

# Effects of Periodontal Therapy on Systemic Markers of Inflammation in Patients With Metabolic Syndrome: A Controlled Clinical Trial

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**Background:** The systemic inflammation in both metabolic syndrome (MetS) and periodontitis is a common denominator of the association of these conditions with higher risk of atherosclerosis. The current study investigates whether periodontal therapy may reduce systemic inflammation in patients with MetS and reduce cardiovascular risk.

**Methods:** A parallel-arm, double-blind, randomized clinical trial of 1-year duration in patients with MetS and periodontitis was conducted. Participants were randomized to an experimental treatment group (ETG) (n = 82) that received plaque control and root planing plus amoxicillin and metronidazole or to a control treatment group (CTG) (n = 83) that received plaque control instructions, supragingival scaling, and two placebos. Risk factors for cardiovascular disease were recorded; serum lipoprotein cholesterol, glucose, body mass index (BMI), C-reactive protein (CRP) and fibrinogen concentrations, and clinical periodontal parameters were assessed at baseline and every 3 months until 12 months after therapy. The primary and secondary outcomes were changes in CRP and fibrinogen levels, respectively.

**Results:** The baseline patients' characteristics of both groups were similar. No significant changes in lifestyle factors, frequency of hypertension, BMI, serum lipoprotein cholesterol, and glucose levels were observed during the study period. The periodontal parameters significantly improved in both groups 3 months after therapy ( $P = 0.0001$ ) and remained lower than baseline up to 12 months. The improvement of periodontal status was significantly greater in the ETG ( $P = 0.0001$ ). A multiple linear regression analysis, controlled for sex, smoking, hypertension, and extent of periodontitis, demonstrated that CRP levels decreased with time and that this reduction was significant at 9 ( $P = 0.024$ ) and 12 ( $P = 0.001$ ) months in both groups, without difference between the groups. Fibrinogen levels significantly decreased in the ETG at 6 and 12 months but not in the CTG.

**Conclusion:** Reduction of periodontal inflammation either with root planing and systemic antibiotics or with plaque control and subgingival scaling significantly reduces CRP levels after 9 months in patients with MetS. *J Periodontol* 2012;83:267-278.

## KEY WORDS

Controlled clinical trial; c-reactive protein; inflammation; metabolic syndrome X; obesity; periodontitis.

Atherogenesis, a critical condition of cardiovascular pathogenesis, is a consequence of systemic and vascular inflammatory processes.<sup>1</sup> Recent evidence suggests that periodontitis is associated with atherosclerotic vascular disease and its sequelae.<sup>2</sup> It has been proposed that the chronic inflammatory burden of periodontitis, characterized by elevated acute-phase response proteins, such as C-reactive protein (CRP)<sup>3-5</sup> and fibrinogen,<sup>5</sup> may represent a possible contributor to atherosclerosis.<sup>6</sup>

The metabolic syndrome (MetS) refers to the clustering of cardiovascular risk factors, including atherogenic dyslipidemia, elevated blood pressure, obesity, hypertension, elevated glucose, a prothrombotic state, and a proinflammatory state.<sup>7</sup> It has been estimated that people with MetS are at twice the risk of developing cardiovascular disease (CVD) than those without the syndrome.<sup>8</sup>

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A positive association between periodontitis and MetS has been found in a large population-based survey<sup>9</sup> and in case-control studies.<sup>10,11</sup> Because atherosclerosis is an inflammatory disease,<sup>1</sup> circulating factors related to inflammation may increase the risk for CVD.<sup>12,13</sup> The chronic state of systemic inflammation that accompanies both periodontitis<sup>4,5,14</sup> and MetS<sup>13</sup> may be a common denominator that underlies the association of these conditions with higher risk of atherosclerosis.<sup>15</sup>

Reduction of systemic inflammation through the elimination of periodontitis in patients with MetS may reduce the CVD risk. Some studies have found that intensive periodontal therapy (PT) results in periodontal and systemic reduction of inflammation in otherwise systemically healthy patients.<sup>6,16</sup> However, a randomized, controlled trial by the same group did not find significant differences in the serum levels of CRP and interleukin-6 (IL-6), 6 months after intensive PT.<sup>17</sup> Other studies using standard non-surgical or surgical PT, in patients of good systemic health, have found either no significant changes in serum levels of CRP, IL-6, or tumor necrosis factor- $\alpha$  after therapy<sup>18</sup> or that the systemic inflammation responses to PT were heterogeneous and inconsistent across patients.<sup>19</sup> Two systematic reviews concluded that there is either modest evidence<sup>20</sup> or no evidence that PT may result in a reduction of serum CRP levels.<sup>21</sup> However, all but three<sup>22-24</sup> of the studies of the effect of PT on systemic inflammation, excluded patients with known propensity for atherosclerosis. Thus, the potential benefits of PT in patients with a high risk of CVD have not been completely explored.

Therefore, a parallel-arm, double-blind, randomized, controlled clinical trial was conducted to determine whether treatment of periodontitis in patients with MetS could reduce the serum CRP and fibrinogen levels and possibly contribute to reduced cardiovascular risk.

## MATERIALS AND METHODS

### *Study Population and Study Design*

The study was performed as a parallel-arm, double-blind, randomized clinical trial of 1-year duration among patients with a diagnosis of MetS in the Dr. Eloisa Diaz Dental Center, San José Hospital, a public health center in Santiago, Chile. From March 2007 to August 2007, 315 patients who were attending for medical treatment to reduce CVD risk were assessed for eligibility. The participants (46 males and 119 females) were enrolled by a periodontist (NL) who was not involved in the treatment of patients. The diagnosis of MetS was made when  $\geq 3$  of the following risk determinants were present: 1) central obesity ( $>102$  cm in males;  $>88$  cm in females) or body mass index (BMI)  $> 30$  kg/m<sup>2</sup>; 2) dyslipidemia defined by plas-

matic triglycerides level  $>150$  mg/dL; 3) high-density lipoprotein cholesterol (HDL)  $<40$  mg/dL in males or  $<50$  mg/dL in females; 4) blood pressure  $\geq 130/85$  mmHg; or 5) fasting glucose  $\geq 110$  mg/dL.<sup>25</sup>

Patients with MetS were eligible for this study if they were aged 35 to 65 years, had periodontitis, and retained  $\geq 14$  teeth. The diagnostic criteria for periodontitis were the presence of four or more teeth with one or more sites with probing depth (PD)  $\geq 4$  mm and concomitant clinical attachment loss of  $\geq 3$  mm.<sup>26</sup> Patients were not eligible if they had a history of PT; had kidney, liver, or lung disease; or had any other chronic or acute infections during the previous 6 months as assessed on clinical examination and routine laboratory testing. Additional exclusion criteria were systemic antibiotic treatment in the past 6 months, regular use of non-steroidal anti-inflammatory drugs, hormone replacement therapy, pregnancy, and breastfeeding.

Information on age, sex, smoking status, physical activity, hypertension, and the use of medications was obtained by means of a questionnaire. Waist circumference and BMI (the weight in kilograms divided by the square of the height in meters) were determined at baseline and at each follow-up visit. Physical activity was defined as participating in any moderate leisure-time physical activity. Blood pressure was measured in triplicate, and the mean of the determinations was recorded. A trained and calibrated examiner (NL) collected a complete medical history and performed all periodontal examinations using a manual periodontal probe.<sup>¶</sup> Intra-examiner calibration was assessed performing test-retest exercises in 10 patients before initiating the study. The reproducibility expressed as proportion of agreement between clinical scores was 91% for PD and bleeding and 89% for clinical attachment level (CAL). During the study period, two recalibration exercises were conducted ranging from 90% to 92% intra-examiner agreement. The same examiner (NL) performed all the periodontal examinations during the entire study period.

The following clinical periodontal parameters were recorded: PD, CAL, and bleeding on probing (BOP) at six sites per tooth. The presence of dental plaque was recorded dichotomously at four sites per tooth.

The primary outcome variable was the change in the serum CRP levels, and the secondary outcome was the change in serum fibrinogen levels. Sample size calculations were made taking into account the change in the CRP level ( $\Delta$ CRP) as a result of therapy. Assuming a mean difference between groups in  $\Delta$ CRP of 0.60,<sup>20</sup> with a standard deviation of 1, it was calculated that with 120 patients, 60 in each group, we would have 90% power to detect this difference in

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$\Delta$ CRP at a significance level ( $\alpha$ ) of 0.05. Because we expected a patient dropout rate of 35%, the sample size was increased to 165. A total of 315 patients was assessed for eligibility from March 2007 to July 2008 to arrive at the target sample size of 165 patients for the study.

The 165 persons underwent randomization to an experimental treatment group (ETG) or a control treatment group (CTG). Randomization was computer generated for the first eight participants enrolled, whereas the group assignment for the following participants was done using the minimization method<sup>27</sup> to prevent an imbalance between the groups related to sex, smoking status (current/former/never), hypertension (yes/no), and the extent of periodontitis, measured as the percentage of sites with PD  $\geq$ 4 mm ( $\leq$ 20 or  $>$ 20%). We selected the threshold of 20% to measure the extent of periodontitis to assign patients to one or the other treatment group because the median of the percentage of sites with PD  $\geq$ 4 mm in the first 20 patients we examined was 20%.

The allocation sequence was generated by a statistician (Benjamin Martinez, University Mayor, Santiago, Chile) without clinical involvement in the study, who also assigned the participants to study groups.

#### PT

Extractions of hopeless teeth and restorative treatments for caries lesions were performed before the commencement of the PT. All patients received oral hygiene instructions, and toothbrushes and toothpaste were provided to the participants during the study. Patients in the ETG received supragingival and subgingival scaling, crown polishing, and root planing under local anesthesia using ultrasound and hand instruments. In addition, patients were given metronidazole (250 mg) and amoxicillin (500 mg) tablets, three times daily, for 7 days, 1 week before beginning root planing.<sup>28,29</sup> Patients in the CTG received a treatment consisting of supragingival scaling with ultrasound instruments, crown polishing, and two placebo tablets three times daily, for 7 days. The metronidazole, amoxicillin, and placebo tablets were identical in appearance. They were prepacked in bottles and consecutively numbered for each participant according to the randomization schedule. Concealment of treatment assignments was obtained by using opaque envelopes with a cardboard inside to render them impermeable to light, and the group allocation was revealed to the therapist on the day the PT began. The examiner, the patients, and the technician who performed the laboratory analyses were all blinded to group assignment. The active PT was performed by three periodontists (AQ, CI, PC) and was completed within 3 to 4 weeks of the baseline visit. Periodontal parameters were reassessed at 3, 6, 9, and 12 months after completion of therapy. At these main-

tenance visits, patients in the ETG received supragingival and subgingival plaque elimination and oral hygiene instructions, and patients in the CTG group received supragingival plaque elimination and oral hygiene instructions. The participants were interviewed at all follow-up visits to determine changes in diet, medication, smoking habits, physical activity, and signs of intercurrent infections.

Participation in the study did not influence the clinical management for MetS. The patients were advised not to suspend any medication they were taking during the study period.

#### Laboratory Assessments

Blood samples were collected after 10 hours of overnight fasting, at baseline, and 3, 6, 9, and 12 months after therapy for the measurement of biochemical markers and to prepare aliquots of serum stored at  $-70^{\circ}\text{C}$  for CRP determinations. Blood samples were always collected 3 to 4 days before periodontal examinations. Total cholesterol, HDL, and low-density lipoprotein (LDL) cholesterol and glucose levels were quantified using standard laboratory procedures. Fibrinogen was assayed by the Clauss method,<sup>30</sup> and high-sensitivity CRP concentrations were determined using a high-sensitivity enzyme-linked immunosorbent assay with a lower detection limit of 10 ng/L.<sup>#</sup>

Before blood sampling, a structured questionnaire was used to obtain information from each patient regarding the presence of intercurrent infections.

The study protocol was approved by the University of Chile, Faculty of Dentistry Ethics Committee. All participants provided written informed consent, and the study was conducted in accordance with the Helsinki Declaration of 1975, as revised in 2000.

#### Statistical Analysis

The primary outcomes were the changes in serum of CRP and fibrinogen levels during the follow-up period. Because of their skewed distributions, the CRP data were logarithmically transformed and analyzed as continuous variables.

Differences between treatment groups in categorical variables at baseline were tested by the  $\chi^2$  test, whereas continuous variables were tested using the Mann-Whitney  $U$  test. Repeated-measures analysis of variance (ANOVA) was used to test between-group differences in the periodontal and the MetS variables during the period from 3 to 12 months. As recommended by Chakraborty and Gu,<sup>31</sup> imputation of missing values was avoided in favor of a statistical analysis based on a mixed-modeling approach. The concept of mixed modeling refers to the fact that measurements are nested (repeated with time) in individuals, a feature

# Quantikine ELISA kit, R & D Systems, Minneapolis, MN.

that can be taken into account using random slope mixed-effects linear regression analysis (using statistical software).\*\* In the present study, the outcomes log(CRP) or fibrinogen level are modeled as a linear function of treatment group and time in months since baseline. Both models were adjusted for the four parameters used in the minimization algorithm for determining patient assignment to the two treatment groups (sex, smoking status, hypertension, and percentage sites with PD  $\geq$ 4 mm). Data to determine the effects of PT on the outcomes were analyzed on an intention-to-treat analysis. Statistical significance was declared when  $P < 0.05$ .

Data of patients with intercurrent systemic infections during the study period were excluded from the analysis after the intercurrent infections were detected. Although this goes against the principles of an intention-to-treat analysis, it was also clear that the presence of acute infections resulted in distorted high values of CRP and fibrinogen and would thereby compromise the objective of the present study.

## RESULTS

Eighty-two patients were assigned to the ETG and 83 to the CTG. Figure 1 shows the flow of participants through the trial progression. In the ETG, one participant was lost to follow-up because he had a fatal myocardial infarction at 6 weeks after finishing therapy. Another three participants in the ETG had cardiovascular events. They included one woman who had an ischemic stroke before the last root planing appointment and another woman 8 weeks after PT. Another woman had several small cerebral hemorrhagic events 7 months after PT. The three patients with vascular events were withdrawn from the study. The data of the participant with the hemorrhagic stroke obtained after the occurrence of the vascular events were excluded from the analysis.

No cardiovascular events occurred in the CTG. One woman in the CTG had a severe allergic reaction to an environmental chemical 2 weeks before the 9-month follow-up visit; the data recorded at 9 months were excluded from the analysis, and the patient was withdrawn from the study. Another patient in the CTG showed progression of periodontitis at the 9-month visit, received conventional periodontal treatment, and was withdrawn from the study. The remaining 79 of 82 ETG patients and 81 of 83 CTG patients finished the study, attending all the study visits.

Concurrently, 43 ETG patients and 50 CTG patients had acute intercurrent infections (mainly upper respiratory, digestive, and urinary infections). Data of patients who had intercurrent infections at any control visit, or between the last control visit and the following visit were excluded from the analysis.

No significant change in diet, smoking habits, and physical activity was determined during the 12-month study period.

### *Patients' Characteristics and Cardiovascular Risk Factors*

The baseline periodontal measurements of both groups were similar (Table 1). At baseline, there were no significant differences between the two groups in terms of age, sex, level of education, medication regimen, BMI, smoking status, mean of lipoprotein cholesterol and CRP levels, percentage of patients with CRP levels  $>3$  mg/L, and physical activity. The mean fibrinogen level was the only variable significantly higher in the ETG at baseline.

Three months after therapy and during the 12-month study period, no significant changes were observed in serum lipids, glucose levels, BMI, and arterial blood pressure whether compared to baseline or whether compared between the patient groups (Fig. 2).

### *Effect of Therapy: Periodontal Outcomes*

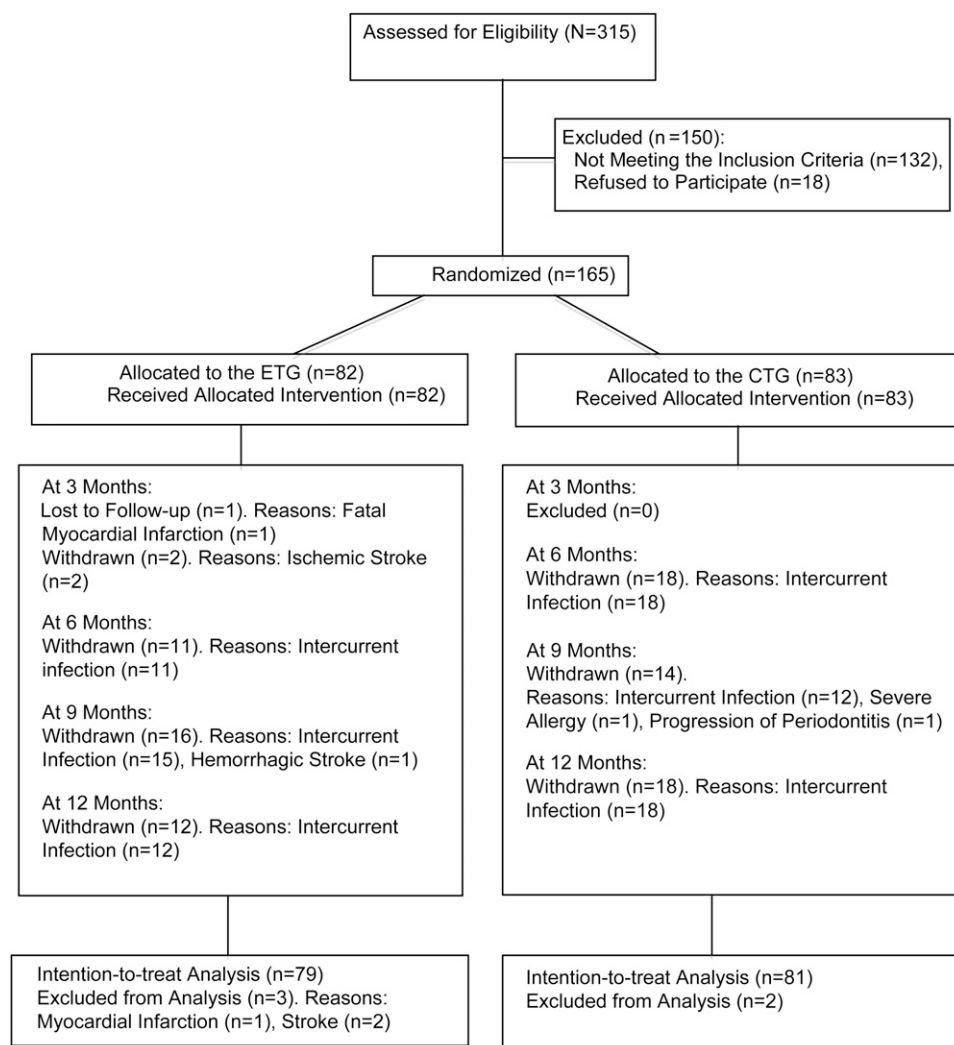
A significant improvement of all the periodontal parameters compared to baseline was observed in the ETG ( $P = 0.0001$ ) and in the CTG ( $P = 0.0001$ ) at 3 months after therapy, and their values remained lower than at baseline up to 12 months in both groups (Fig. 3). The reduction of all the periodontal parameters, except for the mean CAL, was significantly greater in ETG than in CTG at all follow-up assessments ( $P = 0.0001$ ).

### *CRP and Fibrinogen Levels*

The response to PT, measured by the levels of CRP and fibrinogen, was heterogeneous across patients of both groups who showed a tendency for improvement of CRP levels 6 months after PT. The proportion of ETG patients who showed improved CRP levels significantly increased from 54% (43 of 79) at 3 months to 77% (30 of 39) at 12 months ( $P = 0.026$ ) and in the CTG increased from 46% (38 of 83) to 79% (27 of 34) ( $P = 0.002$ ). The proportion of ETG patients with CRP levels  $\geq 3$  mg/L significantly decreased from 63% (50 of 79) at baseline to 30.76% (12 of 39) at 12 months after treatment ( $P = 0.002$ ). In the CTG, the proportion of patients with CRP levels  $\geq 3$  mg/L decreased insignificantly from 60.24% (50 of 83) at baseline to 42.42% (14 of 33) at 12 months ( $P = 0.12$ ). Mean CRP levels decreased from 3 to 12 months in the ETG, and the differences from baseline values were significant at 9 ( $P = 0.024$ ) and 12 ( $P = 0.001$ ) months, without significant differences between the groups. In the CTG, the mean CRP level showed a slight increase at 3 months compared to baseline but decreased thereafter and was significantly lower than baseline at 9 and 12 months ( $P = 0.001$ ) (Fig. 4).

\*\* Procedure xtmixed of Stata statistical software, release 11, StataCorp, College Station, TX.





**Figure 1.**

Flow chart of participants throughout the study.

Fibrinogen levels fell in the ETG from 3 to 12 months, and the values from baseline were significantly lower at 6 ( $P = 0.01$ ) and 12 ( $P = 0.005$ , ANOVA test) months after therapy, whereas no significant changes in CTG occurred. No significant differences in fibrinogen levels between ETG and CTG were found at all follow-up visits (Fig. 4). Obese patients in both treatment groups (logCRP CTG: obese  $0.563 \pm 0.36$  versus non-obese  $0.509 \pm 0.33$ ,  $P = 0.48$ ; logCRP ETG: obese  $0.531 \pm 0.36$  versus non-obese  $0.463 \pm 0.41$ ,  $P = 0.45$ , Mann-Whitney  $U$  test) showed a trend toward higher CRP values compared to non-obese patients at any assessment after therapy, but the differences were not significant.

CTG fibrinogen levels showed significantly higher levels in patients with obesity ( $382 \pm 179$  mg/L) than patients without obesity ( $312 \pm 116$  mg/L) at 6 months ( $P = 0.038$ ) and 12 months ( $387 \pm 162$  mg/L /  $309 \pm$

$392$  mg/L,  $P = 0.031$ , Mann-Whitney  $U$  test), but not in the ETG.

Table 2 shows the results of the regression analyses of the outcomes log(CRP) and fibrinogen, respectively, as a linear function of time and treatment group. The  $\beta$  coefficients and their confidence intervals (CIs) for the effect of treatment group show that treatment group was statistically not significantly associated with the log(CRP) or fibrinogen levels. The  $\beta$  coefficients for time show the average change of the outcomes since the baseline recording, controlled for the effects of sex, smoking, hypertension, percentage sites with PD  $\geq 4$  mm, and treatment group. It is observed that the log(CRP) level continued to decrease with time up to 12 months, although only the 9- and 12-month log(CRP) levels were statistically significantly lower than the baseline levels. These analyses also showed that 24% of the variance of the log(CRP) values, not explained by the covariates, was attributable to time-invariant patient-specific characteristics. There was no

statistically significant effect of treatment group on the fibrinogen levels ( $\beta = 11.0$  95%; CI =  $[-11.8, 33.7]$ ), nor there was an effect of time (Table 2). Approximately 20% of the variance of the fibrinogen levels that was not explained by the covariates was attributable to time-invariant patient-specific characteristics.

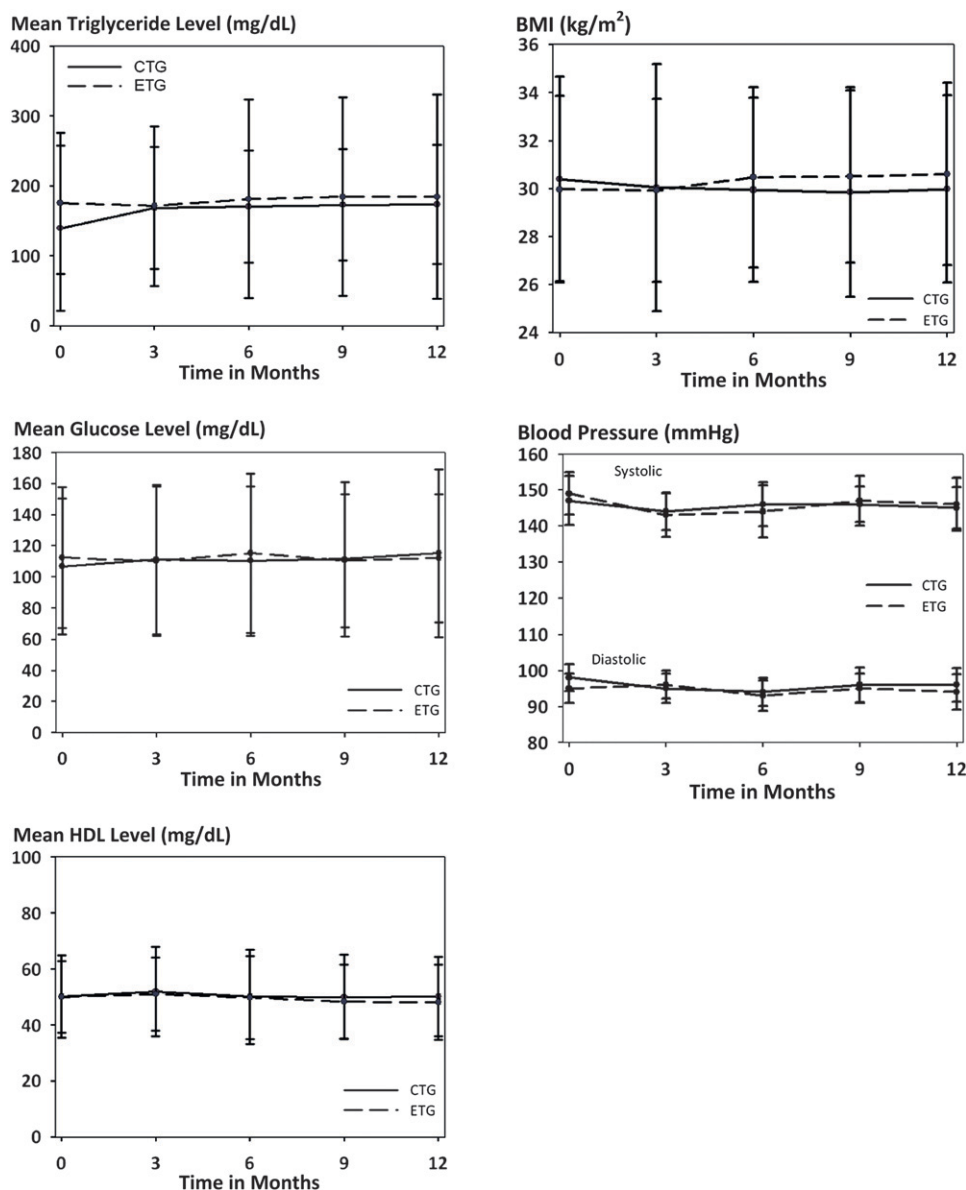
## DISCUSSION

In this study, treatment of periodontitis is associated with a significant reduction in serum CRP in patients with MetS and affected by periodontitis. For ethical reasons, control patients of the current study were not a true untreated control group as used in conventional randomized clinical trials. Patients in the CTG received treatment consisting of plaque control, supragingival scaling, and two placebo tablets, and this treatment significantly improved periodontal

**Table 1.**  
**Characteristics of Patients at Baseline**

Characteristic	CTG (n = 83)	ETG (n = 82)	P Value
Age, years (mean ± SD)	56.33 ± 8.9	54.13 ± 8.8	NS
Males, n (%)	22 (26.5)	24 (29.3)	NS
Education <12 years, n (%)	59 (71.1)	56 (68.3)	NS
Smoking status, n (%)			
Never	33 (39.8)	35 (42.7)	NS
Past	28 (33.7)	22 (26.8)	NS
Current	22 (26.5)	24 (29.3)	NS
Family history of CVD, n (%)	39 (47.0)	43 (52.4)	NS
Physical activity (<3 times/week), n (%)	76 (91.6)	69 (84.1)	NS
Alcohol consumption, n (%)	20 (24.1)	31 (37.8)	NS
Statin use, n (%)	16 (19.3)	16 (19.5)	NS
Aspirin use (100 mg/day), n (%)	31 (37.3)	28 (34.1)	NS
BMI (mean ± SD)	30.39 ± 4.26	29.96 ± 3.89	NS
Central obesity, n (%)	42 (50.6)	37 (45.1)	NS
Systolic blood pressure (mmHg) (mean ± SD)	149 ± 6.9	147 ± 6.8	NS
Diastolic blood pressure (mmHg) (mean ± SD)	98 ± 3.8	97 ± 4.1	NS
Total cholesterol (mg/dL) (mean ± SD)	215.98 ± 48.56	208.0 ± 47.31	NS
LDL (mg/dL) (mean ± SD)	130.72 ± 39.66	124.77 ± 42.75	NS
HDL (mg/dL) (mean ± SD)	50.18 ± 14.63	50.00 ± 12.87	NS
Triglycerides (mg/dL) (mean ± SD)	158.80 ± 118.23	174.90 ± 101.13	NS
Fasting blood glucose (mg/dL) (mean ± SD)	106.60 ± 43.66	112.24 ± 45.16	NS
CRP (mg/L) (mean ± SD)	4.39 ± 3.17	4.43 ± 3.05	NS
Patients with CRP levels ≥3 mg/L, n (%)	50 (60.2)	51 (62.2)	NS
Fibrinogen (mg/dL) (mean ± SD)	337 ± 115	378 ± 102	<0.05
Number of teeth present (mean ± SD)	19.90 ± 4.82	20.51 ± 4.37	NS
PD ≥4 mm (% of sites) (mean ± SD)	30.55 ± 21.37	31.77 ± 19.57	NS
BOP (% of sites) (mean ± SD)	51.22 ± 16.86	51.54 ± 17.13	NS
Mean CAL (mm) (mean ± SD)	3.45 ± 1.36	3.79 ± 1.47	NS
CAL ≥3 mm (% of sites) (mean ± SD)	68.12 ± 28.52	72.04 ± 23.85	NS
Plaque (% of sites) (mean ± SD)	85.15 ± 16.62	87.96 ± 13.89	NS

χ<sup>2</sup> test for categorical variables and Mann-Whitney U test for continuous variables. NS = not significant.



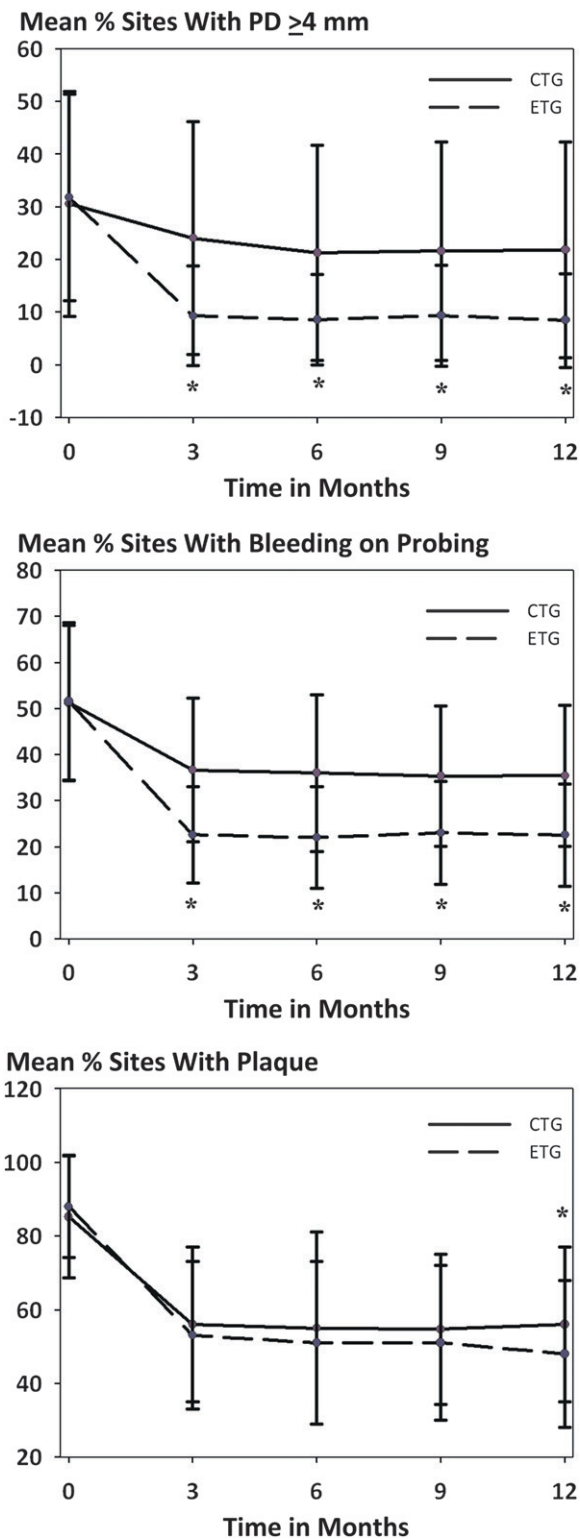
**Figure 2.**

Repeated-measures ANOVA showed that there was no statistically significant difference between treatment groups in the levels of any of the MetS defining criteria triglycerides (top left), glucose (middle left), HDL (bottom left), BMI (top right), or blood pressure (bottom right).

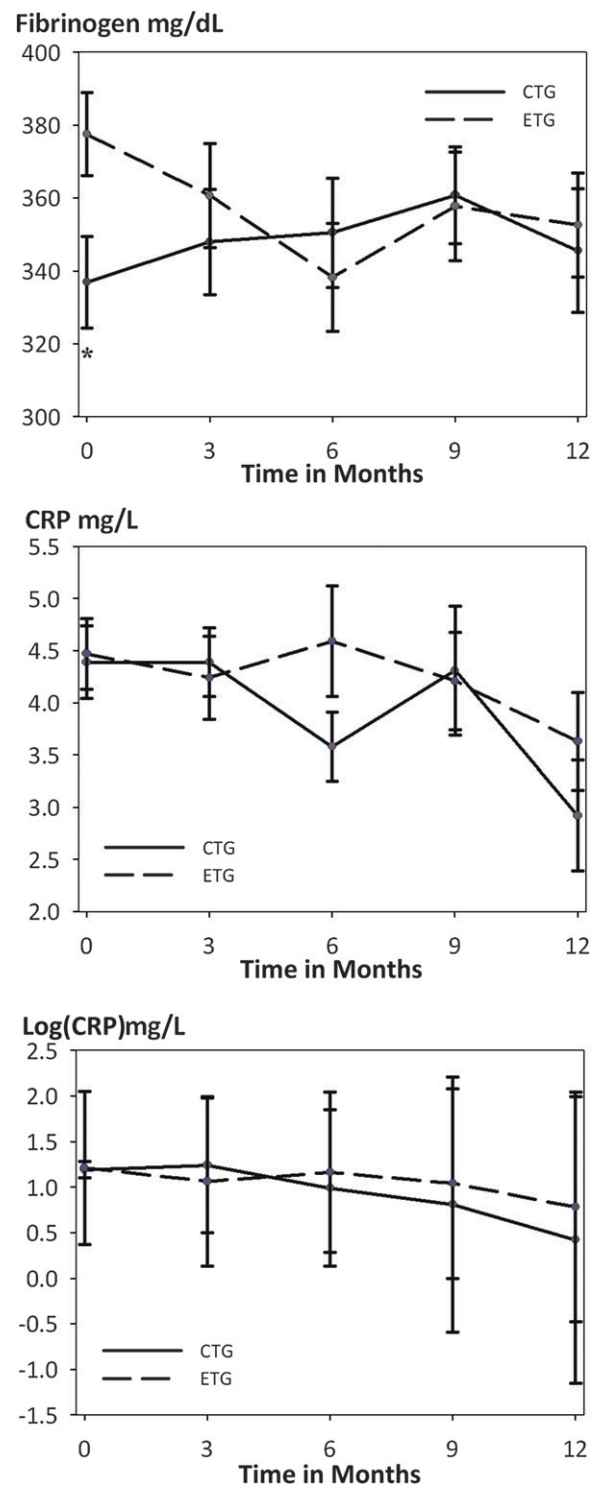
inflammation at 3 months and maintained a low level of inflammation for 12 months. Although the improvement of the periodontal parameters was significantly lower in the CTG than that obtained in the ETG, the treatment given to the CTG had a similar effect in reducing CRP levels. This suggests that the reduction of periodontal inflammation is the key in reducing systemic inflammation in patients with MetS. According to the results of the current study, this may be obtained with plaque control and supragingival scaling, as well as with root planing and systemic antibiotics.

The reduction of CRP levels in the CTG at 9 months was unexpected, although it has been found that persistent supragingival plaque control affects both the amount of subgingival biofilm and its composition<sup>32,33</sup> and results in decreasing signs of gingivitis.<sup>34</sup> Thus, the resolution of the gingival inflammation and consequent pocket reduction in moderately deep pockets in patients in the CTG were probably brought about by shifts in the subgingival microbiota. Only one patient of the CTG showed progression of periodontitis at the 9-month visit, although it has been found that the magnitude of the impact of the elimination of the supragingival biofilm on the subgingival biofilm produced by scaling seems to be insufficient to arrest disease progression.<sup>35</sup> Thus, supragingival plaque control was sufficiently effective to significantly improve periodontal inflammation and to reduce its impact on systemic inflammation as measured by CRP. This agrees with the findings by Offenbacher et al.<sup>23</sup> who reported that any preventive or periodontal treatment showed a significant reduction in the percentage of people with elevated CRP levels at 6 months.

The majority of the patients in the current study had elevated baseline CRP levels because both periodontitis<sup>3,4,14</sup> and MetS<sup>25</sup> are associated with systemic inflammation. No significant changes in lifestyle, habits, or medications were detected throughout the study, and all the other patient characteristics that influence CRP and fibrinogen levels remained unchanged. Therefore, the improvement of CPR values in the ETG and CTG after PT may be ascribed to the reduction of periodontal inflammation. This suggests that moderate-to-advanced periodontitis in MetS patients contributes to their systemic inflammatory burden. The findings that



**Figure 3.** A significant improvement of all the periodontal parameters compared to baseline in the ETG and CTG at 3, 6, 9, and 12 months was found. The improvement of periodontal parameters was significantly greater in the ETG. \* $P \leq 0.05$ , statistically significant difference between groups.



**Figure 4.** Effects of the two regimens of PT on changes in fibrinogen and CRP levels. Fibrinogen levels significantly decreased at 6 ( $P = 0.01$ ) and 12 ( $P = 0.005$ ) months after therapy in the ETG, and no significant changes occurred in the CTG. CRP levels significantly decreased in both groups at 9 ( $P = 0.024$ ) and 12 ( $P = 0.005$ ) months compared to baseline. \* $P \leq 0.05$ , statistically significant difference between groups.



**Table 2.****Results of Mixed-Effects Linear Regression Analysis of the Outcomes Log(CRP) or Fibrinogen Values, Respectively, as a Function of Time and Treatment Group**

Parameter	Log(CRP) (mg/L)		Fibrinogen (mg/dL)	
	$\beta$ Coefficient	95% CI	$\beta$ Coefficient	95% CI
Time				
Baseline	Reference		Reference	
3 months	-0.045	[-0.21; 0.12]	-2.83	[-25.6; 19.4]
6 months	-0.133	[-0.32; 0.05]	-14.89	[-38.9; 8.8]
9 months	-0.313	[-0.54; -0.09]	-4.54	[-30.3; 21.2]
12 months	-0.708	[-0.99; -0.43]	-22.41	[-51.2; 7.0]
Treatment group				
CTG	Reference		Reference	
ETG	-0.002	[-0.19; 0.20]	10.9	[-12.0; 33.8]

The estimates are adjusted for the effects of sex, smoking status, hypertensive status, and percentage sites with PD  $\geq$  4 mm.

PT reduces systemic inflammation are important because increased levels of CRP are good predictors of the development of atherosclerosis and cardiovascular events.<sup>12</sup> Inflammatory markers are highly correlated with obesity, HDL cholesterol, fasting glucose, hypertension, and insulin sensitivity.<sup>36</sup> Twelve months after PT, the mean CRP level remained  $>3$  mg/L in both groups because all the components of the MetS associated with systemic inflammation remained without changes. There is evidence that systemic inflammation burden associated with periodontitis may contribute to atherosclerosis in otherwise healthy individuals.<sup>2</sup> An increase of systemic inflammation in response to periodontitis might increase the metabolic changes in patients with MetS, promoting both insulin resistance and dyslipidemia, increasing the cardiovascular risk. The potential significance of the current study results is in the context that patients with MetS have a doubling in risk for CVD,<sup>8</sup> the prevalence of both periodontitis and MetS is very high in the world population, and the burden of systemic inflammation derived from periodontitis can be controlled with a relatively simple treatment, as shown in the current study.

The strengths of the current study can be summarized as follows: 1) patients with moderate-to-advanced periodontitis are more representative of the population affected by periodontal disease and were thus selected; 2) a well-defined and homogeneous study population that was successfully randomized was selected; 3) treatment protocol used in the ETG is the conventional, non-surgical treatment used to treat periodontitis, as well as the maintenance treatment applied; 4) systemically administered metronidazole and amoxicillin were used because they have been shown to provide better clinical and microbiologic outcomes than scaling and root planing alone;<sup>28,29</sup> 5) PT used in this

study is closer to everyday clinical periodontal practice than treatment protocols used in other studies that included only patients with severe periodontitis and used intensive treatment in a single-appointment full-mouth debridement with adjunctive local delivery of minocycline;<sup>6,17,37</sup> and 6) the study was performed in a public health center and not in a dental school setting, which increases the external validity of the results.

One limitation of the current study is the patient dropout rate, which was higher than expected in both groups. The patient dropout rate was mainly attributable to intercurrent systemic infections that interacted with the main outcome, that is, the CRP levels. Data of patients with intercurrent infections had to be eliminated from the study at the time the infections were detected, which resulted in several missing outcome values in both groups. The missing outcome values were considered to occur at random because they did not depend on the value of the missing outcome after controlling for other variables but were related to intercurrent infections that could be identified. The possibility of introduction of bias in the way we analyzed data is very low because the main cause of dropout was the same in both groups and was not related to a patient's response to treatment. Additionally, the frequency of loss to follow-up did not differ between the groups. Finally, the mixed-model approach used to analyze these randomized controlled trial data does not require the same number of measurements for all participants, and it has been found to yield relatively few statistical errors when missing outcomes occur at random.<sup>31</sup>

The reduction for CRP in the current study agrees with other studies that showed improvement of CRP levels after PT.<sup>6,16,37</sup> However, not all studies show

reduction of CRP levels after PT.<sup>3,17-19,23,38,39</sup> The investigations that found reduction of markers of systemic inflammation after PT studied systemically healthy patients, without apparent CVD risk, and provided data only on the short-term effects of PT.<sup>6,16,37-40</sup> In the present study, there was a considerable inter-patient variability in the CRP levels at baseline and at all the follow-up visits, and the response to PT measured by CRP levels was very heterogeneous among patients. The large variance of CRP levels and the heterogeneous response to PT may be attributable to the effect of the components of the MetS that increase systemic inflammation. However, other studies in systemically healthy patients with periodontitis have also found a large variability of CRP levels and diverse responses to PT in CRP concentrations.<sup>17,19,37,39</sup>

Despite the heterogeneous response to PT and the large variance of CRP levels in our patients, a clear tendency for decreasing CRP levels was observed in ETG and CTG patients, among whom the proportion of patients with improved CRP levels significantly increased from 3 to 12 months after PT.

Apparently, PT may reduce CRP levels in certain circumstances that are influenced by baseline CRP concentrations,<sup>23,39</sup> genetic factors,<sup>41</sup> obesity,<sup>25</sup> use of aspirin,<sup>42</sup> and statins.<sup>43</sup> In the current study, all these variables were well balanced between groups, and no association was found between them and CRP or fibrinogen levels. More than one-third of the patients in both groups used aspirin daily, but the doses received were lower than the one significantly affecting markers of inflammation (300 mg daily).<sup>42</sup> Our finding on reduction in CRP levels at 9 months after eliminating periodontal infection agrees with studies showing that CRP reduction was obtained 1 year after treating *Helicobacter pylori* and *Chlamydia pneumoniae* infections with antibiotics in patients with ischemic heart disease<sup>44</sup> and in patients with unstable angina.<sup>45,46</sup> Thus, a follow-up period of <9 months after periodontal treatment may be too short to demonstrate a clear therapeutic effect of PT on systemic inflammation, although previous pilot studies suggest this possibility.<sup>6,16,40</sup>

Only fibrinogen level was not well balanced between the study groups, in favor of the placebo group at baseline. However, 3 months after therapy, fibrinogen showed a slight and steady increase in the CTG until 9 months and then a decrease at 12 months. The ETG fibrinogen levels significantly decreased at 6 and 12 months, but the results of the linear regression analysis did not show significant changes after treatment in any group. Fibrinogen is an acute-phase protein that functions as a blood coagulation factor, and increased plasma fibrinogen levels are indepen-

dent risk factors for CVD.<sup>12</sup> Fibrinogen levels increase during infections and inflammatory conditions, including periodontal disease,<sup>5</sup> and are influenced by several factors, including smoking, age, sex, and alcohol consumption.<sup>47,48</sup> All the variables that may have influenced fibrinogen levels were well balanced in both groups at baseline and throughout the study, and statistical analyses involving fibrinogen levels controlled for these confounders. Observed variation in fibrinogen levels between individuals is in part genetically determined,<sup>49</sup> and a genetic component may have determined the difference in fibrinogen levels between the groups at baseline.

The occurrence of serious vascular events in four patients in the ETG was unexpected and worrisome. Preliminary results from some studies suggest that intensive periodontal treatment in systemically healthy patients may cause a transient endothelial dysfunction and endothelial activation 1 week after treatment,<sup>17</sup> as well as an acute systemic inflammatory response.<sup>50</sup> Additionally, a self-controlled case series study recently concluded that invasive dental treatment may be associated with a transient increase in the risk of stroke and myocardial infarction.<sup>51</sup> However, although it is tempting to speculate that periodontal interventions may be causally related with these events, the results are not conclusive. The systematic registration and timely report of the occurrence of adverse events in upcoming periodontal intervention studies is warranted and may help to clarify this point. Until this question is resolved, in agreement with the results of this study, it seems advisable to avoid aggressive periodontal treatment before controlling periodontal inflammation with non-invasive methods in patients with MetS.

The results of the current study should encourage additional intervention studies using similar or more specific inflammatory markers to assess the effect of PT on systemic inflammation. Because it is unethical to use a true untreated control group of patients with MetS, a factorial study design would be more appropriate in future studies. These studies may include several treatment groups, such as a group treated with root planing and systemic antibiotics, a control group with supragingival scaling and placebo tablets, and a third group with periodontitis and without MetS to complement the control group.

## CONCLUSIONS

Results from the current study show that elimination of periodontal inflammation by using root planing and systemic antibiotics or using plaque control and supragingival scaling significantly decreases CRP in patients with MetS. The elimination of the inflammatory burden of periodontitis in patients with MetS may contribute to reduce cardiovascular risk.

## ACKNOWLEDGMENTS

This work was supported by National Fund for Scientific and Technological Development Research Grant 1061070. The assistance of Dr. Benjamin Martínez (University Mayor, Santiago, Chile) in the randomization of patients and statistical analysis is gratefully acknowledged. The authors thank Dr. Roberto Avalos (Dr. Eloisa Díaz Dental Center, San José Hospital, Santiago, Chile) for his full cooperation and Unilever Chile (Santiago, Chile) for the donation of toothbrushes and toothpaste for the study patients. The authors report no conflicts of interest related to this study.

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Submitted April 13, 2011; accepted for publication June 14, 2011.