

# Nitric oxide production, systemic inflammation and lipid metabolism in periodontitis patients: possible gender aspect

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## Abstract

**Aim:** Nitric oxide (NO) plays a crucial role in vascular tone regulation and is involved in pathogenesis of periodontitis. In this cross-sectional study, we investigated the serum and saliva levels of NO metabolites in periodontal disease and their relationship with serum C-reactive protein (CRP) levels, lipids metabolism and periodontal disease severity.

**Material and Methods:** Serum and saliva were collected from non-smoking patients with generalized severe periodontitis ( $n = 89$ ) and healthy controls ( $n = 56$ ). Serum and salivary levels of NO metabolites, serum levels of high density lipoproteins (HDL), low density lipoproteins (LDL), triglycerides, cholesterol and CRP were measured. Data were analysed in whole population and in different gender groups.

**Results:** Periodontitis patients exhibited significantly lower serum and saliva levels of NO metabolites and significantly higher LDL, cholesterol and CRP levels than control group. Similar findings were observed within male but not within female population. Serum NO metabolites levels exhibited significant negative correlation with CRP in whole population and in male population. Significant positive correlation of serum NO metabolite levels with HDL levels was observed in whole population.

**Conclusion:** NO production is reduced in periodontitis, especially in male population. Gender might be an important factor in assessing risk of cardiovascular disease in periodontitis.

Key words: cardiovascular disease; C-reactive protein; nitric oxide; periodontitis; serum lipids

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## Conflict of interest and source of funding statement

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Periodontitis is an inflammatory disease, which is initiated by overgrowth of some gram-negative bacteria in the dental pocket and leads to the periodontal tissue destruction and tooth loss. Various clinical and epidemiological studies suggest that periodontitis is associated with an increased risk of

cardiovascular diseases (CVDs) (Friedewald et al. 2009, Kerschull et al. 2010, Schenkein & Loos 2013, Tonetti & Van Dyke 2013).

Nitric oxide (NO) is a physiological messenger molecule involved in various physiological processes, such as the regulation of vascular tone, inhibition of platelet aggregation,

neurotransmission and immune response (Moncada et al. 1991). NO is produced by the specific enzyme nitric oxide synthase (NOS) through the oxidation of L-arginine. There are three distinct NOS isozymes in mammalian tissues: neuronal (nNOS), inducible (iNOS) and endothelial (eNOS). nNOS and eNOS are constitutively expressed in the organism, whereas iNOS is expressed only in response to inflammatory stimuli (Vallance & Chan 2001). The half-life time depends on the environment and usually does not exceed 2 s (Thomas et al. 2001). NO generated by NOS is easily oxidized to nitrite (NO<sub>2</sub>), which, in turn, could be further oxidised to nitrate (NO<sub>3</sub>). The total levels of nitrite and nitrate in biological fluids is generally used for adequate monitoring the NO synthesis (Moshage et al. 1995).

Nitric oxide plays an important role in the progression of periodontal disease. Periodontitis is accompanied by an increase in the expression of iNOS and NO production in gingival tissue (Rausch-Fan & Matejka 2001). Locally produced NO is cytotoxic against periodontal pathogens and the tooth surrounding tissue (Kendall et al. 2001). Previous studies investigated the levels of NO metabolites in saliva of periodontitis patient and their results are controversial: both decreased and increased salivary levels of NO metabolites in periodontitis patients were reported (Aurer et al. 2001, Reher et al. 2007, Ozer et al. 2011, Parwani et al. 2012). Periodontitis is known to be associated with increased risk of CVDs, which are, in turn, usually accompanied by impaired NO production by eNOS (Vallance & Collier 1994, Wennmalm 1994). Therefore, it would be interesting to investigate the changes of the serum levels of NO metabolites in periodontitis patients, but such data are scarce. To date, the only study shows an increase of serum nitrite levels in periodontitis (Menaka et al. 2009). However, measurement of nitrite alone in serum does not represent the levels of NO production, because nitrite is quickly oxidised to nitrate (Moshage et al. 1995).

In this study, we investigated the levels of NO metabolites in serum and saliva of patients with periodontitis in comparison with healthy

individuals. It is well known that NO production is affected by smoking (Node et al. 1997, Tsuchiya et al. 2002), and therefore we included in this study only non-smoking individuals. Furthermore, we investigated the relationship of NO metabolite levels with serum lipids and C-reactive protein (CRP) in periodontitis patients. Changes in levels of serum lipid, namely, high density lipoproteins (HDL), low density lipoproteins (LDL), cholesterol and triglyceride are considered as a risk factor of CVDs, particularly atherosclerosis (Bestermann et al. 2005). CRP was used as a measure of systemic inflammatory reaction, because its levels raise in respond to inflammation (Pepys & Hirschfield 2003). In addition, relationship between the all these parameters and severity of periodontal disease was investigated in periodontitis patients. Finally, since levels of NO metabolites are usually higher in males than in females (Watanabe et al. 2000, Ghasemi et al. 2008), we analysed all parameters in whole population and in different gender groups.

## Materials and Methods

### Patients' selection

This cross-sectional study included 89 periodontitis patients and 54 periodontally healthy volunteers. The patients group consisted of periodontitis patients recruited at the Department of Periodontology of the Bernhard Gottlieb School of Dentistry of the Medical University of Vienna. Clinical history was recorded for all participants (personal data and medical history). Exclusion criteria were defined as follows: smoking, periodontal treatment within the last 3 months,

presence of any systemic disease (e.g. diabetes mellitus, asthma, and malignancies), less than 20 teeth, pregnancy or lactation, acute infection, immune suppressive medication or immunodeficiency, history of radio- or chemotherapy. All study participants did not take any antibiotics, immunomodulatory and anti-inflammatory drugs during 3 months prior to the study. Every participant underwent a panoramic radiographic examination. Bone loss in the periodontitis groups was additionally evaluated with intra-oral radiographs. For periodontal diagnostics, probing pocket depth (PPD), clinical attachment loss and bleeding on probing were recorded at six sites per tooth by experienced periodontologists at the Department of Periodontology. The periodontitis patients group included subjects with severe (loss of supporting bone  $\geq 1/3$  of the root length) and generalized ( $\geq 30\%$  affected sites) periodontal disease with at least five sites with a PPD  $\geq 5$  mm (Armitage 1999). The control group included individuals presenting no radiographic bone loss, PPD  $\leq 3$  mm, and no gingival inflammation. Demographic characteristics and clinical parameters of control group and periodontitis patients are summarized in Table 1. The study was approved by the Ethics Committee of the Medical University of Vienna (Protocol Nr.: 623/2007). All individuals were thoroughly informed about the aims and methods of the study and gave their written agreement.

### Serum and saliva samples collection

Sample collection was carried out between 7:30 and 10:30 hours. Participants were asked to refuse eating, drinking (except water), smoking,

Table 1. Demographic characteristics and clinical parameters of study groups

	Control	Periodontitis
Age, years	34.3 $\pm$ 1.2	42.2 $\pm$ 8.4
Gender, m/w	25/29	53/36
Number of teeth	28.6 $\pm$ 1.6	27.7 $\pm$ 2.6
BoP, %	4.5 $\pm$ 3.7	37.7 $\pm$ 27.9
PD, mm	1.72 $\pm$ 0.28	3.60 $\pm$ 0.94
CAL, mm	1.72 $\pm$ 0.28	4.00 $\pm$ 1.13
Teeth with PD >5 mm	0	16.4 $\pm$ 7.3

Data are presented as mean  $\pm$  SD.

BoP, bleeding on probing; CAL, clinical attachment loss; PPD, probing pocket depth.

chewing gum, brushing teeth and using mouth rinsing solutions from midnight on before the sampling. Saliva was collected using a saliva collection system<sup>®</sup> (Greiner Bio-One, Kremsmuenster, Austria) according to the manufacturer's instructions. The percentage of whole saliva per sample was calculated photometrically using a Saliva Quantification Kit (item number 881010; Greiner Bio-One). Venous blood was drawn from the antecubital vein into serum gel tubes (Vacuette<sup>®</sup>; Greiner Bio-One). After 30 min. to allow blood clotting at room temperature, sera were isolated by centrifugation (10 min. at 2600 g at 4°C). Serum and saliva samples were collected from periodontitis patients before periodontal treatment.

#### NO metabolites, C-reactive protein and serum lipids measurements

Since proteins substantially interfere with nitrite measurements (Moshage et al. 1995), they were removed from the samples by centrifugal ultrafiltration through 10K filter (Amicon Ultra-4; Millipore, Billerica, MA, USA). Levels of NO metabolites in serum and saliva samples were determined by the use of nitrite/nitrate colorimetric assay kit using Griess reaction (Sigma, St. Louis, MO, USA) according to the manufacturer's instruction. Absorbance was measured on a microplate reader (Molecular Device, Sunnyvale, CA, USA) at a wavelength of 540 nm.

Levels of serum lipids and CRP were measured on a Chemistry Immuno-System Olympus AU640. A high sensitive CRP assay with a lower detection limit of 0.08 mg/l

and linear measurements rate 0.08–80 µg/ml was applied.

#### Statistical analysis

The differences between groups were tested by Mann–Whitney *U*-test. The effect of age and gender on the investigated parameters was evaluated using univariate linear models. Correlations between different parameters were checked by Spearman's non-parametric correlation tests. Differences were considered to be statistically significant at  $p < 0.05$ . All statistical analysis was performed using statistical program SPSS 19.0 (SPSS, Chicago, IL, USA).

#### Results

##### Analysis of whole population

Serum and saliva levels of NO metabolites and serum CRP levels in healthy individuals and periodontitis patients are shown in Fig. 1. Periodontitis patients exhibited significantly lower serum NO<sub>x</sub> (NO<sub>2</sub> + NO<sub>3</sub>) and salivary NO<sub>2</sub> levels and significantly higher serum CRP levels than control group. No significant effect of age on the levels of NO metabolites were found ( $p > 0.3$ ). The data on serum lipids level are summarized in Table 2. Periodontitis patients exhibited significantly higher serum levels of LDL and cholesterol as well as significantly higher LDL/HDL ratio than healthy individuals. No significant difference in the serum levels of HDL and triglycerides between control group and periodontitis patients was observed.

The correlations between salivary and serum levels of NO metabolites

on the one hand and serum CRP and lipids levels on the other hand were analysed (Fig. 2). Serum NO<sub>x</sub> levels exhibited significant negative correlation with serum levels of CRP ( $r = -0.181$ ,  $p < 0.05$ ; Fig. 2a) and significant positive correlation with serum HDL levels ( $r = 0.174$ ,  $p < 0.05$ ; Fig. 2b). No other correlation of salivary and serum NO metabolite levels with investigated parameters was found. There was no correlation between levels of NO metabolites in saliva and serum ( $r = -0.02$ ,  $p = 0.82$ ). Within periodontitis patients, no significant correlation of any investigated parameter with periodontal disease severity was found.

##### Analysis in different gender groups

Linear univariate analysis did not observed any significant effect of gender on serum and saliva levels of NO metabolites. However, since gender might influence NO production (Watanabe et al. 2000, Ghasemi et al. 2008), we analysed all parameters depending on gender. The levels of NO metabolites and serum CRP levels in different gender and diagnosis groups are shown in Fig. 3. Within male individuals, periodontitis patients exhibited significantly lower salivary NO<sub>2</sub> and serum NO<sub>x</sub> levels and significantly higher serum CRP than control group ( $p < 0.01$ ). In contrast, no difference in these parameters was found within female individuals. Serum lipid levels in different gender groups are presented in Table 3. Within male individuals, periodontitis patients exhibited significantly higher serum levels of LDL and cholesterol than control

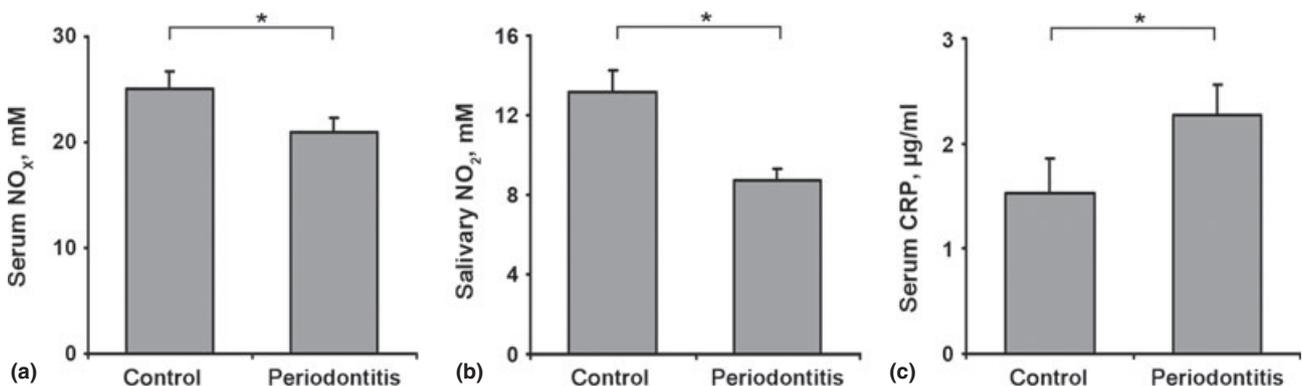


Fig. 1. Levels of serum NO<sub>x</sub> (a), salivary NO<sub>2</sub> (b) and serum CRP (c) in different groups. Data are presented as mean ± SEM \*means significant difference with  $p < 0.01$ , Mann–Whitney *U*-test.

Table 2. Serum lipids levels in control group and periodontitis patients

	Control	Periodontitis	<i>p</i>
HDL, $\mu\text{g}/\text{dl}$	$59.3 \pm 1.9$	$56.3 \pm 1.6$	0.25
LDL, $\mu\text{g}/\text{dl}$	$127.0 \pm 5.4$	$139.9 \pm 3.6$	<0.01
Cholesterol, $\mu\text{g}/\text{dl}$	$205.6 \pm 6.1$	$219.0 \pm 4.0$	<0.05
Triglycerides, $\mu\text{g}/\text{dl}$	$96.3 \pm 7.9$	$113.4 \pm 7.5$	0.12
LDL/HDL	$2.27 \pm 0.12$	$2.66 \pm 0.10$	<0.05

Data are presented as mean  $\pm$  SEM.

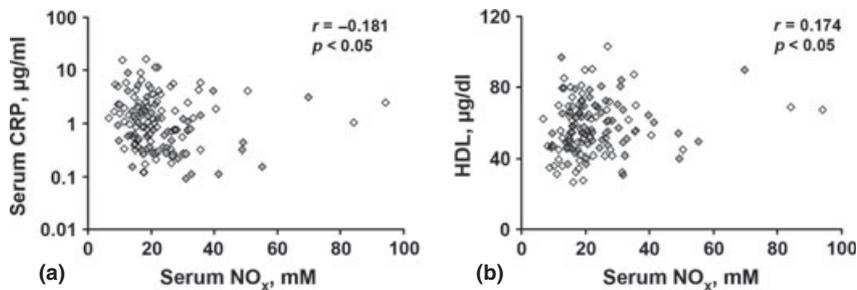


Fig. 2. Relationships between serum  $\text{NO}_x$  versus serum CRP levels (a) and serum  $\text{NO}_x$  versus serum HDL levels (b). Plots include data from healthy (closed symbols) and periodontally diseased (open symbols) individuals. Spearman rank correlation analysis revealed significant negative correlation between serum  $\text{NO}_x$  and serum CRP levels ( $r = -0.181$ ,  $p < 0.05$ ) and significant positive correlation between serum  $\text{NO}_x$  and serum HDL levels ( $r = 0.174$ ,  $p < 0.05$ ).

group ( $p < 0.05$ ). In contrast, no difference in serum lipid levels was found within female individuals. Finally, only in males serum  $\text{NO}_x$  levels exhibited significant negative correlation with serum CRP levels ( $r = -0.28$ ,  $p < 0.05$ ; Fig. 4). No correlation of serum levels of NO metabolites with serum lipid levels was found in both male and female group ( $p > 0.1$ ). No correlation between the levels of NO metabolites in serum and saliva was found in both gender groups (male:  $r = -0.04$ ,  $p = 0.73$ ; female:  $r = 0.01$ ,  $p = 0.94$ ). No significant effect of

age on the levels of NO metabolites were found in both gender groups ( $P > 0.1$ ).

### Discussion

In this study, we investigated the levels of NO metabolites in serum and saliva of periodontitis patients and their relationship with systemic inflammation, risk factors of cardiovascular disease and severity of periodontal disease. There are two possible sources of nitrite and nitrate in serum: NO synthesized from L-arginine by NOS or food. A previ-

ous study show that after 12 h fasting period, serum nitrite and nitrate are originated mainly from L-arginine-NO pathway (Rhodes et al. 1995). During sera isolation, we kept the samples for 30 min. at room temperature for blood clotting, and therefore we measured the total level of nitrite and nitrate in serum samples, which is thought to adequately reflect NO production (Moshage et al. 1995). In contrast, in saliva we measured only nitrite levels, because salivary glands might accumulate nitrate originating from food (Doel et al. 2004), which can lead to an overestimation of NO production levels. Therefore, serum levels of  $\text{NO}_x$  and salivary levels of  $\text{NO}_2$  seem to reflect adequately NO production.

We found that serum levels of NO metabolites in periodontitis patients are lower compared to that of healthy individuals. At a first look, this finding contradicts to the previous study, showing that non-smoking periodontitis patients exhibit increased nitrite levels compared to healthy controls (Menaka et al. 2009). Serum levels of nitrite and nitrate seem to reflect the activity of eNOS, because selective inhibition of eNOS leads to decrease in serum levels of NO metabolites by about 70% (Kleinbongard et al. 2003). Moreover, polymorphism of eNOS gene in human results in significantly different serum  $\text{NO}_x$  levels (Wang et al. 1997). Decreased serum levels of NO metabolites might indicate decreased NO production by eNOS in periodontitis patients. Theoretically, the NO production by iNOS, which is expressed in leucocytes at different pathological conditions, might also contribute to serum  $\text{NO}_x$

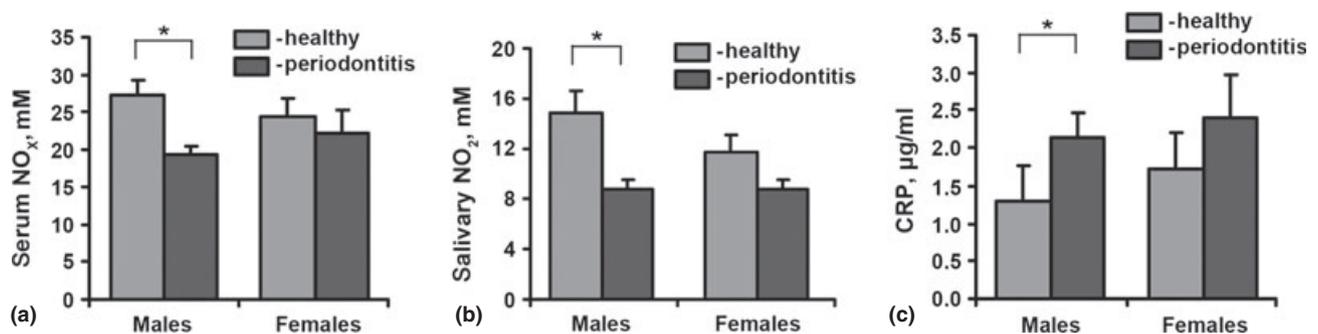


Fig. 3. Levels of serum  $\text{NO}_x$  (a), salivary  $\text{NO}_2$  (b) and serum CRP (c) in different gender and clinical groups. Data are presented as mean  $\pm$  SEM \*means significant difference with  $p < 0.01$ , Mann-Whitney *U*-test.

Table 3. Serum lipid levels in different gender and study groups

	Gender	Control	Periodontitis	<i>p</i> , control versus periodontitis
HDL, $\mu\text{g}/\text{dl}$	M	49.5 $\pm$ 1.7	51.4 $\pm$ 1.9	0.84
	F	67.8 $\pm$ 2.3	64.4 $\pm$ 2.5	0.27
LDL, $\mu\text{g}/\text{dl}$	M	125.6 $\pm$ 7.1	142.1 $\pm$ 5.0	<0.05
	F	128.3 $\pm$ 8.0	136.3 $\pm$ 4.8	0.07
Cholesterol, $\mu\text{g}/\text{dl}$	M	196.5 $\pm$ 8.3	218.5 $\pm$ 5.5	<0.05
	F	213.5 $\pm$ 8.8	219.6 $\pm$ 5.8	0.28
Triglycerides, $\mu\text{g}/\text{dl}$	M	107.3 $\pm$ 13.9	125.1 $\pm$ 10.9	0.28
	F	86.8 $\pm$ 8.3	94.5 $\pm$ 8.1	0.36
LDL/HDL	M	2.62 $\pm$ 0.18	2.92 $\pm$ 0.13	0.43
	F	1.96 $\pm$ 0.14	2.24 $\pm$ 1.42	0.08

Data are presented as mean  $\pm$  SEM.

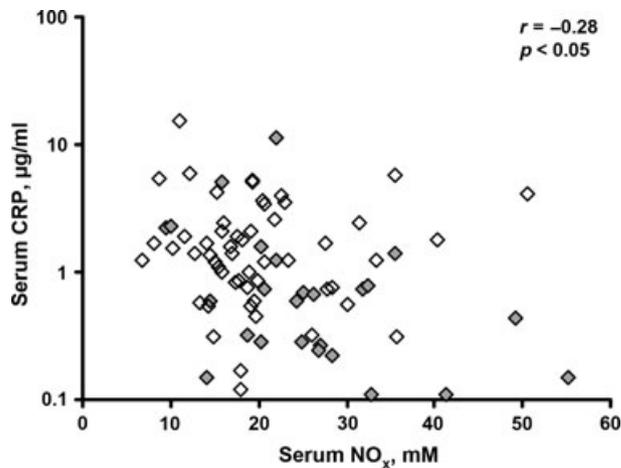


Fig. 4. Relationships between serum  $\text{NO}_x$  and CRP levels within male individuals. Plots include data from healthy (closed symbols) and periodontally diseased (open symbols) individuals. Spearman rank correlation analysis revealed significant negative correlation between serum  $\text{NO}_x$  and serum CRP levels ( $r = -0.28$ ,  $p < 0.05$ )

levels. However, this contribution is significant only systemic inflammatory disorders, for example sepsis, and usually results in increased serum  $\text{NO}_x$  levels (Crawford et al. 2004). In our study, only generally healthy individuals were included, and therefore the contribution of iNOS activity to the changes of serum  $\text{NO}_x$  levels is unlikely.

We further found that salivary  $\text{NO}_2$  levels were decreased in periodontitis patients compared to healthy controls. Our data are in line with some previous studies reporting lower salivary  $\text{NO}_2$  levels in periodontitis patients (Aurer et al. 2001, Ozer et al. 2011). However, there are also some studies reporting an increased salivary  $\text{NO}_2$  levels in periodontitis patients (Reher et al. 2007, Parwani et al. 2012). There are several possible explanations of this discrepancy. First, smoking might have

an effect on salivary  $\text{NO}_2$  levels (Bodis & Haregewoin 1994). In our study, we included only non-smoking individuals, whereas some previous studies included also smokers (Parwani et al. 2012), which might have effect on salivary levels of NO metabolites (Bodis & Haregewoin 1994). Second, the salivary  $\text{NO}_2$  levels could be theoretically influenced by the saliva collection method. Third, salivary proteins might affect measurements of NO metabolites. It is known that periodontitis is associated with increased protein release in saliva and crevicular fluid (Akalin et al. 1993, Henskens et al. 1993). NO produced by salivary gland might be easily scavenged by protein and this might influence detectable NO metabolites levels.

The source of salivary NO is assumed to be the salivary glands, which contain nNOS and eNOS iso-

forms (Bentz et al. 1998, Lomniczi et al. 1998). Therefore, data on salivary  $\text{NO}_2$  levels might suggest decreased NO production by these NOS isoforms in periodontitis patients. Although both serum and salivary levels of NO metabolites were decreased in periodontitis patients, no correlation between their levels in serum and saliva were observed. This could be due to the fact that salivary NO metabolites levels are largely influenced by factors other than NOS activity, for example, oral bacteria (Duncan et al. 1995). Therefore, saliva levels of NO metabolites cannot be used for monitoring their systemic levels.

Our hypothesis on decreased NO synthesis by eNOS in periodontitis patients is supported by previous clinical studies showing that periodontitis is associated with an impaired endothelium-dependent vasodilatation (Amar et al. 2003, Blum et al. 2007, Tonetti et al. 2007, Higashi et al. 2008). Some of these studies have also indirect signs of decreased NO production in periodontitis patients. Particularly, Amar et al. observe a decreased flow-mediated vasodilatation in periodontitis patients compared to the control group, whereas nitro-glycerine administration result in similar vasodilatation in both groups (Amar et al. 2003). Since physiological effects of nitro-glycerine are due to NO release by this substance (Kozlov et al. 2003), authors concluded that impaired vasodilatation in periodontitis patients could be associated with decreased NO production by endothelium. In another study, Higashi et al. (2008) showed that forearm blood flow response to acetylcholine administration was significantly smaller in periodontitis patients than in a control group, whereas administration of NO donor sodium nitroprusside resulted in similar responses in both groups. Since acetylcholine is a well known activator of eNOS (Furchgott & Zawadzki 1980), these data suggest a decreased NO production by the endothelium in periodontitis patients. Many studies suggest that periodontitis as an inflammatory disease is associated with an increased NO production due to induction of iNOS in the gingival tissue (Ugar-Cankal & Ozmeric 2006). However, this increase

in NO production seem to be rather local, and might not be reflected by changes in the systemic NO levels.

In our study, periodontitis patients were older than healthy individuals. We did not find any significant effect of age on the levels of NO metabolites. A previous study of healthy 20- to 69-year-old subject shows an increase in serum NO<sub>x</sub> levels with age in women and no dependency of NO<sub>x</sub> levels on age in men (Watanabe et al. 2000). Other study including over 20-year-old healthy individuals shows a slight increase in NO<sub>x</sub> levels at 50- to 59-year-old in both gender (Ghasemi et al. 2008). Our results showed decreased levels of NO metabolites in periodontitis patients compared to healthy individuals and these differences are unlikely due to age variations between these groups.

The decrease in the levels of NO metabolites in periodontitis patients was accompanied by the changes of other serum parameters. Particularly, serum levels of LDL, cholesterol and CRP as well as LDL/HDL ratio were significantly higher in periodontitis patients compared to control group. These results are in agreement with numerous previous studies, showing altered lipid metabolism and increased serum CRP-levels in periodontitis patients [e.g. (Cutler et al. 1999, Nibali et al. 2007, Buhlin et al. 2009)]. Serum levels of NO<sub>x</sub> exhibited negative correlation with serum levels of CRP. This finding could be interpreted as association between endothelial dysfunction, indicated by lower NO production on the one hand, and increased level of systemic inflammation, indicated by increased CRP, on the other hand. Serum NO<sub>x</sub> level exhibited also positive correlation with serum HDL levels. HDL has a protective effect against atherosclerosis and its low levels are known risk factor for CVDs (Assmann & Gotto 2004). Therefore, decreased NO production was also correlated with other parameters, characterizing increased risk of CVDs. Summarizing, the data of the present study further support the numerous evidences about an existing association between periodontitis and CVDs. However, within the limitation of cross-sectional study design, we cannot determine if the changes in NO

production are caused by periodontitis. The mechanisms underlying association between decreased NO production and periodontitis need to be further investigated in longitudinal studies.

Analysis in different gender groups showed that the decrease in serum and salivary levels of NO metabolites in periodontitis is present only in male but not in female individuals. The reason of this observation could be due to dependency of NO production in women on the day of the menstrual cycle (Rosselli et al. 1994, Ekerhovd et al. 2001), which is probably due to regulation of eNOS activity by oestrogen (Hayashi et al. 1995). These fluctuations in NO production in women make difficult the estimation of the effect of periodontal disease on NO production in female individuals. Our data could suggest that impairment of NO production in periodontitis is characteristic only for male patients. This conclusion is supported by a clinical study suggesting that male gender is a risk factor for endothelial dysfunction in periodontitis patients (Amar et al. 2003). The decreased serum levels of NO metabolites could also be associated with other pathological conditions in male periodontitis patients. Particularly, chronic periodontal disease was shown to be associated with male erectile dysfunction (Zadik et al. 2009). Impaired NO production within male periodontitis patients could be one of the basis for this observation, because erection is crucially dependent on the formation of NO by vascular endothelium (Burnett 2004). The role of NO in the association of periodontitis with erectile dysfunction was proved in the recent study on rat (Zuo et al. 2011), showing that experimentally induced periodontitis resulted in impaired penile erection and decreased eNOS expression.

Gender is a well-known risk factor of both periodontitis and cardiovascular disease. Male individuals usually have a higher prevalence and severity of periodontal disease than females (Shiau & Reynolds 2010a). Male gender is also well-known risk factor of CVDs (Roeters van Lennep et al. 2002). Our data suggest that the relationship between periodontitis and changes of several systemic

parameters, associated with CVDs risk and inflammation might depend on gender and might be more pronounced in males than in females. Particularly, periodontitis-associated changes in the serum levels of NO<sub>x</sub>, cholesterol, triglycerides and CRP, as well as correlation between serum NO<sub>x</sub> and CRP levels were observed only in male but not in female individuals. This gender-dependency is also supported by a clinical study, which shows that long-term periodontitis is related to subclinical atherosclerosis in men but not in women (Desvarieux et al. 2004). The reasons underlying the gender dependency in the association between periodontitis and systemic health are not entirely clear. Numerous previous studies suggest that the intensity of immune response to bacterial pathogen is generally higher in men than in women [for review, see (Marrion & Huet-Hudson 2006, Shiau & Reynolds 2010b)]. In our study, the serum level of C-reactive protein, characterizing systemic inflammation, was significantly increased only in male but not in female periodontitis patients. Therefore, different intensity of inflammatory response could be a possible reason of gender-dependent differences observed in our study. Other possible reasons for gender differences, observed in the present study, might be related to nutrition and sociocultural determinants. However, independently of underlying mechanism, male gender should be considered an important factor by assessing the risk of CVDs in periodontitis patients.

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### Clinical Relevance

*Scientific rationale for the study:* There is no data on the levels of NO metabolites in serum of periodontitis patients, whereas information on these levels in saliva is controversial.

*Principal findings:* Serum and saliva levels of NO metabolites were sig-

nificantly lower, whereas serum levels of CRP, LDL and cholesterol were significantly higher in periodontitis patients than in control group. Serum levels of NO metabolites exhibited correlation with serum CRP and HDL levels. These changes were characteristic for male but not for female population.

*Practical implications:* Data of this study will be useful for exact assessment of the CVDs risk in periodontitis patients and underline the significance of periodontal therapy for prophylaxis of these diseases especially in males.