

## ASSOCIATION BETWEEN PERIODONTAL DISEASE AND ENDOTHELIAL DYSFUNCTION IN SMOKING PATIENTS

Juliana Velosa-Porras<sup>1</sup>, Francina Escobar-Arregoces<sup>1</sup>,  
Catalina Latorre-Uriza<sup>1</sup>, María B. Ferro-Camargo<sup>1</sup>,  
Álvaro J Ruiz<sup>2</sup>, Luis F. Uriza-Carrasco<sup>3</sup>

<sup>1</sup> Department of Periodontal System. School of Dentistry. Pontificia Universidad Javeriana. Bogotá D.C. Colombia.

<sup>2</sup> Department of Clinical Epidemiology. School of Medicine. Pontificia Universidad Javeriana. Bogotá D.C. Colombia.

<sup>3</sup> Department of Radiology. School of Medicine. Pontificia Universidad Javeriana. Bogotá D.C. Colombia.

### ABSTRACT

Over the past two decades, there has been increasing interest in the impact of oral health on cardiovascular disease, particularly regarding the effects of chronic infections such as periodontitis on the endothelium. The aim of this study was to evaluate in healthy smokers whether there are any significant differences in the frequency of endothelial dysfunction between subjects with chronic moderate to severe periodontal disease and periodontally healthy subjects. An observational cross-sectional study was conducted. The target population was adults older than 40 years of age. Blood tests were performed to determine values of CBC, glycaemia, total cholesterol, HDL-C, and LDL-C. Periodontal examinations and probing were conducted with

a Florida Probe®, and standardized procedures were used to measure flow-mediated dilation. Out of 150 subjects [69 male (46%) and 81 female (54%)], 75 (50%) had chronic periodontitis. The mean value for baseline flow-mediated dilation was 4.04% and the mean value for final flow-mediated dilation was 4.66%, with a 0.62% mean difference showing a statistically significant increase ( $p < 0.001$ ). This study found no significant difference in the flow-mediated dilation values between periodontally healthy subjects and those with periodontitis, in contrast to the literature, which suggests a negative impact of periodontal disease on endothelial function.

**Key words:** Atherosclerosis, endothelium, vasodilation.

## ASOCIACIÓN ENTRE ENFERMEDAD PERIODONTAL Y DISFUNCIÓN ENDOTELIAL EN PACIENTES FUMADORES

### RESUMEN

Durante las últimas dos décadas ha venido incrementándose el interés acerca del impacto de la salud oral, sobre las enfermedades cardiovasculares. El objetivo del estudio fue evaluar si en pacientes sistémicamente sanos y con tabaquismo, se encontraban diferencias significativas en la frecuencia de disfunción endotelial entre personas con enfermedad periodontal crónica de moderada a severa y personas con salud periodontal. El diseño empleado fue un estudio observacional de corte transversal. La población blanco pacientes adultos mayores de 40 años de edad. Se tomaron muestras de sangre para obtener los valores de cuadro hemático, glucemia, colesterol total, cHDL y cLDL. Se realizó el examen periodontal diligenciando el anexo de periodoncia de la Facultad de Odontología y de acuerdo con el instructivo. El sondaje se realizó en todos los dientes presentes en boca

utilizando la sonda electrónica periodontal (Florida Probe®). La técnica y procedimientos para la vasodilatación mediada por flujo utilizados fueron los estandarizados internacionalmente. De los 150 participantes, 69 eran hombres (46%) y 81 mujeres (54%) con promedio de edad de 50.2 años. De estos, 75 pacientes (50%) correspondían al grupo de periodontitis crónica. Al evaluar la vasodilatación mediada por flujo el promedio inicial encontrado fue de 4.04% y el final fue de 4.66%, con un 0.62 % de aumento en promedio ( $p < 0.001$ ). Se puede concluir que a pesar de la evidencia en la literatura que sugiere que la enfermedad periodontal impacta negativamente la función endotelial, medida por vasodilatación mediada por flujo, el presente estudio no encontró una diferencia significativa.

**Palabras clave:** aterosclerosis, endotelio, vasodilatación.

### INTRODUCTION

Over the past two decades there has been increasing interest in the impact of oral diseases, (especially periodontitis) on cardiovascular diseases. Cardio-

vascular disease appears today as the main cause of premature death in both developed and developing countries, and as a common disease in adult populations<sup>1</sup>.

The pathophysiological basis of coronary disease is atherosclerosis, which has been defined as a progressive disease that consists of the thickening of the inner and medial layers of arteries, which may be triggered by a cellular reaction as a response to an endothelial injury.

The pathophysiological basis of all proatherogenic changes thus lies in the endothelium. Endothelium physiology, capacity to dilate, response to injury, and protection from coagulation and rupture are all significantly deteriorated by the process of atherosclerosis. This is known as endothelial dysfunction.

Chronic infectious processes such as periodontal disease may exert a proatherogenic effect by acting at a systemic level or at a local level on the vascular wall. The presence of multiple positive serologies (infectious burden) would significantly increase the risk of vascular disease. There is a significant relationship between the number of infectious pathogens to which an individual has been exposed and the extent of the atherosclerotic lesion. Several studies have found a positive association between infectious burden and prevalence of cardiovascular events. Moreover, the risk of vascular death increases with the number of infectious pathogens, especially in patients with advanced atherosclerosis<sup>2,3</sup>.

The literature is still very controversial regarding diseases such as periodontal and cardiovascular diseases, which share some risk factors such as smoking. For example, Beck et al. conducted a study in which they analyzed patients with cardiovascular risk who were smokers or non-smokers. A complete periodontal evaluation was performed and it was found that periodontal disease was not significantly related to cardiovascular disease, regardless of smoker status<sup>4</sup>.

Based on the aforementioned context, the goal of this study was to evaluate in healthy smokers whether there are any significant differences in the frequency of endothelial dysfunction between subjects with chronic to moderate periodontal disease and subjects with normal periodontal health.

## MATERIALS AND METHODS

An observational, cross-sectional study was conducted. The population was made up of adult patients, 40 years of age or older, living in Bogotá, who were receiving healthcare services at the Hospital Universitario de San Ignacio and the

Dental clinics at the Dental School in the Pontificia Universidad Javeriana.

Comparisons were made between subjects older than 40, smokers (5 to 15 cigarettes a day), who had a mild – severe chronic periodontitis diagnosis (PD) and smokers (5-15 cig/day) older than 40, who were not diagnosed with PD. Subjects with diabetes, high blood pressure or who took any drugs that modify endothelial function (aspirin, statins, ACE inhibitors, ARBs, calcium antagonists, sildenafil, L-arginine and antioxidants, corticosteroids or estrogens) were excluded from the study. For both groups, PD was diagnosed according to the Armitage classification (1999).

Sample size was calculated using data from the CANDEV study (López PJ, 2001), with the software TAMAMU 1.1<sup>®</sup>. The calculated sample was 150 patients: 75 with periodontitis and 75 in periodontal health.

## METHOD

Each patient that met the inclusion criteria was asked to sign a written Consent Form. Blood samples were taken to determine values of CBC, glycaemia, total cholesterol, HDL-C, and LDL-C, which were processed at the Hospital Universitario de San Ignacio. Patients were required to fast for at least 12 hours before sample collection.

Periodontal examination was conducted according to the Dental school formats, and each tooth in the mouth was probed using a periodontal electronic probe (Florida Probe<sup>®</sup>). Once the subject was identified and assigned to one of the two groups, the FMD was performed after measuring blood pressure and calculating arterial index. Internationally standardized techniques and procedures for FMD were used and the results are provided in millimeters. Since smoking is a significant risk factor for endothelial dysfunction, smokers were selected in order to increase the probability of finding dysfunction; otherwise the sample size needed would have increased significantly. There was an accepted risk that the strong effect of smoking could minimize the impact of periodontal disease, but the main comparison between patients with and without periodontitis, within the moderate risk produced by smoking, can show differences if they are significant enough to be detected.

The endothelial function was evaluated by means of the non-invasive Flow Mediated Dilation (FMD)

test of the brachial artery; ultrasound images were obtained from the brachial artery in the cubital fossa using a 14 MHz transducer. Initial images were obtained after 10 minutes' rest in supine position. FMD was determined as the change in arterial diameter responding to a reactive hyperemia (final image vs. initial image). Reactive hyperemia was induced by inflating a pneumatic cuff around the arm (beside the segment where the image was to be taken) up to a pressure of 200 mmHg for 5 minutes. Images were taken 2 minutes after deflating the cuff (final image). Percentage of FMD through the brachial artery was calculated according to the following formula:

$$\%VMF = ((\text{Average Final score} - \text{Average Initial score}) / \text{Average Initial score}) * 100\%$$

The reference value in the literature is 4%. Any result having a dilation percentage equal to or less than 4 was considered as endothelial dysfunction. According to established regulations (Resolution 8430, Colombian Ministry of Health) and the CIOMS, the study was classified as minimal risk research. Approval was obtained from the IRBs at Schools of Dentistry and Medicine at Javeriana University.

### STATISTICAL ANALYSIS

Descriptive data were obtained for demographics, periodontal evaluation results and FMD, through means, medians, ranges, standard deviations and 95% confidence intervals. FMD was reported in absolute values, percentage of change, and in categorical terms as normal or abnormal, using international standards. In the analysis of outcomes, odds ratios were used for the analysis of likelihood of having dysfunction between both groups and controls. Adjustments were made for age and sex. T-Student or chi-square tests were used for comparisons between groups, when appropriate. A difference that had a value of  $p < 0.05$  (two tails) was considered significant.

### RESULTS

Out of the 150 participants, 69 were male (46%) and 81 female (54%), with mean age 50.2 years. Of these, 75 patients (50%) had chronic periodontitis. They smoked an average 10.28 cigarettes/day, with a minimum of 5 and a maximum of 40. The average laboratory values found for these patients were:

glucose 95.8 mg/dL, total cholesterol 202 mg/dL, triglycerides 147.4 mg/dL, HDL-C 42.8 mg/dL and LDL-C 129.5 mg/dL. Regarding exercise, 5.3% exercised at least 30 minutes, 5 times a week; 22% exercised between 1 and 4 times a week, 5.3% exercised less than once a week, 24% did not exercise but had physical activity and 43.3% neither exercised nor engaged in physical activity.

Average initial flow-mediated dilation (Initial FMD) was 4.04 and average final flow-mediated dilation (final FMD) was 4.66, with a difference of 0.62 average increase, which was found to be statistically significant ( $p < 0.001$ ). The comparison of the initial and final percentage changes showed 16.07% at the beginning and 16.97% at the end, with a difference of 0.89%, which was not statistically significant ( $p = 0.284$ ).

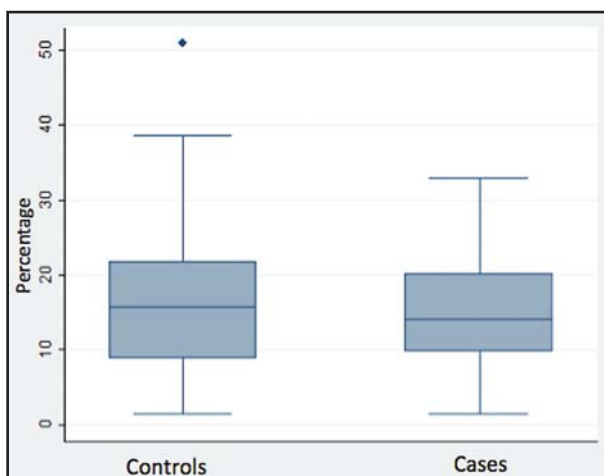
Analysis of variables according to sex showed a statistically significant difference for glucose, triglycerides, HDL-C, and Initial FMD and final FMD. With the exception of HDL-C, all values were higher in males (Table 1). A statistically significant difference according to gender, with less vasodilation response in women, was also observed. The analysis according to periodontitis showed that the mean age for the group with periodontitis was 49.85 years (S.D. 7.9) and for the periodontally healthy group 50.60 years (S.D. 7.09), with no statistically significant difference ( $p = 0.545$ ). No statistically significant difference was found in smokers between groups ( $p = 0.128$ ). Significant differences were found in exercise done less than once a week, favoring the periodontally healthy group, and in non-exercise but with physical activity, favoring the periodontal disease group (Table 2).

In the periodontally healthy group, mean initial flow-mediated dilation (Initial FMD) was 3.97%, and mean final flow-mediated dilation (Final FMD) was 4.57%, with 0.60% difference in average increase, which was statistically significant ( $p < 0.0001$ ). In the group with periodontitis, mean initial flow-mediated dilation (Initial FMD) was 4.10% and mean final flow-mediated dilation (Final FMD) was 4.75%, with 0.65% difference in average increase, which was also statistically significant ( $p < 0.001$ ). This shows that there was a significant increase in FMD in both groups, with an apparent greater change in the group with periodontitis.

Mean initial FMD was 4.10% for the group with periodontitis and 3.97% for the periodontally healthy

**Table 1: Variable distribution by gender.**

Variable		Female	Male	P value
		81	69	
Age ( $\bar{x}$ )		50.95	49.38	0.202
Number of cigarettes/day ( $\bar{x}$ )		10.28	10.28	0.991
Group	Periodontitis	37 (45.6%)	38 (55%)	0.326
	Periodontally Healthy	44 (54.3%)	31 (44.9%)	
Glycemia ( $\bar{x}$ )		91.14	101.4	0.000*
Cholesterol ( $\bar{x}$ )		206.14	197.2	0.111
Triglycerides ( $\bar{x}$ )		124.3	174.6	0.000*
cHDL ( $\bar{x}$ )		46.8	38.15	0.000*
cLDL ( $\bar{x}$ )		133	125.3	0.090
Initial FMD ( $\bar{x}$ )		3.69	4.44	0.000*
Final FMD ( $\bar{x}$ )		4.23	5.17	0.000*
% FMD ( $\bar{x}$ )		15.19	17.69	0.191
Endothelial Dysfunction	Yes	9 (11.1%)	1 (1.4%)	0.021*
	No	72 (88.8%)	68 (98.5%)	
Exercise 30 minutes 5 times a week (n)		3 (3.7%)	5 (7.2%)	0.471
Exercise 1 to 4 times a week (n)		11 (13.5%)	22 (31.8%)	0.010*
Exercise less than 1 time a week (n)		5 (6.17%)	3 (4.3%)	0.726
No exercise, but with physical activity		20 (24.7%)	16 (23.1%)	0.850
No exercise or physical activity		43 (51.8%)	23 (33.3%)	0.031*

*Fig. 1: Percentage of Vessel Dilation.*

group, with a difference of 0.13%, which was not statistically significant ( $p = 0.309$ ). This shows that the groups were comparable. Mean final FMD was 4.75% for the group with periodontitis and 4.57% for the periodontally healthy group, with a difference of 0.182%, which was not statistically significant

( $p = 0.209$ ), meaning that the responses in the groups were similar (Fig. 1).

The risk of developing endothelial dysfunction according to gender showed an OR=8.5 CI 95% (1.04 – 68.8) ( $p=0.0450$ ) for women.

## DISCUSSION

Endothelial dysfunction is the vascular event preceding atherogenesis and is caused by an early lesion in the endothelium. This deterioration in endothelium physiology constitutes the basic mechanism of the onset of atherosclerosis and is the pathophysiological basis of cardiovascular disease.

In addition to the traditional risk factors for cardiovascular disease, which are also related to endothelial dysfunction, such as smoking, age, hypercholesterolemia, hypertension and hyperglycemia, there are others such as excess weight, insulin resistance, inflammation and chronic infections, which can be detected by tests such as C-reactive protein and have been added as risk

**Table 2: Variable distribution by group.**

Variable		Periodontitis group	Periodontally healthy group	P value
		75	75	0.545
Age ( $\bar{x}$ )		50.6	49.85	0.128
Number of cigarettes/day ( $\bar{x}$ )		10.85	9.71	0.679
Glycemia ( $\bar{x}$ )		95.29	96.48	0.915
Cholesterol ( $\bar{x}$ )		202.3	201.7	0.530
Tryglicerides ( $\bar{x}$ )		151.8	143.0	0.200
cHDL ( $\bar{x}$ )		41.6	44.0	0.539
cLDL ( $\bar{x}$ )		130.9	128.1	0.310
VMF Inicial ( $\bar{x}$ )		4.10	3.97	0.209
VMF Final ( $\bar{x}$ )		4.75	4.57	0.721
% VMF ( $\bar{x}$ )		16.68	16.0	1.0
Endothelial Dysfunction	Yes	5 (6.6%)	5 (6.6%)	1.0
	No	70 (93.3%)	70 (93.3%)	
Exercise 30 minutes 5 times a week (n)		4 (5.3%)	4 (5.3%)	0.431
Exercise 1 to 4 times a week (n)		14 (18.6%)	19 (25.3%)	0.006*
Exercise less than 1 time a week (n)		0 (0%)	8 (10.6%)	0.000*
No exercise, but with physical activity		30 (40%)	6 (5%)	0.099
No exercise or physical activity		27 (36%)	38 (50.6%)	

factors<sup>5</sup>. Although men in this study showed more risk factors as glycaemia, cholesterol, tryglicerides and cLDL, women showed less initial and final FMD than men. It is important to take into account that exercise and smoking may have influence, either positive or negative, on this finding. Moreover, 9 women, but only 1 man was found to have endothelial dysfunction. These women had other potentially related factors such as smoking and periodontal status; however, conclusions cannot be drawn for any association because the sample is too small. A new study is currently underway to enlarge the sample and find more conclusive results.

This study analyzed 150 patients, 75 with periodontitis and 75 periodontally healthy, all smokers. The patients were healthy and comparable in age, smoking, blood sugar and cholesterol levels. Mean age was 49.85 years for the group with periodontitis and 50.60 years ( $p = 0.545$ ) for the periodontally healthy group. When smoking status was compared between groups, no statistically significant difference ( $p = 0.128$ ) was found. Smokers were selected on the basis that they are assumed to have some degree of basal endothelial dysfunction that might

be increased by adding another risk factor such as periodontitis. Since smoking is a strong predictor of endothelial dysfunction, only smokers were selected, in order to increase the chances of finding dysfunction. Smoking status may have masked a few of the possible differences, but the groups with and without periodontitis differed significantly in the factor to be evaluated.

Nevertheless, our results showed that of the 150 patients analyzed, all smokers, only 10 presented endothelial dysfunction (6.6%).

These results contrast with those of several studies, which have been emphatic in stating that smoking causes peripheral vascular endothelial dysfunction and that smokers have a greater impairment of endothelium-dependent vasodilation. Heffernan et al.<sup>6</sup> examined the association between endothelial function and exercise capability in chronic smokers versus non-smokers, concluding that FMD was significantly lower in smokers than in nonsmokers ( $8.9 \pm 0.9$  vs.  $12.6 \pm 0.7\%$ ,  $p < 0.05$ ).

Similarly, Wiesmann et al.<sup>7</sup> observed a reduction in brachial artery VMF in smokers compared to non smokers ( $7.5 \pm 2.7\%$  vs.  $15.5 \pm 2.0\%$ ,  $p = 0.03$ ),

which was related to impaired endothelium-dependent dilation. Barua et al.<sup>8</sup> reported that smoking was associated with decreased endothelium-dependent vasodilation and reduction in oxide production.

This study only found statistically significant differences in subjects who exercised less than once a week, in favor of the periodontally healthy group, and no exercise but physical activity favoring the group with periodontitis. Nevertheless, the two groups had identical endothelial dysfunction frequencies of 6.6% (5 periodontitis patients and 5 periodontally healthy patients). This contrasts with results reported in human studies in which strict exercise regimes under supervision improved endothelial function in coronary and brachial function. The low number of periodontal groups found with endothelial dysfunction may be responsible for the lack of differences, mainly due to lack of statistical power.

Heffernan et al.<sup>6</sup> reported a positive association for vasodilation and exercise for both non-smokers and smokers. Braga et al.<sup>9</sup> reported that exercise, even in estrogen deficiency conditions, can improve endothelial dependent vasodilation in rat aorta via enhanced nitric oxide (NO) bioavailability and reduced reactive oxygen (ROS) species levels. De Souza et al.<sup>10</sup> reported that endothelial dysfunction was greater in sedentary groups when comparing FMD of the brachial artery in sedentary young men, sedentary old men, young and old men in physical training.

This study found that when patients with periodontitis were compared to periodontally healthy patients, periodontitis and chronic infections did not impact or add a risk factor to endothelial dysfunction with OR = 1.0 95% CI (0.277 - 3.6) (p = 1.0).

These results are consistent with those reported by Aristizabal, Gomez et al.<sup>11</sup>, who in a systematic review of the literature found that there is little evidence for the direct relation of the periodontal disease with endothelial dysfunction in patients aged between forty and eighty. The relationship between periodontal disease and endothelial dysfunction was supported by the effect of periodontal treatment on endothelial dysfunction. The previous systematic review also reported that endothelial dysfunction was analyzed differently in studies by clinical, subclinical, and through biomarkers of systemic inflammation. They noted that it cannot be concluded that periodontal disease

is a risk factor for endothelial dysfunction. However, based on the findings reported in the selected articles it was concluded that intensive periodontal treatment in the long term (over six months) improved endothelial function; but the degree of improvement was not directly correlated with the change in inflammatory biomarkers.

Regarding the effect of periodontal treatment on endothelial dysfunction, the literature is controversial. There is evidence as reported by D' Aiuto et al.<sup>12</sup>; Blum et al.<sup>13</sup>; Tonetti et al.<sup>14</sup>, and Piconi et al.<sup>15</sup> in which the treatment of periodontal disease significantly improves endothelial function, and it was reported that better results were obtained with intensive periodontal therapy (antibiotic) than with basic periodontal therapy<sup>12-15</sup>. However, in contrast to the above, Li et al.<sup>16</sup> noted that periodontal treatment had a neutral effect on peripheral endothelial function. Aristizabal Gomez et al.<sup>11</sup> justified these controversial findings by the strict criteria for patient selection and the method for measuring endothelial function. Moreover, they considered that if patients had an underlying disease that predisposed to endothelial dysfunction, it was unlikely that they could achieve an immediate benefit on endothelial function related only to periodontal treatment. The analysis of the controversial results of this research could therefore be aligned with the issues raised by Aristizabal Gomez et al.<sup>11</sup> because in this study, patients were strictly selected, excluding other systemic diseases such as diabetes, hypertension or dyslipidemia, which may have been the reason why the results showed neither the presence of endothelial dysfunction even though patients were smokers, nor a difference in dysfunction between patients with periodontitis and periodontally healthy subjects.

## CONCLUSIONS

Despite the evidence in the literature suggesting that periodontal disease has a negative impact on endothelial dysfunction, as measured by FMD, this study found no significant difference between periodontitis and periodontally healthy subjects. The pathophysiology of the disease, however, suggests the rationale for further studies to show that chronic infection and inflammation caused by periodontal disease may disrupt endothelial function and become athero thrombotic risk factors for disease, and show that controlling periodontal disease may reduce the risk of atherosclerosis.

The findings may be explained by lack of statistical power, or they may point to racial or local differences in the weight of the influence of periodontal disease on the endothelium, or even that the effect exists but is so small that the results were difficult to detect in the study population.

Some other interesting explanations, to be further explored in future studies, include protective factors

related to demographic characteristics, or even that the international standards for endothelial dysfunction do not correctly validate in our population.

The results are a contribution to the understanding of the complex relationship between local chronic inflammation and endothelium function. Further studies are needed in the region, exploring the different risk and protective factors.

#### ACKNOWLEDGEMENT

This work was founded by the Pontificia Universidad Javeriana.

#### CORRESPONDENCE

Dr. Juliana Velosa Porras  
Cra. 7 No. 40 –62. Facultad de Odontología  
Bogotá D.C., Colombia. 110311.  
juliana.velosa@javeriana.edu.co

#### REFERENCES

- Persson GR, Persson RE. Cardiovascular disease and periodontitis: an update on the associations and risk. *J Clin Periodontol* 2008; Sep;35 (8 Suppl):362-379.
- Mattila KJ, Valtonen VV, Nieminen MS, Asikainen S. Role of infection as a risk factor for atherosclerosis, myocardial infarction, and stroke. *Clin Infect Dis* 1998;26:719-734.
- Espinola-Klein C, Rupprecht HJ, Blankenberg S, Bickel C, Kopp H, Rippin G, Victor A, Hafner G, et al. Impact of infectious burden on extent and long-term prognosis of atherosclerosis. *Circulation*. 2002 1;105:15-21.
- Beck JD, Eke P, Heiss G, Madianos P, Couper D, Lin D, Moss K, Elter J, et al. Periodontal disease and coronary heart disease: a reappraisal of the exposure. *Circulation*. 2005 5;112:19-24.
- Landmesser U, Drexler H. The clinical significance of endothelial dysfunction. *Curr Opin Cardiol* 2005; Nov;20: 547-551.
- Heffernan KS, Karas RH, Patvardhan EA, Kuvin JT. Endothelium-dependent vasodilation is associated with exercise capacity in smokers and non-smokers. *Vasc Med* 2010;15:119-125.
- Wiesmann F, Petersen SE, Leeson PM, Francis JM, Robson MD, Wang Q, Choudhury R, Channon KM, et al. Global impairment of brachial, carotid, and aortic vascular function in young smokers: direct quantification by high-resolution magnetic resonance imaging. *J Am Coll Cardiol* 2004;44: 2056-2064.
- Barua RS, Ambrose JA, Eales-Reynolds LJ, DeVoe MC, Zervas JG, Saha DC. Dysfunctional endothelial nitric oxide biosynthesis in healthy smokers with impaired endothelium-dependent vasodilatation. *Circulation* 2001; 104:1905-1910.
- Braga VA, Couto GK, Lazzarin MC, Rossoni LV, Medeiros A. Aerobic exercise training prevents the onset of endothelial dysfunction via increased nitric oxide bioavailability and reduced reactive oxygen species in an experimental model of menopause. *PLoS One*. 2015; 10:e0125388. *PLoS One*. 2015 Apr 29;10:e0125388. DOI: 10.1371/journal.pone.0125388. eCollection 2015.
- De Souza CA, Shapiro LF, Clevenger CM, Dinenna FA, Monahan KD, Tanaka H, Seals DR. Regular aerobic exercise prevents and restores age-related declines in endothelium-dependent vasodilatation in healthy men. *Circulation* 2000; 102: 1351-1357
- Aristizábal PA, Gómez MP, Escobar F, Velosa J. Asociación entre enfermedad periodontal y disfunción endotelial. Revisión sistemática de la literatura. *UnivOdontol* 2013; 32:147-160. <http://revistas.javeriana.edu.co/index.php/revUnivOdontologica/article/view/6650>
- D'Aiuto F, Parkar M, Nibali L, Suvan J, Lessem J, Tonetti MS. Periodontal infections cause changes in traditional and novel cardiovascular risk factors: results from a randomized controlled clinical trial. *Am Heart J* 2006; 151: 977-984.
- Blum A, Kryuger K, Mashiach Eizenberg M, Tatour S, Vigder F, Laster Z, Front E. Periodontal care may improve endothelial function. *Eur J Intern Med* 2007;18: 295-298.
- Tonetti MS. Periodontitis and risk for atherosclerosis: An update on intervention trials. *J Clin Periodontol* 2009; 36: 15-9.
- Piconi S, Trabattoni D, Luraghi C, Perilli E, Borelli M, Pacci M, Rizzardini G, Lattuada A, et al. Treatment of periodontal disease results in improvements in endothelial dysfunction and reduction of the carotid intima-media thickness. *FASEB J* 2009; 23:1196-1204.
- Li X, Tse HF, Yiu KH, Li LS, Jin L. Effect of periodontal treatment on circulating CD34(+) cells and peripheral vascular endothelial function: a randomized controlled trial. *J Clin Periodontol* 2011;38:148-156.