# The Effect of Delayed Versus Early Loading on Nitric Oxide Metabolism Around Dental Implants: An 18-month Comparative Follow-up Study

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***Purpose:*** *Nitrite is a stable end-product of nitric oxide oxidation. The aim of the present study was to quantitatively analyze peri-implant sulcular fluid (PISF) nitrite levels in a longitudinal study design to evaluate the potential changes in nitric oxide metabolism in relation to the clinical status of the peri-implant site and the loading style of the dental implants.* ***Materials and Methods:*** *A total of 34 implants, either early loaded (EL) or delayed loaded (DL), in 17 patients were followed up for a period of 18 months. Clinical parameters were recorded, PISF samples were obtained, and PISF nitrite levels were spectrophotometrically determined. Clinical measurements and nitrite analysis were repeated at 1, 3, 6, 9, 12, and 18 months.* ***Results:*** *Despite the gradual decrease in clinical parameters, fluctuations in PISF total nitrite levels were observed during follow-up. The pattern of nitric oxide metabolism, as reflected by PISF nitrite levels, also demonstrated differences between EL and DL implants that diminished toward the end of the experimental period.* ***Discussion:*** *Although the presence of clinical and subclinical gingival inflammation contributes to the PISF total nitrite levels, nitric oxide metabolism is also associated with healing and bone remodeling, and the pattern of loading seemed to have an impact on nitric oxide production at dental implant sites.* ***Conclusion:*** *Nitric oxide production at dental implant sites seems to be tightly regulated to enable the maintenance of peri-implant bone.* Int J Oral Maxillofac Implants 2007;22:53–62

**Key words:** *comparative studies, dental implants, loading, longitudinal study, nitric oxide, nitrite*

The free radical nitric oxide is synthesized from arginine via the activity of constitutive and inducible isoforms of nitric oxide synthases (cNOS and iNOS).[1–4] Since nitric oxide is a highly reactive molecule, it has many potential target molecules and is involved in the regulation of many physiologic processes, such as inhibition of platelet adhesion and aggregation, vasodilation, host defense against infectious agents such as fungi and parasites, neurotransmission, and cell-to-cell communication.[1–4] However, nitric oxide is also regarded as harmful because of its direct cytotoxic or cytostatic actions, such as stimulation of the release of proinflammatory mediators such as peroxynitrite, which mediates cytotoxic effects of nitric oxide, and interleukin-6, tumor necrosis factor, and interferon gamma, which are capable of stimulating nitric oxide production in bone cells.[5–7]

The role of nitric oxide in the pathogenesis of inflammation is well-documented.[1–3,7] Various studies have demonstrated increased iNOS expression in gingivitis and periodontitis, the contribution of macrophages and endothelial cells to nitric oxide production,[8,9] and periodontal destruction resulting from high levels of iNOS from macrophages in localized aggressive periodontitis.[5] Polymorphonuclear

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cells have also been reported to express significant iNOS, and it has been suggested that they act as an important source of nitric oxide in gingivitis and localized chronic periodontitis.[10]

End metabolites of nitric oxide metabolism are nitrate (formed by reaction with oxyhemoglobin) and nitrite (formed from spontaneous and rapid autoxidation reactions in aerobic solutions). While cNOS isoforms produce low nitric oxide concentrations for a short period of time, iNOS, which is expressed in response to inflammatory stimuli, results in the production of high amounts of nitric oxide production for long periods.[1,2,8,10] Thus, autoxidation of nitric oxide is dependent upon its own concentration, and the end-product nitrite predominates at inflammatory sites.[11] Unlike its constitutive forms, the activity of iNOS is not regulated, and therefore much of the nitrite of body fluids is formed from oxidation of nitric oxide produced by iNOS.[1–3,10]

Nitric oxide affects bone cell function, bone maintenance, and remodeling.[1–3,12] There is considerable evidence that fluid flow-induced shear stress subsequent to mechanical strain or stress rapidly stimulates nitric oxide release from osteoblasts, preosteoclastlike cells, and osteocytes.[12,13] A nitric oxide precursor (L-arginine) injection, together with the application of orthodontic force, results in significant increase in tooth movement and number of osteoclasts, whereas an NOS inhibitor (L-NAME) injection reduces tooth movements.[14,15] After rat molar tooth movement, iNOS activity increases in periodontal ligament and connective tissue between the roots of the moved teeth as well as around blood vessels.[16,17] This suggests that nitric oxide is an important biochemical mediator in response of periodontal tissue to orthodontic forces and has a primary role in the bone remodeling cycle.[14–17] With respect to hip replacement and aseptic loosening of orthopedic implants, moderate increases in the number of immunohistochemically iNOS+ cells have been detected in tissues containing particulate wear and implant debris, and a statistical correlation has been found between iNOS and the severity of osteolysis around prosthetic hip implants.[7] Despite differences in study designs and purposes, all of these studies support the idea that nitric oxide plays a role in bone metabolism.[7,12–15,17]

Based on considerable data suggesting the role of nitric oxide in the pathogenesis of inflammation and in bone metabolism, the aim of the present study was to quantitatively analyze the nitrite levels of peri-implant sulcular fluid (PISF), a stable end-product of nitric oxide oxidation, in a longitudinal study design to evaluate the potential changes in nitric oxide metabolism in relation to the clinical status of the peri-implant site and the loading style of dental implants.

## MATERIALS AND METHODS

### Patient Selection and Evaluation

Seventeen completely edentulous patients (9 female, 8 male) seeking prosthodontic rehabilitation were included in the study. The medical history of these patients was unremarkable; none were known to have allergies or metabolic bone diseases. They ranged in age from 42 to 65 years (mean age, 53 years). Patients were provided with surgical treatment consisting of dental implants and, subsequently, a complete mandibular prosthesis with 2 ball attachments. Ideal dental implant sites in the anterior mandibular area were determined by use of dental computerized tomography (CT) prior to surgical procedure. All patients received two 15-mm-long and 3.75-mm-diameter mandibular endosseous dental implants (Brånemark System; Nobel Biocare, Göteborg, Sweden). The same oral surgeon performed all dental implant surgery. All patients were advised of the treatment protocol and signed an informed consent form.

### Surgical Procedure, Prosthodontic Rehabilitation, and Onset of Loading

Local anesthesia was induced in the anterior mandible with Ultracain D-S (Hoechst Marion Roussel, Frankfurt, Germany). Full-thickness flaps were reflected, and implant sites were drilled 5 mm anterior to the mental foramina. The locations of the mental foramina were determined on dental CT scans obtained prior to the surgical procedure. Following the placement of implants in the sockets, resonance frequency analysis was used to evaluate both implants (Osstell, Integration Diagnostics, Sweden).[18] The transducer was mounted on the implants orthoradially with the upright part on the oral side and was tightened with a screw by hand. Implants with a stability quotient value greater than 65 (ie, those with high primary stability) were included in the study to provide a standardized methodology.[19] The flaps were subsequently closed with 4-0 sutures. Following surgery, patients were given a cold compress extraorally to minimize swelling and bleeding. A week after surgery, uneventful healing was observed in all cases.

A total of 34 mandibular dental implants were randomly divided into 2 groups based on the protocol used for loading: the delayed loading (DL) group (n = 16) or the early loading (EL) group (n = 18). The EL implants were connected to abutments immediately after surgery, and a definitive mandibular prosthesis was introduced within 5 days. DL implants were connected to abutments and loaded 3 months after surgery. All subjects were provided with prostheses by the same prosthodontist.

### Clinical Evaluation of Dental Implants

To determine the clinical status of the dental implant sites, probing depth (PD)[20] was measured and Plaque Index (PI),[21] Gingival Index (GI),[22] and the Gingival Bleeding Time Index (GBTI)[23] scores were recorded. To reduce the risk of any mechanical irritation at the sampling site, all clinical measurements were carried out after PISF sampling.[24] For further standardization, all clinical measurements were performed by the same periodontist. Clinical measurements were recorded at 1 (baseline), 3, 6, 9, 12, and 18 months of follow-up.

### PISF Sampling

PISF samples were obtained using standardized paper strips (Periopaper no. 593525, Ora Flow, Amityville, NY) according to the sampling method described by Rüdin and colleagues.[25] Briefly, following the isolation of the sampling area with cotton rolls, supragingival plaque was removed, and the site was gently air-dried to reduce any contamination with plaque and/or saliva. Special care was taken to minimize the level of mechanical irritation during PISF sampling, as this is known to affect the actual fluid volume in a given site. For sample collection, paper strips were placed at the entrance of the peri-implant sulcus and were inserted to a standardized depth (1 mm) at each site regardless of the PD. A standard sampling time of 30 seconds was also used. Samples with evidence of gingival bleeding were not included. These measures were considered necessary for the standardization of clinical sampling procedure.

To eliminate the risk of evaporation, paper strips with PISF were immediately transported to a previously calibrated Periotron 8000 (Ora Flow) for volume determination. Prior to sampling, the Periotron 8000 was switched on and allowed to warm up. A blank Periopaper strip was placed in the device, and the reading dial was set to 0.[26] The calibration of the Periotron 8000 was checked at periodic intervals and performed by triplicate readings as previously described.[27,28] The collected PISF was measured with the Periotron 8000, and Periotron units were converted to microliters by MLCONVERT.EXE software (Ora Flow).[27] Then, PISF samples were placed in sterile, firmly wrapped Eppendorf tubes and stored at –20°C until the day of laboratory analysis. PISF sampling was repeated with the same protocol at 3, 6, 9, 12, and 18 months during the follow-up period. The same periodontist conducted PISF collection for each patient at each follow-up visit.

### Determination and Quantification of Nitrite in PISF

To each PISF sample in the Eppendorf tube 130 mL of distilled water was added, and the samples were vigorously mixed for the extraction of nitrite into water. Then 100 mL of the extract were mixed with 0.5 mL of freshly prepared Griess reagent. After 10 minutes of incubation at room temperature, the absorbance of each sample was determined at 540 nm.[29] A standard curve was prepared using sodium nitrite to calculate nitrite concentration in PISF.

### Statistical Analysis

SPSS 11.5.0 software for Windows (SPSS, Chicago, IL) was used for all statistical analysis.

*Clinical Parameters*. The Shapiro-Wilk test was used to test the normality of distribution for the total sample and for DL and EL implants.[30] Since data were not normally distributed, the Friedman test was performed for comparison of all experimental time points.[31] For pairwise comparisons, the Wilcoxon signed rank test with Bonferroni correction was used.[32]

*Total Nitrite Levels and Nitrite Concentration*. The Shapiro-Wilk test was used to test the normality of distribution for the total sample and for DL and EL implants.[30] Since data were normally distributed (after logarithmic transformation), repeated-measures analysis of variance (ANOVA) was performed for the comparison of all experimental time points.[33] For pairwise comparisons, the Bonferroni test was used.[33] Further, sphericity assumption was not satisfactory, and Greenhouse-Geisser correction was used for total nitrite levels. *P* values less than .005 were considered statistically significant for clinical parameters, while *P* values less than .05 were considered statistically significant for total nitrite level and nitrite concentration.

## RESULTS

The results of the study are summarized in Tables 1 to 3. Descriptive data for clinical parameters and nitrite levels during 18 months of follow-up are given in Tables 1a and 1b, 2a and 2b, and 3a and 3b for all implants, DL implants, and EL implants, respectively. Comparative statistical analyses and the actual *P* values are provided in Tables 1c, 2c, and 3c for all implants, DL implants, and EL implants, respectively.

### Clinical Parameters

*All Implants.* The highest mean GI score for all dental implants was found at baseline. Significant reductions in mean score were observed at all experimental time intervals ($P < .005$). PD was signifcantly reduced at 6, 9, and 12 months compared to baseline ($P = .0001$), while GI score was significantly reduced at 9 and 12 months compared to baseline ($P = .001$).

During follow-up no statistically significant differences were observed for mean GBTI scores; however, the lowest GBTI score was recorded after 12 months. When compared to baseline PI scores, PI scores were not significantly different at any time interval ($P > .005$). Stability in all clinical parameters was observed between 12 and 18 months.

*DL Implants*. Except for PD and GI, the highest scores for all clinical parameters were observed at baseline. A general pattern of decrease was observed during follow-up. PD was significantly higher at baseline than at all subsequent experimental time points ($P < .005$), except the 18th month. Compared to baseline, GI scores demonstrated a pattern of decrease until 12 months. The lowest GBTI and GI scores were observed at 12 months. Generally, clinical parameters presented stability between 12 and 18 months.

*EL Implants*. The highest GI score was demonstrated at baseline. The lowest mean GI score was observed at 18 months; this was significantly lower than the baseline GI score ($P = .004$). PD decreased at all time intervals compared to baseline; the difference between PD at baseline and at 18 months was significant ($P = .003$). The highest mean GBTI score was found at baseline, and the lowest was observed at 18 months; mean GBTI scores stayed relatively stable throughout the 18-month follow-up period. The lowest mean PI scores were observed at 12 and 18 months. Further, the PI score demonstrated a pattern of decrease at 18 months compared to all other time intervals (not significant; $P > .05$). Between 12 and 18 months, stability was observed for all clinical parameters.

### Total Nitrite Levels and Nitrite Concentration

*All Implants*. The mean total nitrite level decreased from baseline to 3 months; thereafter, the level remained relatively stable until the 12th month. A significant decrease was observed at both the 12- and 18-month follow-ups ($P = .0001$). The lowest nitrite levels were observed at the 12th and 18th months. The lowest nitrite concentration was found at baseline ($P < .05$); the concentration increased over the 18-month follow-up period. Compared to the baseline level, nitrite concentration was significantly greater at the 3-, 6-, 9-, and 12-month follow-ups ($P < .05$).

*DL Implants*. Figure 1 demonstrates the pattern of nitrite levels increases and decreases for DL implants. Total nitrite levels demonstrated stability between 1 and 9 months, followed by a significant decrease at the 12th month ($P = .021$). The lowest nitrite level was observed at the 12th month; this decrease was significant compared to the 1st ($P = .021$) and 3rd months ($P = .016$). Nitrite level remained relatively stable from 12 to 18 months.

Nitrite concentration was significantly increased at all time points postbaseline; the increase was significant for 3rd month ($P = .001$). Moreover, nitrite concentration was significantly lower at 18 months compared to 3, 6, and 12 months of follow-up ($P < .05$).

*EL Implants*. When compared to baseline, a pattern of decrease at the 3rd month was observed in total nitrite levels, followed by a significant increase at the 6th month compared to the 3rd month ($P = .018$) (Fig 1). The highest nitrite level was observed at the 9th month, and this increase was significant when compared to the 3rd month ($P = .002$). The lowest nitrite levels were observed at the 12th ($P = .047$) and 18th ($P = .017$) months; nitrite levels at these points were significantly lower than baseline values. Total nitrite levels were stable between 12 and 18 months. Generally, nitrite concentration was lowest at baseline. Nitrite concentration was significantly greater at the 9-month follow-up ($P = .004$). Nitrite concentration dropped at the 18-month follow-up, although the drop was not statistically significant compared to 3, 6, 9, and 12 months ($P > .05$). Interaction between change in time intervals and loading type was found to be statistically significant for total nitrite levels ($P = .005$). However, interaction between time intervals and loading type for nitrite concentration was not statistically significant ($P = .173$).

## DISCUSSION

The presence of various cell types; the complexity of the mechanisms involved in the maintenance of bone mass, architecture, and remodeling; and the tight coordination of simultaneous bone formation and resorption make bone a very complex tissue.[3,12,34] In this regard, the impact of a mechanical stimulus on bone metabolism is of particular concern, since mechanical stimulation is essential for maintaining the homeostasis and architecture of bone.[3,12,34] Further, bone cells are highly responsive to mechanical stimuli, as they can be influenced differently by fluid shear, tension, and compression.[35]

Nitric oxide is among the various signaling molecules released in response to strain,[35] and bone anabolic responses to mechanical load involve cNOS.[3,12,13] While nitric oxide that diffuses to the alveolar bone influences osteoclastic differentiation, nitric oxide–dependent mechanisms also modulate the function of osteoblasts.[14] During orthodontic treatment, increased tooth movement after nitric oxide precursor application and reduction of tooth movement with nitric oxide inhibitors support the role of nitric oxide in bone remodeling.[14,15] As mechanical strain stimulates NOS activity in

**Table 1a Descriptive Data Regarding Clinical Parameters of All Dental Implants for 18 Months of Follow-up**

| | PI | | | PD (mm) | | | GBTI | | | GI | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Mo | Mean ± SD | Median | Min–Max | Mean ± SD | Median | Min–Max | Mean ± SD | Median | Min–Max | Mean ± SD | Median | Min–Max |
| 1 | 0.8529 ± 1.050 | 0.125 | 0.00–3.00 | 2.3456 ± 0.743 | 2.125 | 1.00–3.75 | 0.4926 ± 0.635 | 0.00 | 0.00–2.00 | 0.9653 ± 0.751 | 1.00 | 0.00–2.00 |
| 3 | 1.0143 ± 1.088 | 1.00 | 0.00–3.00 | 1.6857 ± 0.562 | 2.00 | 1.00–2.50 | 0.4763 ± 0.726 | 0.00 | 0.00–2.25 | 0.6571 ± 0.761 | 0.00 | 0.00–2.00 |
| 6 | 0.5242 ± 0.895 | 0.00 | 0.00–3.00 | 1.4839 ± 0.547 | 1.00 | 1.00–2.75 | 0.5081 ± 0.662 | 0.00 | 0.00–2.25 | 0.5806 ± 0.699 | 0.00 | 0.00–2.00 |
| 9 | 0.5714 ± 0.947 | 0.00 | 0.00–3.00 | 1.5625 ± 0.488 | 1.50 | 1.00–2.50 | 0.4018 ± 0.590 | 0.00 | 0.00–1.50 | 0.4554 ± 0.649 | 0.00 | 0.00–2.00 |
| 12 | 0.1875 ± 0.535 | 0.00 | 0.00–2.00 | 1.4062 ± 0.534 | 1.00 | 1.00–2.75 | 0.2891 ± 0.531 | 0.00 | 0.00–1.75 | 0.4453 ± 0.728 | 0.00 | 0.00–2.00 |
| 18 | 0.3077 ± 0.751 | 0.00 | 0.00–2.00 | 1.5769 ± 0.534 | 1.50 | 1.00–2.50 | 0.3077 ± 0.597 | 0.00 | 0.00–1.50 | 0.4808 ± 0.780 | 0.00 | 0.00–2.00 |

**Table 1b Nitrite Levels of All Dental Implants for 18 Months of Follow-up**

| | Total nitrite level (nmol) | | | Nitrite concentration (nmol/µl) | | |
|---|---|---|---|---|---|---|
| Months | Mean ± SD | Median | Min–Max | Mean ± SD | Median | Min–Max |
| 1 | 0.1478 ± 0.046 | 0.1357 | 0.085–0.246 | 0.3980 ± 0.2267 | 0.3849 | 0.119–1.118 |
| 3 | 0.1309 ± 0.042 | 0.1217 | 0.074–0.280 | 0.7250 ± 0.4840 | 0.5939 | 0.148–2.280 |
| 6 | 0.1334 ± 0.028 | 0.1357 | 0.085–0.195 | 0.9582 ± 0.9460 | 0.6787 | 0.247–4.667 |
| 9 | 0.1566 ± 0.053 | 0.1633 | 0.065–0.301 | 0.8826 ± 0.7600 | 0.6376 | 0.168–3.564 |
| 12 | 0.1034 ± 0.019 | 0.1009 | 0.074–0.143 | 0.7522 ± 0.6060 | 0.5568 | 0.185–2.895 |
| 18 | 0.1131 ± 0.018 | 0.1128 | 0.089–0.156 | 0.6244 ± 0.4978 | 0.4949 | 0.145–1.731 |

**Table 1c Comparative Statistical Data Regarding Clinical Parameters and Nitrite Levels of All Implants During Follow-up**

| | Months | | | | |
|---|---|---|---|---|---|
| | 3 | 6 | 9 | 12 | 18 |
| **1** | | | | | |
| PI | 0.5750 | 0.4020 | 0.4050 | 0.0210 | 0.3060 |
| PD | 0.0001* | 0.0001* | 0.0001* | 0.0001* | 0.0010* |
| GBTI | 0.4960 | 0.7200 | 0.3870 | 0.0350 | 0.9090 |
| GI | 0.0200 | 0.0350 | 0.0040* | 0.0010* | 0.0130 |
| Total nitrite level | 0.6440 | 1.0000 | 1.0000 | 0.0001* | 0.0001* |
| Nitrite concentration | 0.0001* | 0.0010* | 0.0001* | 0.0020* | 1.0000 |
| **3** | | | | | |
| PI | | 0.028 | 0.068 | 0.002* | 0.037 |
| PD | | 0.147 | 0.188 | 0.040 | 0.659 |
| GBTI | | 0.897 | 0.626 | 0.142 | 0.600 |
| GI | | 0.471 | 0.314 | 0.143 | 0.951 |
| Total nitrite level | | 1.000 | 1.000 | 0.970 | 0.118 |
| Nitrite concentration | | 1.000 | 1.000 | 1.000 | 0.022* |
| **6** | | | | | |
| PI | | | 0.905 | 0.106 | 0.8540 |
| PD | | | 0.746 | 0.657 | 0.0820 |
| GBTI | | | 0.554 | 0.068 | 0.8390 |
| GI | | | 0.638 | 0.406 | 0.6340 |
| Total nitrite level | | | 1.000 | 0.001* | 0.0001* |
| Nitrite concentration | | | 1.000 | 1.000 | 0.0150* |
| **9** | | | | | |
| PI | | | | 0.003* | 0.944 |
| PD | | | | 0.348 | 0.096 |
| GBTI | | | | 0.139 | 0.467 |
| GI | | | | 0.329 | 0.362 |
| Total nitrite level | | | | 0.004* | 1.000 |
| Nitrite concentration | | | | 1.000 | 0.015* |
| **12** | | | | | |
| PI | | | | | 0.118 |
| PD | | | | | 0.018 |
| GBTI | | | | | 0.053 |
| GI | | | | | 0.140 |
| Total nitrite level | | | | | 1.000 |
| Nitrite concentration | | | | | 0.015* |

Actual *P* value is given. * Indicates statistical significance.

**Table 2a Descriptive Data Regarding Clinical Parameters of DL Implants for 18 Months of Follow-up**

| | PI | | | PD (mm) | | | GBTI | | | GI | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Mo | Mean ± SD | Median | Min–Max | Mean ± SD | Median | Min–Max | Mean ± SD | Median | Min–Max | Mean ± SD | Median | Min–Max |
| 1 | 0.8472 ± 1.018 | 0.625 | 0.00–3.00 | 2.4027 ± 0.707 | 2.375 | 1.25–3.75 | 0.5694 ± 0.611 | 0.500 | 0.00–1.75 | 0.9750 ± 0.687 | 1.000 | 0.00–2.00 |
| 3 | 0.7375 ± 0.915 | 0.000 | 0.00–2.50 | 1.7875 ± 0.488 | 2.000 | 1.00–2.50 | 0.5875 ± 0.731 | 0.125 | 0.00–2.25 | 0.7375 ± 0.758 | 1.000 | 0.00–2.00 |
| 6 | 0.2656 ± 0.478 | 0.000 | 0.00–1.25 | 1.4843 ± 0.566 | 1.250 | 1.00–2.75 | 0.5312 ± 0.729 | 0.000 | 0.00–2.25 | 0.5937 ± 0.644 | 0.500 | 0.00–1.75 |
| 9 | 0.5357 ± 0.745 | 0.000 | 0.00–2.00 | 1.4821 ± 0.566 | 1.375 | 1.00–2.00 | 0.6250 ± 0.691 | 0.375 | 0.00–1.50 | 0.5357 ± 0.634 | 0.125 | 0.00–1.75 |
| 12 | 0.1250 ± 0.341 | 0.000 | 0.00–1.00 | 1.4843 ± 0.566 | 1.250 | 1.00–2.75 | 0.1250 ± 0.341 | 0.000 | 0.00–1.00 | 0.2812 ± 0.576 | 0.000 | 0.00–2.00 |
| 18 | 1.0000 ± 1.154 | 1.000 | 0.00–2.00 | 1.8750 ± 0.478 | 1.750 | 1.50–2.50 | 0.7500 ± 0.866 | 0.750 | 0.00–1.50 | 0.9375 ± 1.087 | 0.875 | 0.00–2.00 |

**Table 2b Nitrite Levels of DL Implants for 18 Months of Follow-up**

| | Total nitrite level (nmol) | | | Nitrite concentration (nmol/µl) | | |
|---|---|---|---|---|---|---|
| Months | Mean ± SD | Median | Min–Max | Mean ± SD | Median | Min–Max |
| 1 | 0.1408 ± 0.034 | 0.135 | 0.085–0.208 | 0.4136 ± 0.226 | 0.377 | 0.153–1.118 |
| 3 | 0.1455 ± 0.049 | 0.125 | 0.086–0.280 | 0.7716 ± 0.526 | 0.550 | 0.304–2.280 |
| 6 | 0.1279 ± 0.032 | 0.127 | 0.085–0.195 | 1.2613 ± 1.220 | 0.796 | 0.247–4.667 |
| 9 | 0.1392 ± 0.061 | 0.129 | 0.065–0.301 | 0.8712 ± 0.885 | 0.754 | 0.168–3.564 |
| 12 | 0.1028 ± 0.019 | 0.102 | 0.077–0.143 | 0.8651 ± 0.522 | 0.676 | 0.185–1.930 |
| 18 | 0.1158 ± 0.019 | 0.118 | 0.089–0.137 | 0.3850 ± 0.259 | 0.348 | 0.145–0.699 |

**Table 2c Comparative Statistical Data Regarding Clinical Parameters and Nitrite Levels of DL Implants During Follow-up**

| | Months | | | | |
|---|---|---|---|---|---|
| | 3 | 6 | 9 | 12 | 18 |
| **1** | | | | | |
| PI | 0.731 | 0.298 | 0.752 | 0.080 | 0.316 |
| PD | 0.002* | 0.004* | 0.001* | 0.001* | 0.095 |
| GBTI | 0.860 | 0.806 | 0.906 | 0.010 | 0.233 |
| GI | 0.363 | 0.219 | 0.049 | 0.005 | 0.813 |
| Total nitrite level | 1.000 | 1.000 | 1.000 | 0.021* | 0.053 |
| Nitrite concentration | 0.001* | 0.071 | 0.151 | 0.085 | 1.000 |
| **3** | | | | | |
| PI | | 0.051 | 0.253 | 0.011 | 0.943 |
| PD | | 0.056 | 0.014 | 0.026 | 0.323 |
| GBTI | | 0.813 | 0.937 | 0.011 | 0.236 |
| GI | | 0.372 | 0.289 | 0.032 | 0.464 |
| Total nitrite level | | 1.000 | 1.000 | 0.016* | 0.137 |
| Nitrite concentration | | 1.000 | 1.000 | 1.000 | 0.013* |
| **6** | | | | | |
| PI | | | 0.086 | 0.180 | 0.006 |
| PD | | | 0.857 | 0.959 | 0.009 |
| GBTI | | | 0.573 | 0.049 | 0.102 |
| GI | | | 0.643 | 0.103 | 0.189 |
| Total nitrite level | | | 1.000 | 0.337 | 1.000 |
| Nitrite concentration | | | 1.000 | 1.000 | 0.023* |
| **9** | | | | | |
| PI | | | | 0.014 | 0.055 |
| PD | | | | 0.730 | 0.007 |
| GBTI | | | | 0.017 | 0.137 |
| GI | | | | 0.121 | 0.065 |
| Total nitrite level | | | | 0.807 | 1.000 |
| Nitrite concentration | | | | 1.000 | 0.560 |
| **12** | | | | | |
| PI | | | | | 0.006 |
| PD | | | | | 0.012 |
| GBTI | | | | | 0.007 |
| GI | | | | | 0.016 |
| Total nitrite level | | | | | 0.611 |
| Nitrite concentration | | | | | 0.035* |

Actual *P* value is given. * Indicates statistical significance.

**Table 3a Descriptive Data Regarding Clinical Parameters of EL Dental Implants for 18 Months of Follow-up**

| | PI | | | PD (mm) | | | GBTI | | | GI | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Mo | Mean ± SD | Median | Min–Max | Mean ± SD | Median | Min–Max | Mean ± SD | Median | Min–Max | Mean ± SD | Median | Min–Max |
| 1 | 0.8088 ± 1.102 | 0.000 | 0.00–3.00 | 2.2941 ± 0.766 | 2.00 | 1.00–3.75 | 0.5588 ± 0.731 | 0.00 | 0.00–2.00 | 1.0441 ± 0.786 | 1.000 | 0.00–2.00 |
| 3 | 1.2205 ± 1.243 | 1.750 | 0.00–3.00 | 1.5441 ± 0.632 | 1.00 | 1.00–2.50 | 0.3482 ± 0.708 | 0.00 | 0.00–2.00 | 0.5441 ± 0.761 | 0.000 | 0.00–2.00 |
| 6 | 0.8000 ± 1.146 | 0.000 | 0.00–3.00 | 1.4833 ± 0.546 | 1.00 | 1.00–2.25 | 0.4833 ± 0.608 | 0.00 | 0.00–1.50 | 0.5666 ± 0.776 | 0.000 | 0.00–2.00 |
| 9 | 0.5312 ± 1.083 | 0.000 | 0.00–3.00 | 1.5937 ± 0.515 | 1.50 | 1.00–2.50 | 0.2500 ± 0.483 | 0.00 | 0.00–1.50 | 0.4375 ± 0.727 | 0.000 | 0.00–2.00 |
| 12 | 0.2352 ± 0.664 | 0.000 | 0.00–2.00 | 1.3382 ± 0.522 | 1.00 | 1.00–2.50 | 0.3382 ± 0.572 | 0.00 | 0.00–1.75 | 0.4705 ± 0.780 | 0.000 | 0.00–2.00 |
| 18 | 0.0000 ± 0.000 | 0.000 | 0.00–0.00 | 1.5000 ± 0.547 | 1.50 | 1.00–2.00 | 0.1666 ± 0.408 | 0.00 | 0.00–1.00 | 0.4166 ± 0.664 | 0.250 | 0.00–1.75 |

**Table 3b Nitrite Levels of EL Dental Implants for 18 Months of Follow-up**

| | Total nitrite level (nmol) | | | Nitrite concentration (nmol/µl) | | |
|---|---|---|---|---|---|---|
| Months | Mean ± SD | Median | Min–Max | Mean ± SD | Median | Min–Max |
| 1 | 0.1578 ± 0.053 | 0.144 | 0.086–0.246 | 0.3711 ± 0.225 | 0.342 | 0.119–0.848 |
| 3 | 0.1112 ± 0.021 | 0.110 | 0.074–0.144 | 0.6310 ± 0.422 | 0.579 | 0.148–1.838 |
| 6 | 0.1391 ± 0.024 | 0.135 | 0.102–0.191 | 0.6350 ± 0.327 | 0.543 | 0.266–1.364 |
| 9 | 0.1649 ± 0.043 | 0.176 | 0.086–0.221 | 0.8506 ± 0.623 | 0.550 | 0.234–2.302 |
| 12 | 0.1086 ± 0.023 | 0.108 | 0.074–0.156 | 0.7653 ± 0.740 | 0.474 | 0.196–2.895 |
| 18 | 0.1049 ± 0.007 | 0.105 | 0.095–0.113 | 0.4450 ± 0.141 | 0.429 | 0.272–0.653 |

**Table 3c Comparative Statistical Data Regarding Clinical Parameters and Nitrite Levels of EL Dental Implants During Follow-up**

| | Months | | | | |
|---|---|---|---|---|---|
| | 3 | 6 | 9 | 12 | 18 |
| **1** | | | | | |
| PI | 0.211 | 0.786 | 0.645 | 0.131 | 0.017 |
| PD | 0.004* | 0.012 | 0.006 | 0.005 | 0.003* |
| GBTI | 0.400 | 0.823 | 0.150 | 0.228 | 0.084 |
| GI | 0.018 | 0.084 | 0.029 | 0.027 | 0.004* |
| Total nitrite level | 0.112 | 1.000 | 1.000 | 0.047* | 0.017* |
| Nitrite concentration | 0.410 | 0.116 | 0.004* | 0.188 | 1.000 |
| **3** | | | | | |
| PI | | 0.218 | 0.302 | 0.035 | 0.006 |
| PD | | 0.692 | 0.841 | 0.261 | 0.572 |
| GBTI | | 0.753 | 0.528 | 0.906 | 0.340 |
| GI | | 0.905 | 0.698 | 0.721 | 0.152 |
| Total nitrite level | | 0.018* | 0.002* | 1.000 | 1.000 |
| Nitrite concentration | | 1.000 | 1.000 | 1.000 | 1.000 |
| **6** | | | | | |
| PI | | | 0.504 | 0.167 | 0.0170 |
| PD | | | 0.524 | 0.473 | 0.8420 |
| GBTI | | | 0.234 | 0.418 | 0.1060 |
| GI | | | 0.858 | 0.964 | 0.5000 |
| Total nitrite level | | | 1.000 | 0.001* | 0.0001* |
| Nitrite concentration | | | 1.000 | 1.000 | 1.0000 |
| **9** | | | | | |
| PI | | | | 0.066 | 0.0410 |
| PD | | | | 0.249 | 0.5970 |
| GBTI | | | | 0.553 | 0.7260 |
| GI | | | | 0.943 | 0.5270 |
| Total nitrite level | | | | 0.025* | 0.0001* |
| Nitrite concentration | | | | 1.000 | 0.1370 |
| **12** | | | | | |
| PI | | | | | 0.157 |
| PD | | | | | 0.705 |
| GBTI | | | | | 0.389 |
| GI | | | | | 0.752 |
| Total nitrite level | | | | | 1.000 |
| Nitrite concentration | | | | | 1.000 |

Actual *P* value is given. * Indicates statistical significance.

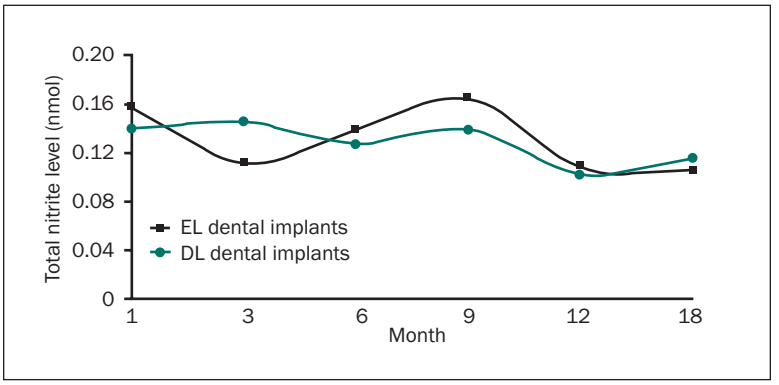


**Fig 1** Total nitrite levels (nmol) of DL and EL dental implants for 18 months of follow-up.

osteoblasts and osteocytes,[12,34] shear stress rapidly stimulates cNOS in osteoblasts. The small amounts of nitric oxide produced by osteoblasts act as an autocrine stimulator of osteoblast growth,[3] and nitric oxide serves as an essential regulatory molecule in both bone formation and resorption induced by mechanical stimuli.[14,15,36] Nitric oxide is an important autocrine/paracrine factor modulating the process of bone remodeling and is essential for mechanically-induced anabolic bone response.[12,36]

When all dental implants, including DL and EL implants, were examined, generally a gradual decrease in all of the clinical parameters was observed during follow-up. This can be interpreted as a sign of uneventful healing and clinical improvement at the peri-implant sites. However, such a gradual trend of decrease was not observed for the mean total nitrite levels, which presented clear fluctuations throughout the follow-up period. A reduction at the 3rd month was followed by relative stability until the 6th month and a slight increase at the 9th month. Significant reductions were then observed at the 12th and 18th months, and these experimental time points provided the lowest levels. When the potential source of nitrite in PISF is questioned, the contribution of gingival inflammation cannot be excluded. As cNOS is involved in short-lasting and low-level synthesis of nitric oxide, iNOS is responsible for long-lasting and high-level synthesis, and autoxidation of nitric oxide is dependent upon its own concentration, the end-product nitrite predominates at inflammatory sites.[11] Unlike constitutive forms, the activity of iNOS is not regulated, and therefore much of the nitrite of body fluids is formed from the oxidation of nitric oxide produced by iNOS.[1,2,10] iNOS is shown to be expressed in bone only in response to inflammatory stimuli[3] and PISF nitrite levels were suggested to depend on the severity of gingival inflammation at the dental implant sites.[37] Although severe gingival inflammation was not present at any experimental time period, the presence of slight clinical inflammation and associated subclinical inflammation at the peri-implant sites needs to be considered as a contributor of PISF nitrites to a certain extent, via the activity of iNOS.[3]

However, because of the presence of cNOS in bone tissue, this does not seem to be the sole source. The role of the widely expressed endothelial NOS (eNOS) includes modulation of the effect of mechanical loading on the skeleton to promote bone formation and suppress bone resorption.[3] Many previous studies have proposed an increase in nitric oxide production in bone remodeling and bone repair, mechanical loading, mechanical strain and shear stress, and the nitric oxide-dependent activities of osteoblasts and osteoclasts.[3,12,14,17,34-36,38,39] Defective bone formation and osteoporosis have occurred in eNOS knockout mice,[3] and the impact of loading of dental implants on nitric oxide metabolism has also been demonstrated previously.[37] Together with these studies, the fluctuations of PISF total nitrite levels observed in the present study suggest bone tissue and metabolism as an important source of the nitrite content of PISF.

There is considerable evidence that fluid flow-induced shear stress rapidly stimulates nitric oxide release from osteoblasts, preosteoclastlike cells, and osteocytes in response to mechanical strain or stress during bone remodeling.[12,13] Fluid flow-induced shear stress has been shown to induce nitric oxide production and thus to play a primary role in bone maintenance and remodeling.[12] Therefore, the present longitudinal study of dental implants supports the suggestions that nitric oxide plays a role in bone metabolism[14,16,38] and that force application has a clear impact on nitric oxide metabolism and bone remodeling.[3,12,14,34–36,38,39]

As nitric oxide is involved in both bone formation and bone resorption, and the role of nitric oxide in bone remodeling may be concentration-dependent,[3,35] tight regulation of nitric oxide production is needed.[3] It has been shown that osteoblasts and osteocytes under noninflammatory conditions

express only the cNOS isoform. Mechanical loading results in a greater increase in nitric oxide production in osteocytes than in osteoblasts; this supports the notion that osteocytes are the principal cells that react to the mechanical stress in bone.[3] Furthermore, high nitric oxide levels have been shown to inhibit bone resorption and formation and may act to suppress bone turnover in the presence of severe inflammation, indicating a regulatory function in bone maintenance.[3] Thus, the pattern of PISF nitrite levels may reflect the presence of a mechanism for the regulation of nitric oxide production through the processes of bone repair, bone remodeling, and adaptation to forces that enables the maintenance of the supporting bone around dental implants. The significant reduction of nitrite levels at the end of the experimental period, together with the relative stability between 12 and 18 months, may be interpreted as a reflection of the achievement of stability in nitric oxide metabolism around dental implants regardless of the timing of loading of dental implants. It seems that despite the clearly apparent clinical improvement of peri-implant sites and stability of dental implants during the first year postplacement, more time (ie, 12 to 18 months) is required for the stability of nitric oxide metabolism at the tissue level.

Although the lowest levels of total nitrite levels were observed at the 12th and 18th months for both DL and EL implants, the nitric oxide metabolism pattern was different for the 2 dental implant groups regarding the experimental time intervals. In general, stability between 1 and 9 months, followed by a significant decrease at the 12th month, was observed for DL implants. However, EL implants presented a pattern of decrease at the 3rd month followed by an increase at month 6, and again the same pattern of increase at the 9th month compared to the 6th month. While orthodontic forces may induce the formation of cNOS and iNOS during the early phases of orthodontic treatment,[38] occlusal force was shown to have a significant place in the formation of NOS, since occlusal forces were shown to induce the formation of NOS expression in the periodontal ligament compared to a group in which occlusal forces were eliminated.[17] Based on the well-demonstrated impact of force and mechanical stimulus on bone and nitric oxide metabolism,[3,12,14,16,34,36] the difference in the pattern of EL and DL implants may be attributed to the differences in the timing of prosthodontic rehabilitation and the subsequent application of occlusal forces.

However, despite the different patterns, comparable total nitrite levels in PISF were achieved in both implant groups at the end of the follow-up period, and the stability observed between 12 and 18 months may indicate the diminishing of differences between the patterns and the presence of stability in nitric oxide metabolism at the tissue level, regardless of the differences in the treatment models. Moreover, the interaction between time intervals and loading demonstrated statistical significance for total nitrite levels, where nitrite concentration did not present any significance except from baseline to 3 months. Conversely, many studies are available that highlight the discrepancy between the total amount and concentration as modes of data presentation for various gingival crevicular fluid components and the clear impact of the fluid volume on the concentration expression.[40,41] The findings of the present study confirm a discrepancy between the 2 modes of data presentation for PISF nitrite levels. However, the statistically significant interaction demonstrated between loading and time intervals for total nitrite levels may emphasize the importance of total activity of biologic markers in PISF.

Aurer and colleagues[42] reported on the difficulty of direct measurement of nitric oxide from body fluids because of the reactivity of nitric oxide and its short life but introduced measurement of nitrite as a much easier method.[42] This was the reason that the stable end-product nitrite was preferred as the measure of nitric oxide metabolism in the present study. However, analysis of nitrite in PISF can only serve as a general measure of nitric oxide metabolism around dental implants, as the availability of cofactors may limit enzyme activity so that the amount of nitrite does not always reflect the amount of the present NOS.[3] Further studies that differentiate between various isoforms of NOS and analysis of the role of nitric oxide precursors and inhibitors may help to clarify the distinct role of nitric oxide in bone metabolism around dental implants.

## CONCLUSIONS

The findings of the present longitudinal study suggest that nitric oxide is involved in bone repair and remodeling around dental implants, that mechanical loading influences nitric oxide metabolism, and that nitric oxide production at dental implant sites is closely regulated.

## REFERENCES

1. Kendall HK, Marshall RI, Bartold PM. Nitric oxide and tissue destruction. Oral Dis 2001;7:2–10.
2. Brennan PA, Thomas GJ, Langdon JD. The role of nitric oxide in oral diseases. Arch Oral Biol 2003;48:93–100.

3. Van't Hof RJ, Ralston S. Nitric oxide and bone. Immunology 2001;103:255–261.
4. Lowenstein CJ, Snyder SH. Nitric oxide, a novel biologic messenger. Cell 1992;70:705–707.
5. Gaspirc B, Masera A, Skaleric U. Immunolocalization of inducible nitric oxide synthase in localized juvenile periodontitis patients. Connect Tissue Res 2002;43:413–418.
6. Bartosz G. Peroxynitrite: Mediator of the toxic action of nitric oxide. Acta Biochim Pol 1996;43:645–659.
7. Stea S, Visentin M, Donati ME, et al. Nitric oxide synthase in tissues around failed hip prostheses. Biomaterials 2002;23:4833–4838.
8. Hirose M, Ishihara K, Saito A, et al. Expression of cytokines and inducible nitric oxide synthase in inflamed gingival tissue. J Periodontol 2001;72:590–597.
9. Lappin DF, Kjeldsen M, Sander L, Kinane DF. Inducible nitric oxide synthase expression in periodontitis. J Periodontal Res 2000;35:369–373.
10. Batista AC, Silva TA, Chun JH, Lara VS. Nitric oxide synthesis and severity of human periodontal disease. Oral Dis 2002;8:254–260.
11. Wink DA, Mitchell JB. Chemical biology of nitric oxide: Insights into regulatory, cytotoxic, and cytoprotective mechanisms of nitric oxide. Free Radic Biol Med 1998;25:434–456.
12. Johnson DL, McAllister TN, Frangos JA. Fluid flow stimulates rapid and continuous release of nitric oxide in osteoblasts. Am J Physiol 1996;271:E205–E208.
13. McAllister TN, Du T, Frangos JA. Fluid shear stress stimulates prostaglandin and nitric oxide release in bone marrow-derived preosteoclast-like cells. Biochem Biophys Res Commun 2000;270:643–648.
14. Shirazi M, Nilforoushan D, Alghasi H, Dehpour AR. The role of nitric oxide in orthodontic tooth movement in rats. Angle Orthod 2002;72:211–215.
15. Akin E, Gurton AU, Ölmez H. Effects of nitric oxide in orthodontic tooth movement in rats. Am J Orthod Dentofacial Orthop 2004;126:608–614.
16. Brogan JM, Jin K, Eiki K, Tuncay KOC. Localization of nitric oxide sythase in the periodontal tissues of orthodontically moved and stationary teeth. Progress in Orthodontics 2002;3:12.
17. Warita H, Watarai H, Soma K. Nitric oxide synthase expression is increased by occlusal force in rat periodontal ligament. Orthod Craniofacial Res 2004;7:122–126.
18. Balleri P, Cozzolino A, Ghelli L, et al. Stability measurements of osseointegrated implants using Osstell in partially edentulous jaws after 1 year of loading: A pilot study. Clin Implant Dent Relat Res 2002;4:128–132.
19. How to interpret obtained data. Osstell Clinical Manual 2004;10.
20. Gargiulo AW, Wentz FM, Orban B. Dimensions and relations of dentogingival junction in humans. J Periodontol 1961;32:261–267.
21. Sillness J, Löe H. Periodontal disease in pregnancy. II. Correlation between oral hygiene and periodontal condition. Acta Odontol Scand 1964;22:121–135.
22. Löe H, Sillness J. Periodontal disease in pregnancy. I. Prevalance and severity. Acta Odontol Scand 1963;21:533–551.
23. Nowicki D, Vogel RI, Melcer S, Deasy MJ. The gingival bleeding time index. J Periodontol 1981;52:260–262.
24. Atici K, Yamalik N, Eratalay K, Etikan I. Analysis of gingival crevicular fluid intracytoplasmic enzyme activity in patients with adult periodontitis and rapidly progressive periodontitis. A longitudinal study model with periodontal treatment. J Periodontol 1998;69:1155–1163.
25. Rüdin HJ, Overdiek HF, Rateitschak KH. Correlation between sulcus fluid rate and clinical and histological inflammation of the marginal gingiva. Helv Odontol Acta 1970;14:21–26.
26. Chapple IL, Landini G, Griffiths GS, et al. Calibration of the Periotron 8000 and 6000 by polynomial regression. J Periodontal Res 1999;34:79–86.
27. Deinzer R, Mossanen BS, Herforth A. Methodological considerations in the assessment of gingival crevicular fluid volume. J Clin Periodontol 2000;27:481–488.
28. Tözüm TF, Hatipoğlu H, Yamalik N, et al. Critical steps in electronic volume quantification of gingival crevicular fluid: The potential impact of evaporation, fluid retention, local conditions and repeated measurements. J Periodont Res 2004;39:344–357.
29. Grisham MB, Johnson GG, Gautreaux MD, Beng RD. Measurement of nitrate and nitrite in extracellular fluids: A window to systemic nitric oxide metabolism. Methods: A companion to methods in enzymol 1995;7:84–89.
30. Conover WJ. Practical Nonparametric Statistics, ed 2. New York: John Wiley & Sons, 1980:363.
31. Dawson B, Trapp, RG. Nonparametric ANOVA. In: Basic & Clinical Biostatistics, ed 3. Singapore: Lange Medical Books/McGraw-Hill, 2001:179.
32. Dawson B, Trapp, RG. The Wilcoxon signed rank test for the mean difference. In: Basic & Clinical Biostatistics, ed 3. Singapore: Lange Medical Books/McGraw-Hill, 2001: 92–131.
33. Dawson B, Trapp, RG. Research questions about means in three or more groups. In: Basic & Clinical Biostatistics, ed 3. Singapore: Lange Medical Books/McGraw-Hill, 2001: 161–182.
34. Zaman G, Pitsillides AA, Rawlison SCF, et al. Mechanical strain stimulates nitric oxide production by rapid activation of endothelial nitric oxide synthase in osteocytes. J Bone Miner Res 1999;14:1123–1131.
35. Mullender M, El Haj AJ, Yang Y, et al. Mechanotransduction of bone cells in vitro: Mechanobiology of bone tissue. Med Biol Eng Comput 2004;42:14–21.
36. Kunnel JG, Igarashi K, Gilbert JL, Stern PH. Bone anabolic responses to mechanical load in vitro involve COX-2 and constitutive NOS. Connect Tissue Res 2004;45:40–49.
37. Tözüm TF, Türkyilmaz I, Yamalik N, et al. Analysis of the possible impact of the inflammation severity and early and delayed loading on nitric oxide metabolism around dental implants. Int J Oral Maxillofac Imp 2005;20:547–556.
38. D'Attillio M, Di Maio F, D'Arcangela C, et al. Gingival endothelial and inducible nitric oxide synthase levels during orthodontic treatment: A cross-sectional study. Angle Orthod 2004;74:851–858.
39. Bakker AD, Klein-Nulend J, Tanck E, et al. Additive effects of estrogen and mechanical stress on nitric oxide and prostaglandin E2 production by bone cells from osteoporotic donors. Osteoporos Int 2005;16:983–989.
40. Yamalik N, Çaglayan F, Kilinc K, et al. The importance of data presentation regarding gingival crevicular fluid myeloperoxidase and elastase-like activity in periodontal disease and health status. J Periodontol 2000;71:460–467.
41. Lamster IB, Oshrain RL, Fiorello LA, et al. A comparison of 4 methods of data presentation for lysosomal enzyme activity in gingival crevicular fluid. J Clin Periodontol 1988;15:347–352.
42. Aurer A, Aleksic J, Ivic-Kardum M, et al. Nitric oxide synthesis is decreased in periodontitis. J Clin Periodontol 2001;28:565–568.