

The interplay between surgical technique, implant macrogeometry, implant surface topography and chemical properties may affect the healing pattern, rate and extent of osseointegration, mainly in low-quality bone and in systemically-compromised patients [29–33]. Screw-type implant design with large-pitch dual thread with an outer-to-inner thread diameter difference have been engineered to allow a mismatch between the instrumented bone and implant surface, creating healing chambers [32]. Such a design provides a favorable primary stability at the outer thread tip and immediate blood clot filling at the healing chambers, which rapidly evolves towards osteogenic tissue promoting hastened osseointegration in a hybrid healing pathway [29–33]. This scenario may compensate for the implant stability loss due to compression where implant threads contact bone for primary stability and maximize the interaction of the newly-developed complex implant surfaces, such as the bioactive calcium phosphate (CaP) coated nanostructured surfaces, resulting in early secondary stability with improved bone tissue mechanical properties [29–34].

Given the sparse clinical evidence concerning the influence of metabolically compromised systemic conditions on dental implant therapy [10,13], especially on the osseointegration parameters of complex design implants, the aim of the current study was to evaluate clinical outcomes, histomorphometric and nanomechanical properties of human retrieved implant/bone interface around a large-pitch dual-thread implant macrogeometry with a bioactive nanostructured CaP coated surface placed in the edentulous mandible of healthy and metabolic syndrome patients. The postulated null hypotheses were that (i) metabolic syndrome would not influence osseointegration clinical outcomes and (ii) metabolic syndrome would not influence osseointegration histological parameters nor bone nanomechanical properties of human retrieved implants.

2. Materials and methods

2.1. Clinical analysis

This prospective controlled clinical study was approved by the Ethics and Research Committee of the University of Grande Rio (Unigranrio) (Report number 70214017.0.00005283).

Healthy and metabolic syndrome (MS) patients under dental treatment at the School of Dentistry (Unigranrio) were recruited in the current study between 08/2017 and 07/2018, to evaluate peri-implant healing outcomes (n = 12 patients/condition). The patient's inclusion criteria were: 35 years of age or older (male or female), edentulous mandible with a desire to receive an implant-supported prosthesis, and sufficient bone volume for implant placement without the need for bone augmentation: at least a 5.0 mm diameter and 10.0 mm length. The exclusion criteria were alcoholism, smoking, use of illicit drugs, heart diseases, diabetes, previous bone regenerative procedures, bleeding disorders, compromised immune system, irradiated patients, treatment with steroids or bisphosphonates in the past 12 months. Each patient received detailed description of the study protocol, signed the inform consent form and gave written approval to be included in the study population.

Anthropometric measurements, blood pressure monitoring, and blood analyses were obtained from all patient venous blood drawn early in the morning after an overnight fast for systemic condition evaluation. MS patients were diagnosed by the evidence of 3 or more risk factors [16,17]: waist circumference - ethnicity specific values - as a measure of central obesity (males: > 90 cm and females: > 80 cm), elevated triglyceride levels (> 150 mg/dl, or specific treatment for this lipid abnormality); reduced HDL cholesterol levels (< 40 mg/dl in males and < 50 mg/dl in females, or specific treatment for this lipid abnormality); elevated blood pressure (systolic > 130 mmHg or diastolic > 85 mmHg, or treatment of previously diagnosed hypertension), elevated plasma glucose (fasting plasma glucose > 100 mg/dl, or specific treatment for elevated glucose).

All patients underwent cone-beam computerized tomography (CBCT) scans prior to implant placement for surgical planning and assessment of bone dimensions around the implantation site. A grade IV titanium implant (3.5 × 10 mm) with macrogeometry comprised of a large-pitch dual-thread implant with a bioactive nanostructured CaP coated surface over dual acid etched (Unitite U, S.I.N Implant System, Sao Paulo, SP, Brazil) was utilized for the current study, which has previously demonstrated high initial stability within the healing chambers [32]. A prototype implant (3.0 × 5 mm) with the same macrogeometry and surface treatment was used for retrieval for histologic and nanomechanical evaluation.

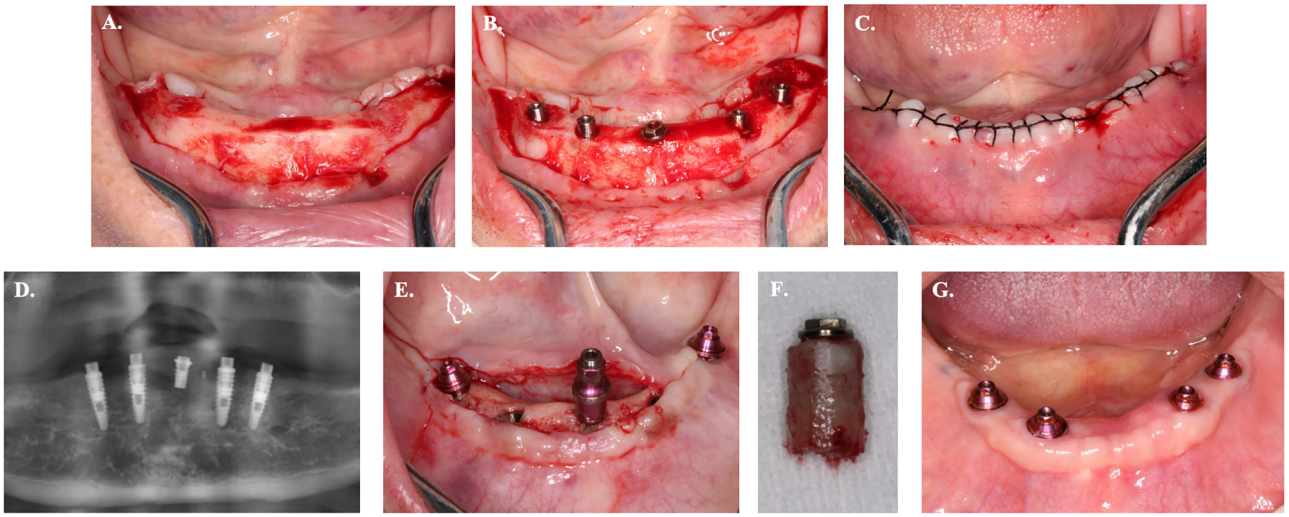

Fig. 1. Figure 1. Representative images of implant surgery (A-C), panoramic radiograph (D) of implants at the time of reopening surgery (E), retrieved implant (F), and abutment installation for prosthesis fabrication (G).

Fig. 1 (A–C) illustrates location of implant placement for restorative and retrieval purposes. Four conventional implants were placed between the mental foramina; the prototype implant was installed in the central region of the anterior mandible using a single-stage surgical protocol. After anesthesia, full-thickness flap elevation and implant osteotomy were performed following the manufacturer's recommendation under continous irrigation. The insertion of the implants was performed without irrigation at 20 rpm and installation was finalized with a surgical torque wrench. Insertion torque (IT) values were recorded as the maximum torque value (N.cm) reached at the end of implant insertion. IT data were dichotomized as values ≤ 30 Ncm or > 30 Ncm.

Subsequently to final seating of the implant, a device (type 16 Smartpeg®, Article no.100388, Ostell/Integration Diagnostics, Gothenburg, Sweden) was attached to each implant and a resonance frequency analysis (RFA) was performed (OsstellMentor device, OstellIntegration Diagnostics, Gothenburg, Sweden) to record implant stability quotient (ISQ) values of all implant surfaces. Healing abutments were placed after implant installation, and the incision was sutured closed th and sutures removed 1 week after surgery. All patients were instructed to follow a doughy and cold diet for the first 3 days after surgery, along with instructions for oral hygiene. They received a prescription with amoxicillin 500 mg, one tablet every 8 h for 7 days, starting 1 h before surgery. Additional prescriptions included anti-inflammatory and analgesic drugs for 3 days, nimesulide 100 mg every 12 h and paracetamol every 8 h.

After 60 days of healing, the implants were reopened, and healing abutments replaced by implant abutments for prostheses fabrication. At this stage, ISQ values were also recorded, the prototype implant was retrieved and placed in 10 % formaldehyde before histological preparation (Fig. 1 D–G).

2.2. Histological analysis

The histology processing followed a step-by-step dehydration protocol in ethanol and methyl salicylate, as previously reported in preclinical *in vivo* models [34,35]. The retrieved samples were stored in 70 % ethanol for 24 h and subsequently washed under running water for an additional for 24 h. Thereafter, progressive dehydration was performed through a series of alcohol solutions ranging from 70 % to 100 % ethanol and methyl salicylate. All samples were embedded in a methacrylate-based resin according to the manufacturer's instructions (Technovit 9100, Heraeus Kulzer GmbH, Wehrheim, Germany).

The resin blocks were sectioned longitudinallyinto slices using a low-speed precision diamond saw (Isomet 2000, Buehler Ltd. Lake Bluff, IL, USA) in order to provide non-decalcified histological sections of approximately 300 µm thickness. Each section was glued to an acrylic plate by a photolabile acrylate-based adhesive (Technovit 7210 VLC adhesive, Heraeus Kulzer GMBH, Wehrheim, Germany). Grinding and polishing process was performed under constant water irrigation with grit silicon carbide (SiC) abrasive papers (600, 800, and 1200) (Metaserv 3000, Buehler Ltd., Lake Bluff, IL, USA) to a thickness of approximately 100 µm. Subsequently, the samples were stained with Stevenel's Blue and Van Giesons's Picro Fuschin (SVG) stains and scanned via an automated slide scanning system and specialized computer software (Aperio Technologies, Vista, CA, USA).

For histomorphometry, an imaging analysis software (ImageJ, NIH, Bethesda, MD, USA) was used to quantify and evaluate osseointegration parameters around peri-implant surface, as previously detailed [33,36,37]. The dependent variables of the present study were percentage of bone-to-implant contact (%BIC) and bone area fraction occupancy (%BAFO). All evaluations were performed in a blinded manner.

2.3. Nanomechanical analysis

For nanomechanical analysis, indentation was performed in the histological sections, with 30 indentations per sample, using a nanoindenter (Hysitron nanoindenter, Minneapolis, MN, USA) equipped with a Berkovich diamond 3-sided pyramid probe. Mechanical testing was performed within the threaded regions between the first and second plateau or the initial set of inter-plateau spaces containing new bone. Bone tissue within these regions was initially detected via imaging using the optical microscope of the nanoindenter (Hysitron nanoindenter). A loading profile was established after pilot tests with a peak load of 300 µN at a rate of 60 µN/sec, followed by a holding time of 10 s and an unloading time of 5 s. The extended holding period allowed bone to relax to a linear response, so that no tissue creep effect occurred in the unloading portion of the profile [38].

Therefore, from each indentation, a load-displacement curve was generated allowing the calculation of the reduced elastic modulus, Er (GPa), and hardness, H (GPa), of the bone tissue via software (Hysitron TriboScan, Hysitron nanoindenter) using the following equation, respectively:

$$E_r = \frac{\sqrt{\pi}}{2\sqrt{A(h_c)}} \times S \quad H = \frac{P_{max}}{A(h_c)}$$

where S is the stiffness, hc is the contact depth, Pmax is the maximum

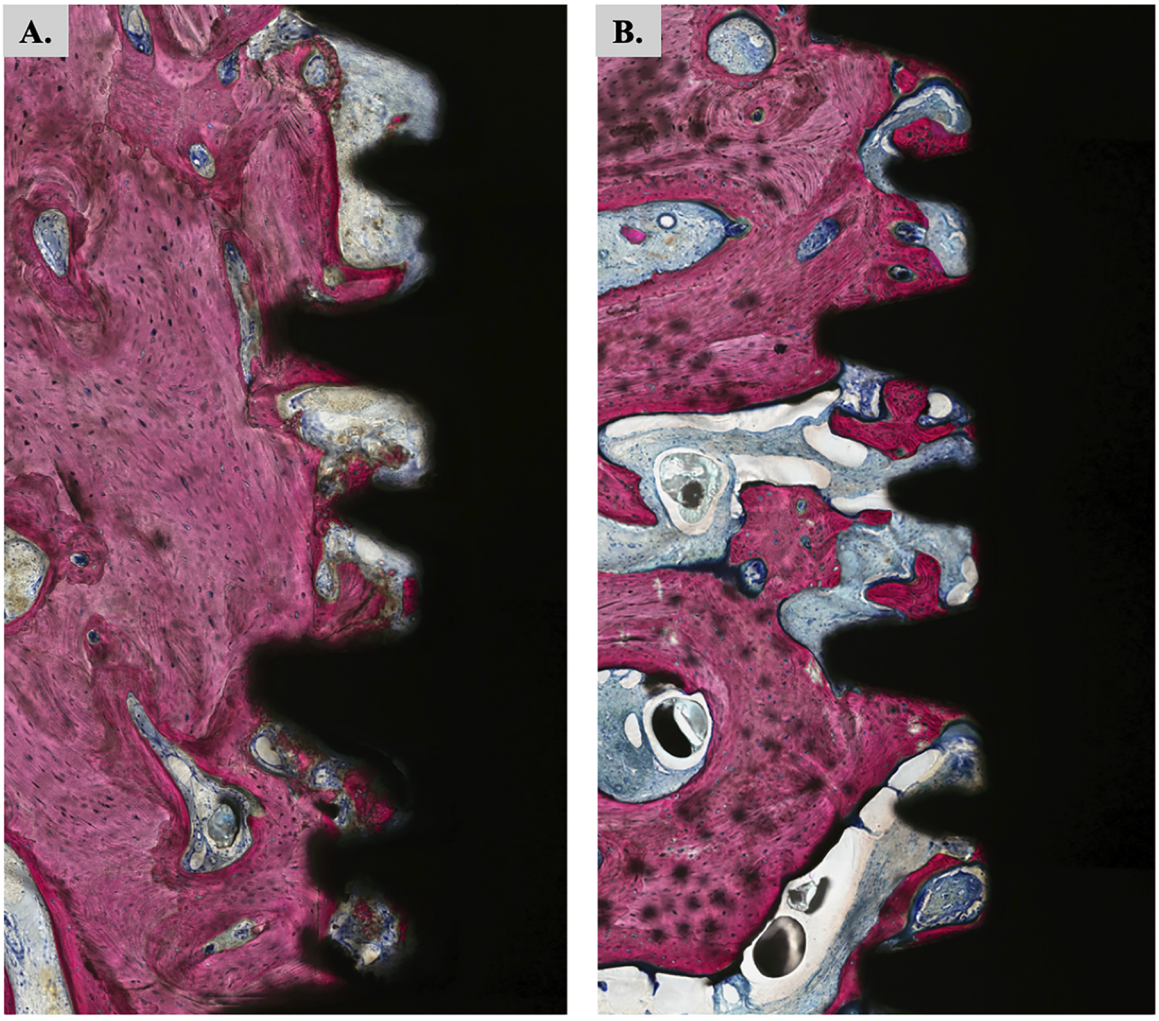

Fig. 2. Histological micrographs of implants retrieved from healthy (A) and MS patients (B).

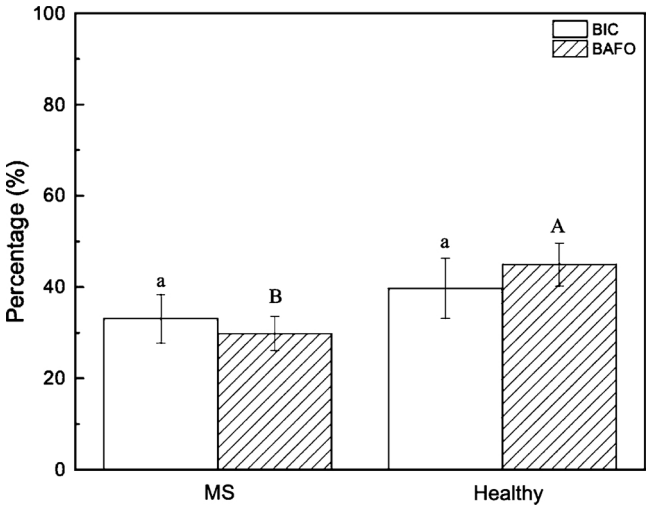

Fig. 3. Percentage of bone to implant contact (%BIC) and bone area fraction occupancy (%BAFO) of MS and healthy individuals. Different letters indicate statistically significant difference ($p < 0.05$).

applied force (300 μN), and A(hc) is the contact area computed from the TriboScan software utilizing the area function with respect to the contact depth. Through the reduced elastic modulus Er, the corresponding elastic modulus Em (GPa) could be calculated using the following equation:

$$\frac{1}{E_r} = \frac{1 - v_b^2}{E_m} + \frac{1 - v_i^2}{E_i}$$

where vb (0.3) is the Poisson's ratio for cortical bone, Ei (1140 GPa) is the elastic modulus of the indenter, and vi (0.07) is the Poisson's ratio for the indenter [38]. Bone mechanical properties using nanoindentation has previously been detailed [34,35,38].

2.4. Statistical analysis

A sample size calculation was performed based on the preliminary data obtained in current study for clinical and histological parameters associated with implant stability, IT and ISQ, and bone formation, %BIC and %BAFO, respectively. The minimum sample size calculated to obtain a statistical test power of 80 %, a 5% alpha error, and an effect size of 0.31 for IT and ISQ were 82 and 64, respectively. Similarly, the minimum sample size to obtain a power of 80 %, a 5% alpha error within an effect size of 0.67 and 2.2 for %BIC and %BAFO was 16 and 4 implants, respectively. Altogether, the study should evaluate at least 82 and 16 implants for clinical and histological parameters, respectively (G*Power 3.1, HHU University, Germany). Descriptive statistics including mean values and the corresponding standard deviation or 95 % confidence interval were calculated for each variable. Analyses of waist circumference and blood tests data, %BIC, %BAFO, Em and H data have demonstrated normal distribution (Shapiro Wilk test, all $p > 0.05$),

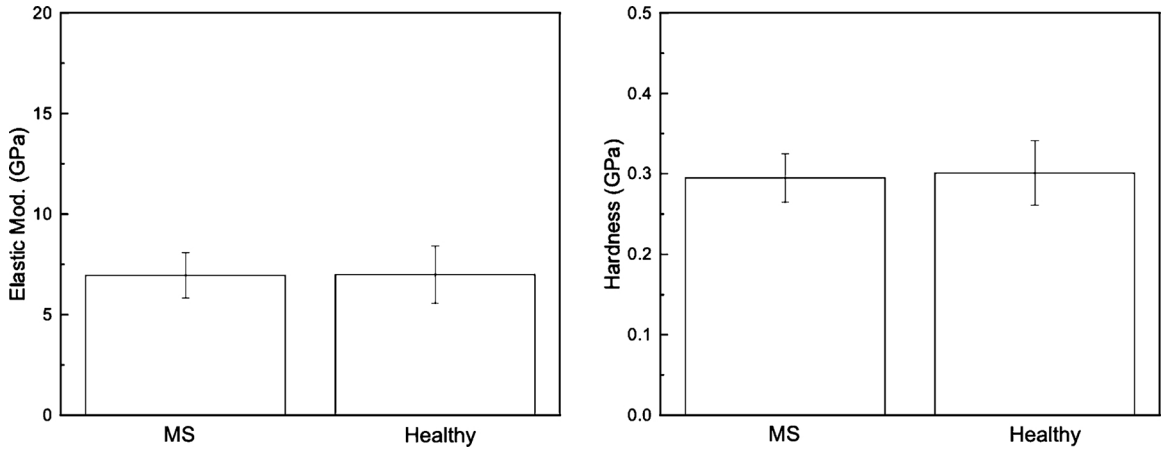

Fig. 4. Nanomechanical properties, Elastic Modulus and Hardness (GPa), of MS and healthy individuals. Groups were statistically homogeneous ($p > 0.804$).

and systemic conditions (MS versus Healthy) outcomes comparisons were gathered using Student's t test. Repeated-measures analysis of variance was used for ISQ data analysis with fixed factors of health condition and healing time. Chi-square test was used to investigate the association between IT (≤ 30 or > 30 Ncm) and blood pressure ($> 130 \times 85$ or $< 130 \times 85$ mm Hg) values and systemic condition. Kaplan-Meier analysis was performed for implant osseointegration survival estimate. The analyses were accomplished using a significance level of 5% (IBM SPSS 23, IBM, Armonk, NY).

3. Results

This study initially included 24 patients; however, 3 patients were excluded from the study (2 from the metabolic syndrome - MS group and 1 from the healthy group) because they missed implant retrieval and reopening surgery time. The final study population comprised of 10 female (healthy: 4 and MS: 6) and 11 males (healthy: 7 and MS: 4) patients, with an average age of 64 years (healthy: 63 years and MS: 66 years). A total of 105 implants were placed in the anterior mandible, being 21 retrieved for histology.

The anthropometric measurement of waist circumference, blood pressure and blood tests results are depicted in Table 1. Although healthy patients presented more positive results for waist circumference, triglycerides and high-density lipoprotein cholesterol (HDL-c) levels, they were not significantly different from MS individuals ($p > 0.516$). Sixty percent of individuals included in the MS group present blood pressure greater than 130×85 mm Hg, while 40 % of healthy patients were included in this category, all under drug treatment for hypertension. Statistically significant higher glucose levels were recorded for MS relative to healthy individuals ($p < 0.001$).

Overall, the current clinical findings demonstrated an uneventful healing, with no signs and symptoms of peri-implant tissue inflammation and/or infection and implant mobility at the time of implant reopening surgery. Only one implant was lost during the healing period in the healthy group, showing no correlation with compromised metabolic conditions, which led to an implant survival rate of 99 % for healthy and 100 % for MS groups.

The insertion torque (IT) registered by the surgeon showed a non-significant association with systemic condition ($p = 0.216$), with a similar percentage of MS and healthy patients presenting IT ≤ 30 Ncm (-64 %) and ≥ 30 Ncm (-36 %). Similarly, resonance frequency analysis (RFA) showed no significant effect of systemic condition and healing time on the ISQ values ($p > 0.181$). Thus, healthy (baseline: 66 and 60 days: 89) and MS (baseline: 64 and 60 days: 72) individuals presented high ISQ values, irrespective of healing period.

Histological micrographs of retrieved implants depicted absence of epithelial and connective tissue apical migration and similar bone morphology for both systemic conditions, woven bone formation in intimate contact with the implant surface and within the healing chambers (Fig. 2). Histomorphometric measurements of %BIC showed no statistically significant difference between both systemic conditions, MS (33 ± 5.3 %) and healthy (39 ± 6.5 %) ($p > 0.116$), whereas significantly higher %BAFO was observed for healthy (45 ± 4.6 %) relative to MS (30 ± 3.8 %) patients ($p < 0.001$) (Fig. 3).

Nanomechanical results showed no significant influence of health systemic condition on bone mechanical properties ($p > 0.804$), with both groups presenting hardness of approximately 0.3 (± 0.02) GPa and elastic modulus of 6.9 (± 1.2) GPa (Fig. 4).

4. Discussion

The current study evaluated clinical outcomes, nanomechanical and histomorphometric osseointegration parameters of human retrieved implants presenting large-pitch dual-thread macrogeometry with a bioactive nanostructured CaP coated surface placed in the edentulous mandible of patients with healthy and metabolic syndrome (MS) systemic conditions. The clinical outcomes demonstrated no significant association of health condition on the insertion torque (IT) and implant stability quotient (ISQ) values, as well as a typical healing process with no signs and symptoms of peri-implant tissue inflammation or infection and similar ISQ values to baseline for both systemic conditions, leading to high implant success rate (approximately 99 %). Therefore, the first postulated null hypothesis that metabolic syndrome would not influence osseointegration clinical outcomes was not rejected. While the metabolically compromised systemic condition showed no significant influence on new bone morphology, amount of bone-to-implant contact (%BIC) and new bone mechanical properties, the histomorphometric parameter of osseointegration, bone area fraction occupancy (%BAFO),

Table 1
Waist circumference and blood test data for both patient groups (mean ± standard deviation).

	Waist Circumference (cm)	Triglycerides (mg/dl)	HDL-c (mg/dl)	Blood Pressure > 130 × 85 mm Hg	Glucose (mg/dl)
MS	101 (± 10) a	151 (± 69) a	51 (± 11) a	60 % a	111 (± 27) a
Healthy	96 (± 11) a	141 (± 72) a	51 (± 10) a	40 % a	92 (± 6) b

Different letters indicate statistically significant difference between health conditions ($p < 0.05$).

exhibited significantly lower percentage for patients with metabolic syndrome relative to healthy. Therefore, the second postulated null hypothesis that metabolic syndrome would not influence osseointegration histological parameters nor bone nanomechanical properties of human retrieved implants was rejected.

Although implant-supported reconstructions have demonstrated reliable long-term clinical outcomes [5,8], their predictability chiefly relies on the achievement of a successful implant osseointegration [6,7], which is a highly dynamic and continuous process dependent on a healthy bone metabolism that dictates immune-inflammatory response and, consequently, the rate and extent of peri-implant new bone formation [21,22]. The kinetics of immune-inflammatory response around dental implants, including the hemostasis, inflammation, proliferation and remodeling processes, has shown to be impaired in metabolically compromised health conditions [21,22], however, the main body of literature has focused on the influence of Diabetes Melittus (DM), especially type 2 DM, on osseointegration establishment and its maintenance over time [12,39]. Given the parallel pathophysiology between MS and type 2 DM (about 90 % of type 2 DM has been associated with MS progression) and the results of highly-translational preclinical animal studies showing a similar level of osseointegration impairment for both metabolic diseases (MS and type 2 DM) [15,16,29], clinical concerns arise when planning implant-supported rehabilitations in individuals with MS, which are frequently treated as healthy obese from the standpoint of local and systemic disturbances before and after surgical procedures [14]. Such assumptions suggest that clinicians should also consider MS patients as high-risk for implant failure and support the current investigation of clinical and human retrieved histological outcomes of osseointegration to clarify the effect of MS health condition on peri-implant tissue healing, even from a preliminary perspective.

The present clinical outcomes yielded with one implant lost during the healing period in the healthy group, leading to implant survival rates of at least 99 % for both groups. Similarly, the IT and ISQ data analyses demonstrated no significant association of health systemic condition on the results, with a similar percentage of MS and healthy patients presenting IT values ≤ 30 Ncm (-64 %) and > 30 Ncm (-36 %) and ISQ values higher than 60, irrespective of healing period. Also, IT and primary stability registered by ISQ showed no correlation, which corroborates with previous studies findings and indicates that attempts to perform direct correlation between methods may frequently be inaccurate [40,41]. In fact, implant primary stability is a prerequisite for successful osseointegration and prevention of biomechanically-induced early failures, irrespective of systemic condition [40,42]. IT is the numerical expression of bone-to-implant interlocking and implant primary stability, which has been significantly associated with bone availability and quality, surgical protocol, and implant design [29,30,40,42]. It is noteworthy that the majority of patients included in the current investigation presented IT values equal or lower than 30 N.cm, which despite being considered adequate for primary stability and for osseointegration success it is not usually classified as a high IT (IT $\geq$ 45 Ncm) [40,42]. The rationale for the moderate IT for both systemic conditions may lie on implant macrogeometry, which has previously demonstrated to achieve lower primary stability relative to tight-fit implants due to the osteotomy-implant walls mismatch and healing chambers formation [29–34]. Nonetheless, such empty spaces are promptly filled with blood clot that will be the precursor of the new bone formation through intramembranous-like pathway promoting rapid secondary stability [29–34], as corroborated by the similar ISQ values at baseline and after 60 days of healing. Tight-fit placed implants (IT $\geq$ 50 cm) might show significant differences in the biological response, with bone tissue evolving into a purely interfacial bone remodeling healing pathway that is a cell-mediated resorption as a result of bone compression and subsequent bone apposition [29–34]. Approximately 10 % reduction has been reported in the ISQ values after 60–90 days for IT $\geq$ 45 Ncm [40]. Despite sparse clinical investigations comparing the benefits of the interplay between macrogeometry, chemically/topographically complex surfaces, and osteotomy dimensions on implant survival rates, *in vitro* and preclinical biomechanical results have motivated their use for compromised systemic conditions that affect bone healing kinetics [29–34].

Histological micrographs of human retrieved implants depicted a similar bone morphology for both systemic conditions, with woven bone formation within the healing chambers. Such observations corroborate with the nanomechanical results where no significant influence of systemic condition on bone mechanical properties was observed, with both groups presenting hardness of approximately 0.3 ($\pm$ 0.02) GPa and elastic modulus of 6.9 ($\pm$ 1.2) GPa, similarly to previous data obtained for woven bone formation around bioactive CaP coated nanostructured surfaces using the same nanoindentation protocol [34]. Previous histological analyses of human retrieved implants evaluating different timepoints have exhibited the maturation progress of such clinical scenario, where the woven bone formed within the healing chambers was replaced by lamellar bone and evolved towards a cortical-like configuration that only increased bone mechanical properties over time [43,44].

Histomorphometric parameters indicate similar %BIC between both systemic conditions, whereas significantly higher %BAFO was calculated for healthy relative to MS patients. %BIC and %BAFO are well-established histological parameters for osseointegration evaluation in the scientific literature [29,30,34,35,43,45,46]. While %BIC quantifies the degree of osseointegration derived from primary stability by measuring the percentage of bone along with implant surface perimeter, %BAFO evaluates the degree of osseointegration derived from secondary stability by measuring the percentage of bone within the implant threads [46]. Therefore, the decreased %BAFO in metabolically compromised relative to healthy individuals may be associated with the delayed bone healing reported in previous *in vitro* and preclinical studies as a result of vascular supply reduction secondary to microangiopathies, perpetuated pro-inflammatory state and decreased host immune resistance, and accumulation of toxic metabolites in MS [23–26]. Such metabolically triggered disturbances directly affect bone formation processes, including compromised collagen structure, downregulation of mesenchymal stem cell differentiation, reduced osteoblast proliferation and function and enhanced osteoclast-related bone resorption [23–28]. Although not reflected in some clinical outcomes, critical anabolic events required for new bone formation seems to be suppressed in MS, leading to an overall delaying in robust implant integration relative to healthy individuals. Such premises, along with the fact that IT values were chiefly lower than 30 N.cm [40,42], suggest that clinicians should allow longer healing time before loading implants in systemically compromised patients. The ideal time for prosthetic loading after implant placement where increased bone amount in the peri-implant area is favorable for long-term bone level maintenance still demands further investigations.

Anthropometric measurements and blood assays similarity between both systemic conditions may be associated with the included population, which was particularly composed of overweight elderly people (mean waist circumference > 90 cm and -65 years). Given the MS diagnosis criteria adopted in the current study, where the presence of any 3 of the 5 proposed risk factors constituted MS diagnosis, many individuals in the control group presented 2 or less comorbidities, affecting the average value calculated for each risk factor. The only factor that was systematically considered as an exclusion factor for the inclusion of individuals in the control group was glucose level, as there is substantial evidence regarding bone healing impairment in hyperglycemic individuals [23,45]. In fact, it has been well established the association of aging and the presence of various comorbidities, especially in overweight individuals [47,48], which indicates the need for a more distinct patient selection in future studies to estimate and compare implant survival and peri-implant tissue stability.

In fact, the current clinical and histological outcomes provided an

initial understanding of the osseointegration kinetics in patients diagnosed with MS, thus similar studies with long-term follow-ups are required to compare implant survival and peri-implant tissue maturation over time to estimate the ideal loading time. Moreover, prospective clinical studies, encompassing surgical and prosthodontics factors associated with pro-inflammatory metabolic conditions, which have shown to lead to increased levels of onset and progression of peri-implant diseases through severe tissue breakdown with time, are warranted [9–14,49].

5. Conclusion

Although no significant influence on clinical parameters and bone nanomechanical properties was observed, MS significantly reduced the amount of bone formation in the peri-implant area in the short-term.

CRediT authorship contribution statement

Rodrigo Granato: Conceptualization, Funding acquisition, Investigation, Writing - review & editing. **Edmara T.P. Bergamo:** Investigation, Formal analysis, Writing - original draft. **Lukasz Witek:** Investigation, Formal analysis, Writing - review & editing. **Estevam A. Bonfante:** Conceptualization, Investigation, Writing - review & editing. **Charles Marin:** Investigation, Writing - review & editing. **Michael Greenberg:** Investigation. **Gregory Kurgansky:** Investigation. **Paulo G. Coelho:** Conceptualization, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jdent.2020.103436.

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