Journal of
PERIODONTAL RESEARCH

J Periodont Res 2013; 48: 308–314

JOURNAL OF PERIODONTAL RESEARCH
doi:10.1111/jre.12009

# Periodontal therapy decreases serum levels of adipocyte fatty acid-binding protein in systemically healthy subjects: a pilot clinical trial

**X. Li[1], H. F. Tse[2], K. H. Yiu[2] C. Zhang[1], L. J. Jin[1]**

[1]Faculty of Dentistry, The University of Hong Kong, Hong Kong SAR, China and [2]Division of Cardiology, Department of Medicine, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong SAR, China

*Li X, Tse HF, Yiu KH, Zhang C, Jin LJ. Periodontal therapy decreases serum levels of adipocyte fatty acid-binding protein in systemically healthy subjects: a pilot clinical trial. J Periodont Res 2013; 48: 308–314. © 2012 John Wiley & Sons A/S. Published by John Wiley & Sons Ltd*

*Background and Objective:* Adipocyte fatty acid-binding protein (A-FABP) is expressed in adipocytes, macrophages and microvascular endothelial cells, and it plays a central role in inflammation, atherosclerosis and metabolic responses. This pilot study investigated the effect of nonsurgical periodontal therapy on the serum levels of A-FABP in subjects with chronic periodontitis.

*Material and Methods:* A pilot clinical trial was conducted in 24 otherwise healthy Chinese subjects with moderate to severe chronic periodontitis. The treatment group ($n$ = 12) received nonsurgical periodontal therapy immediately, whereas in the control group ($n$ = 12) the treatment was delayed for 3 months. The serum levels of A-FABP were measured by ELISAs. Other inflammatory and endothelial biomarkers and periodontal conditions were evaluated at baseline and at the 3-month follow-up appointment.

*Results:* A-FABP levels decreased significantly in the treatment group compared with the control group (treatment effect: −1.7 ng/mL; 95% confidence interval: −2.8 to −0.6; $p$ = 0.003). The treatment also significantly improved periodontal conditions but had no significant effect on other biomarkers. In the multivariable regression model, the change in the percentage of sites with detectable plaque was significantly associated with the change in the level of A-FABP (beta: 0.04, 95% confidence interval: 0.01–0.06, $p$ = 0.004).

*Conclusion:* Within the limitations of this pilot study, the current findings suggest that treatment of periodontitis may significantly decrease the serum levels of A-FABP. Further longitudinal study with a large sample size is warranted to confirm this finding and elaborate the relevant clinical implications.

Lijian Jin, DDS, PhD, Odont Dr, Hon FDSRCS (*Edin*), Faculty of Dentistry, Periodontology, The University of Hong Kong, Prince Philip Dental Hospital, 34 Hospital Road, Hong Kong SAR, China
Tel: (852) 2859 0302
Fax: (852) 2858 7874
e-mail: ljjin@hkucc.hku.hk

Key words: adipocyte fatty acid-binding protein; atherosclerosis; inflammation; periodontal disease; type 2 diabetes

Accepted for publication August 21, 2012

Adipocyte fatty acid-binding protein (A-FABP) is produced by adipocytes, macrophages and microvascular endothelial cells, and it accounts for around 6% of the total cellular protein (1). It is a member of the family of intracellular lipid transport proteins and plays a crucial role in the evolutionary crossroads of inflammation,

atherosclerosis and metabolic responses (2). It is closely associated with atherosclerotic inflammation, as assessed by positron emission tomography (3). Recent Chinese cohort studies showed that A-FABP was independently associated with carotid atherosclerosis, as measured using carotid intima-media thickness (4), as well as with ischemic stroke and mortality in stroke patients (5). Animal studies found that deletion of A-FABP-related genes could prevent or reduce the development of atherosclerosis in the atherosclerosis-prone apo-E-deficient mice (6–8). In addition, the serum levels of A-FABP were closely associated with obesity and metabolic syndrome (9,10), and they also predicted the development of type 2 diabetes mellitus (DM) and metabolic syndrome in 5- or 10-year prospective studies (11,12). Furthermore, A-FABP was reported to be significantly correlated with nephropathy staging and macrovascular complications in patients with type 2 DM (13). Overall, A-FABP is a novel and promising biomarker in cardiovascular disease (CVD) and DM, and is currently receiving much attention in medical fields.

Since 1989, a number of studies have been undertaken in the area of periodontal medicine to investigate the relationship between periodontal diseases and systemic diseases or disorders (14). It has been well documented that periodontal treatment could reduce the systemic level of inflammation and improve endothelial function (15–17). In these studies, high-sensitivity C-reactive protein (hs-CRP) has been widely used and considered as a biomarker of inflammation. However, two studies failed to use CRP to explain the established relationship between periodontal disease and CVD (15,18). Emerging evidence shows that several novel biomarkers including circulating progenitor cells and A-FABP may be more closely associated, than CRP, with the risk of developing CVD (4,19,20). Our recent studies showed that the level of circulating endothelial progenitor cells was related to the severity of chronic periodontitis and that periodontal treatment reduced the level of these cells (20,21). Therefore, these newly defined biomarkers could be used in the research on periodontal disease and CVD (22).

Based upon the current understanding of A-FABP and its crucial roles in inflammation, atherosclerosis and metabolism, it is hypothesized that A-FABP may be an alternative biomarker in clinical research investigating the association of periodontal diseases with CVD or DM. In this pilot study, we investigated the effect of nonsurgical periodontal treatment compared with no periodontal treatment on the serum levels of A-FABP in subjects with chronic periodontitis.

## Material and methods

### Study design and subjects

A pilot clinical trial was conducted in 24 systemically healthy subjects with moderate to severe chronic periodontitis. These subjects had received a health-screening programme to exclude CVD and DM. None of the subjects had received antibiotics, anti-inflammatory or immunosuppressant agents in the last 3 months, and they were never-smokers. This study was approved by the Institutional Review Board of The University of Hong Kong/Hospital Authority Hong Kong West Cluster, and was registered at http://www.hkclinicaltrials.com (number HKCTR-458). Oral and written informed consent was obtained from all participants before the study.

These 24 subjects were placed in either a treatment group ($n = 12$) or a control group ($n = 12$), avoiding an imbalance of age and gender between the two groups (23,24). The treatment group received periodontal treatment immediately, whereas periodontal treatment in the control group was delayed by 3 months. Blood sampling and periodontal examination were conducted at baseline and at the 3-month follow-up. All examiners responsible for periodontal and medical examinations, and dental hygienists who provided the periodontal treatment, were blinded to the group assignments.

### Periodontal examination and nonsurgical periodontal therapy

Comprehensive periodontal examination at six sites per tooth, excluding the third molars, was undertaken by a single investigator at baseline and at the 3-month follow-up. The intra-examiner reproducibility was good, with a κ-value of 0.762. The number of missing teeth, presence or absence of plaque, bleeding on probing, probing depth and gingival recession were recorded. Interproximal clinical attachment loss was calculated on the basis of probing depth and gingival recession (20). In this study, moderate to severe chronic periodontitis was diagnosed using the following criteria (20): (i) more than six sites with a probing depth of $\geq$ 4 mm; and (ii) over 25% of sites with interproximal clinical attachment loss of $\geq$ 5 mm, or more than eight missing teeth, excluding third molars.

After baseline examination, each subject received professional oral-hygiene instructions. One course of nonsurgical periodontal therapy, including supragingival and subgingival scaling and root debridement, using both hand instruments and a piezoelectric ultrasonic scaler, was provided to subjects in the treatment group by senior dental hygienists. At the end of each session, Corsodyl mint mouthwash (300 mL containing 0.2% w/v chlorhexidine digluconate; GlaxoSmithKline, Middlesex, UK) was given to each subject for 1 wk of use. Hopeless teeth were extracted, and restoration and endodontic therapy were conducted for caries and pulpal conditions, as necessary. In the control group, similar treatment was provided after the 3-month follow-up appointment.

### Medical assessment and sample assays

Body mass index was calculated by dividing the subject's weight (in kg) by the square of their height (in m). Waist circumference was measured at the smallest circumference of the natural waist, and hip circumference was measured around the widest

portion of the buttocks. For diastolic blood pressure and systolic blood pressure, two measurements, taken with a 10-min intervening period, were averaged. A 10-mL sample of peripheral blood was collected from each subject for the analysis of serum levels of creatinine, albumin, total protein, urea, and glucose and lipid profiles using standard biochemical approaches. The level of hs-CRP was evaluated using a particle-enhanced immunoturbidimetric assay (Roche Diagnostics, Mannheim, Germany) with a chemistry analyser (Hitachi 747 Analyzer; Boehringer Mannheim, Mannheim, Germany). Commercial ELISA kits were used to measure asymmetric dimethylarginine (Diagnostika Gmbh, Hamburg, Germany), adiponectin and A-FABP (Antibody and Immunoassay Services, The University of Hong Kong, Hong Kong SAR, China).

Flow cytometry (Elite; Beckman Coulter, Inc., Brea, CA, USA) was used to measure the number of kinase insert domain containing receptor positive cells ($KDR^+$) in blood, as previously described (20,21). In brief, 100 μL of peripheral blood was incubated with human KDR antibody (Sigma, St Louis, MO, USA). Fluorescein isothiocyanate-labelled IgG1a (Beckman Coulter) and phycoerythrin-labelled IgG2b (Becton Dickinson, Franklin Lakes, NJ, USA) served as the isotypic controls for colour compensation. An automated counter (Elite; Beckman Coulter) was used to count the number of $KDR^+$ cells in every 1,000,000 events in the lymphocyte gate.

### Statistical analysis

The primary outcome measure was the change of A-FABP levels, and the secondary outcome measures included the change of other biomarkers and the change in periodontal conditions. Based on the available evidence on the change in serum levels of A-FABP (25), it was decided to recruit at least 10 subjects in each group to permit the detection of a difference in A-FABP levels of 2.0 ng/mL, with a standard deviation of approximately 1.5, at a 5% level of significance and with a power of 85%. The difference between the groups was compared using the independent *t*-test, chi-square analysis, Fisher's test or a nonparametric test, as appropriate. The analysis of covariance was employed to make the comparisons after adjusting for the baseline values concerned. Univariable and multivariable linear regression models were used to identify variables that might influence the change of A-FABP. A two-sided *p*-value of $< 0.05$ was considered statistically significant using a software program (SPSS 14.0; SPSS Inc, Chicago, IL, USA).

## Results

The recruitment of subjects started in early 2009 and the follow up of the last participant finished at the end of 2009. The baseline demographic data and clinical characteristics of the subjects showed no significant difference between the two groups (Table 1). There was no significant difference in periodontal conditions and serum biomarkers between the groups (Table 2). Periodontal treatment significantly improved the periodontal conditions, as demonstrated by reductions in plaque level, bleeding on probing and probing depth ($p < 0.05$). A significant reduction in the A-FABP level was observed in the treatment group after adjustment for baseline values (treatment effect: −1.7 ng/mL; 95% CI: −2.8 to −0.6; $p = 0.003$). After adjustment for the baseline value, the hs-CRP level decreased in the treatment group compared with the control group (treatment effect: −0.5; 95% CI: −2.0 to 1.1; $p = 0.54$). No statistically significant difference in adiponectin or other inflammatory and endothelial biomarkers was noted (Table 2).

Using the univariable linear model (Table 3), changes in the concentration of asymmetric dimethylarginine, the percentage of $KDR^+$ cells, the percentage of sites with detectable plaque and the number of sites with a probing depth of $\geq 4$ mm, as well as the baseline level of A-FABP, were found to be potentially related to a change in A-FABP ($p < 0.10$). These risk variables were further included in the multivariable regression model. In the final model, baseline A-FABP (beta: −0.2; 95% CI: −0.3 to 0.03; $p = 0.02$), the percentage change in the number of $KDR^+$ cells (beta: −0.5; 95% CI: −0.8 to −0.1; $p = 0.02$) and the percentage change in the number of sites with detectable plaque (beta: 0.04; 95% CI: 0.01 to 0.06; $p = 0.004$) remained significant, with an adjusted $R^2$ of 58.2%.

*Table 1.* Baseline demographic data and clinical characteristics of patients

| Variable[a] | Control group ($n = 12$) | Treatment group ($n = 12$) | *p*-value |
|---|---|---|---|
| Age (years) | 57.6 ± 13.6 | 55.8 ± 13.4 | 0.74[b] |
| Male | 6 (50.0) | 4 (33.3) | 0.41[b] |
| Body mass index (kg/m$^2$) | 24.6 ± 4.3 | 25.9 ± 3.0 | 0.42 |
| Waist–hip ratio | 0.9 ± 0.07 | 0.9 ± 0.09 | 0.79 |
| Mean DBP (mm Hg) | 85.5 ± 13.9 | 84.1 ± 6.6 | 0.76 |
| Mean SBP (mm Hg) | 140.8 ± 17.5 | 131.7 ± 16.7 | 0.23[b] |
| Serum total protein (mM) | 78.3 ± 3.2 | 80.4 ± 3.3 | 0.11 |
| Albumin (mM) | 43.6 ± 2.2 | 43.5 ± 2.2 | 0.93 |
| Fasting glucose (mM) | 5.3 ± 0.6 | 4.9 ± 0.4 | 0.12 |
| Serum urea (mM) | 5.2 ± 1.1 | 5.9 ± 2.5 | 0.37 |
| Serum creatinine (mM) | 69.2 ± 15.7 | 74.9 ± 24.1 | 0.50[b] |
| Total cholesterol (mM) | 4.9 ± 0.7 | 5.0 ± 0.4 | 0.73 |
| Triglycerides (mM) | 1.7 ± 1.2 | 1.2 ± 0.5 | 0.22[b] |
| Low-density lipoprotein (mM) | 2.8 ± 0.8 | 3.1 ± 0.6 | 0.30 |
| High-density lipoprotein (mM) | 1.4 ± 0.3 | 1.3 ± 0.4 | 0.55 |

[a]Values are given as mean ± SD or *n* (%).
[b]Nonparametric test or Fisher's test, as appropriate. Other *p*-values were calculated using the independent *t*-test.
DBP, diastolic blood pressure; SBP, systolic blood pressure.

*Table 2.* Clinical conditions and blood biomarkers at baseline and at the 3-month follow-up appointment

| | Control group ($n$ = 12) | Treatment group ($n$ = 12) | Treatment effect (95% CI) | $p$-value |
|---|---|---|---|---|
| Clinical conditions | | | | |
| No. of missing teeth | | | | |
| Baseline | 3.4 ± 3.8 | 3.7 ± 4.7 | | 0.89 |
| 3-month follow-up | 3.4 ± 3.8 | 4.2 ± 5.4 | | 0.70 |
| Change[a] | 0 ± 0 | 0.5 ± 0.9 | | 0.07 |
| Change[b] | 0.01 ± 0.5 | 0.5 ± 0.5 | 0.5 (0.02 to 0.9) | 0.04 |
| Sites with detectable plaque (%) | | | | |
| Baseline | 47.9 ± 15.8 | 53.9 ± 22.7 | | 0.32 |
| 3-month follow-up | 44.8 ± 15.8 | 24.5 ± 22.9 | | 0.02 |
| Change[a] | −3.1 ± 9.6 | −29.4 ± 17.6 | | 0.001 |
| Change[b] | −3.9 ± 13.4 | −28.6 ± 13.4 | −24.7 (−36.2 to −13.2) | 0.000 |
| Sites with bleeding on probing (%) | | | | |
| Baseline | 45.5 ± 17.0 | 43.7 ± 18.5 | | 0.81 |
| 3-month follow-up | 31.5 ± 16.1 | 23.5 ± 11.9 | | 0.18 |
| Change[a] | −14.0 ± 9.3 | −20.3 ± 10.4 | | 0.13 |
| Change[b] | −13.7 ± 8.0 | −20.5 ± 8.0 | −6.9 (−13.7 to −0.05) | 0.04 |
| Sites with probing depth $\geq$ 4 mm (%) | | | | |
| Baseline | 10.0 ± 8.2 | 10.1 ± 8.4 | | 0.96 |
| 3-month follow-up | 7.7 ± 6.5 | 2.2 ± 2.7 | | 0.01 |
| Change[a] | −2.2 ± 3.6 | −7.9 ± 8.7 | | 0.04 |
| Change[b] | −2.3 ± 4.1 | −7.9 ± 4.1 | −5.6 (−2.1 to −9.0) | 0.003 |
| Inflammatory biomarkers | | | | |
| hs-CRP (mg/L) | | | | |
| Baseline | 2.8 ± 3.1 | 1.8 ± 1.0 | | 0.31 |
| 3-month follow-up | 2.3 ± 2.3 | 1.8 ± 1.0 | | 0.43 |
| Change[a] | −0.5 ± 2.9 | −0.1 ± 0.8 | | 0.72 |
| Change[b] | −0.05 ± 3.2 | −0.5 ± 3.2 | −0.5 (−2.0 to 1.1) | 0.54 |
| Endothelial biomarkers | | | | |
| ADMA (μM) | | | | |
| Baseline | 0.6 ± 0.1 | 0.7 ± 0.2 | | 0.39 |
| 3-month follow-up | 0.7 ± 0.1 | 0.7 ± 0.2 | | 0.85 |
| Change[a] | 0.05 ± 0.09 | 0 ± 0.2 | | 0.34 |
| Change[b] | 0.04 ± 0.2 | 0.01 ± 0.2 | −0.03 (−0.1 to 0.07) | 0.58 |
| $KDR^+$ cells (%) | | | | |
| Baseline | 2.9 ± 1.5 | 2.0 ± 0.6 | | 0.07 |
| 3-month follow-up | 2.0 ± 0.9 | 1.6 ± 0.4 | | 0.23 |
| Change[a] | −0.8 ± 1.7 | −0.3 ± 0.6 | | 0.32 |
| Change[b] | −0.4 ± 0.8 | −0.7 ± 0.8 | −0.3 (−1.0 to 0.4) | 0.39 |
| Adipokines | | | | |
| Adiponectin (ng/mL) | | | | |
| Baseline | 618.7 ± 680.4 | 963.0 ± 1624.9 | | 0.51 |
| 3-month follow-up | 823.7 ± 829.5 | 912.8 ± 1130.7 | | 0.83 |
| Change[a] | 205.0 ± 636.3 | −50.2 ± 546.3 | | 0.30 |
| Change[b] | 154.1 ± 478.5 | 0.7 ± 478.5 | −153.3 (−561.7 to 255.0) | 0.44 |
| A-FABP (ng/mL) | | | | |
| Baseline | 6.9 ± 3.4 | 9.3 ± 3.3 | | 0.11 |
| 3-month follow-up | 6.1 ± 3.6 | 6.5 ± 2.5 | | 0.75 |
| Change[a] | −0.8 ± 0.9 | −2.8 ± 1.5 | | 0.001 |
| Change[b] | −0.9 ± 1.2 | −2.7 ± 1.2 | −1.7 (−2.8 to −0.6) | 0.003 |

A-FABP, adipocyte fatty acid-binding protein; ADMA, asymmetric dimethylarginine; CI, confidence interval; hs-CRP, high-sensitivity C-reactive protein; KDR, kinase-insert-domain-containing receptor.
[a]Without adjustment for baseline value.
[b]With adjustment for baseline value.

## Discussion

The present study shows, for the first time, that nonsurgical periodontal therapy significantly decreases the serum levels of A-FABP, in conjunction with improved periodontal conditions. Emerging evidence on the relationship between A-FABP and inflammation may support the current findings. It has been shown that lipopolysaccharide significantly increases the production of A-FABP by macrophages through the toll-like receptor pathways (26). Periodontal infection could induce significant bacteraemia or the release of virulence factors of periodontal pathogens, such as

*Table 3*. Univariable and multivariable model of risk variables with the change in adipocyte fatty acid-binding protein (A-FABP)

| | Univariable[b] | | Multivariable[c] | |
|---|---|---|---|---|
| Risk variables[a] | Beta (95% CI) | *p*-value | Beta (95% CI) | *p*-value |
| Age (years) | −0.001 (−0.06 to 0.05) | 0.96 | | |
| Gender (male vs. female) | 0.4 (−1.0 to 1.8) | 0.55 | | |
| Baseline A-FABP (ng/mL) | −0.2 (−0.4 to −0.05) | **0.01** | −0.2 (−0.3 to −0.03) | 0.02 |
| hs-CRP (mg/L) | −0.05 (−0.2 to 0.3) | 0.72 | | |
| Adiponectin (ng/mL) | 0 (0 to 0.002) | 0.46 | | |
| ADMA (μM) | 4.5 (−0.6 to 9.6) | **0.08** | | |
| $KDR^+$ cells (%) | −0.5 (−1.0 to −0.01) | **0.047** | −0.5 (−0.8 to −0.1) | 0.02 |
| Sites with detectable plaque (%) | 0.05 (0.03 to 0.08) | **0.001** | 0.04 (0.01 to 0.06) | 0.004 |
| Sites with bleeding on probing (%) | 0.04 (−0.03 to 0.1) | 0.23 | | |
| Sites with probing depth $\geq$ 4 mm (%) | 0.1 (0.02 to 0.2) | **0.02** | | |

[a]Risk variables mean the change of the variable concerned except age, gender and baseline A-FABP.
[b]*p*-values in bold indicate potential variables that might influence the change of A-FABP and were included in the multivariate linear regression analysis.
[c]Adjusted $R^2$ = 58.2% in the final regression model.
ADMA, asymmetric dimethylarginine; CI, confidence interval; hs-CRP, high-sensitivity C-reactive protein; KDR, kinase-insert-domain-containing receptor.

lipopolysaccharide, into the circulation (27–31). The present study found that detectable plaque decreased in parallel with the significant reduction of A-FABP levels, which implies that a dynamic change of the local periodontal bacterial burden may, to some extent, modulate the systemic levels of A-FABP.

Circulating $KDR^+$ cells represent mature endothelial cells, endothelial progenitor cells and other stem or progenitor cells (32,33). The present study found that changes in the levels of $KDR^+$ cells were significantly related to the change in the A-FABP level after adjusting for other variables. This observation suggests that A-FABP may have a close association with endothelial cells or endothelial progenitor cells. Emerging evidence shows that A-FABP is present in regenerated endothelial layers and is associated with endothelial dysfunction (34,35). In diabetic patients, the level of A-FABP is associated with endothelial function, as measured by the reactive hyperemia index (36). Interestingly, after including various conventional variables, such as CRP, A-FABP remained the only variable related to endothelial function in the final regression model (36). Moreover, inhibition of A-FABP could improve endothelial function both *in vivo* and *in vitro*, through increased production of nitric oxide and nitric oxide synthase (35). Taken together, it is conceivable that A-FABP may be used to elaborate the association of periodontal disease with endothelial cells or dysfunction in future studies.

In addition to its role in endothelial dysfunction, a number of animal studies found that deficiency of A-FABP could prevent mice from developing insulin resistance (37,38), an unbalanced lipid metabolism (39) as well as early and advanced atherosclerosis (6,40). As a lipid-binding chaperone, A-FABP enhances the transport of fatty acids into cells and modulates downstream lipid-signalling cascades (41). It may also exacerbate a lipopolysaccharide-induced inflammatory response through a positive feedback loop involving JNKs (42). It has been shown that increased expression of chemoattractant and inflammatory cytokines in macrophages may accelerate the formation of foam cells, thereby leading to the formation of atherosclerosis (8,40). Notably, inhibition of A-FABP expression by macrophages could decrease the levels of various inflammatory cytokines, including tumour necrosis factor, interleukins 1 and 6, and monocyte chemoattractant protein (40). Based upon the current understanding of A-FABP and the present findings, the role of A-FABP is further elaborated and presented in Fig. 1.

Several limitations of this study need to be addressed. Firstly, the sample size of this pilot study is small, and it limits, to some extent, the strength of the findings. As a result of the small sample size, subgroup analysis is impossible, limiting the conclusions drawn from this study. Secondly, although the difference in the change of A-FABP between the two groups reached statistical significance, its clinical relevance and implications remain to be determined in these otherwise systemically healthy subjects. More meaningful clinical outcomes, such as endothelial function and carotid intima-media thickness, could have been analysed, and the subject groups may be extended to include medically compromised cohorts, such as diabetic patients. Lastly, in the present study the change in the level of A-FABP was not significantly associated with inflammatory biomarkers or lipid profiles (unreported data), as suggested in a previous study (43). No significant difference was observed between the two study groups in the change of levels of other blood biomarkers. These negative data may, in part, be a result of the small sample size of the study or related data variation. According to available data on A-FABP (25), its variation is small and its level remains relatively stable within months that is consistent with the result of this pilot study. The above critical points need to be considered and incorporated in future studies.

Within the limitations discussed above, the present study shows that nonsurgical periodontal therapy may significantly decrease the serum levels of A-FABP. The current findings could enhance our understanding of the mechanisms accounting for the

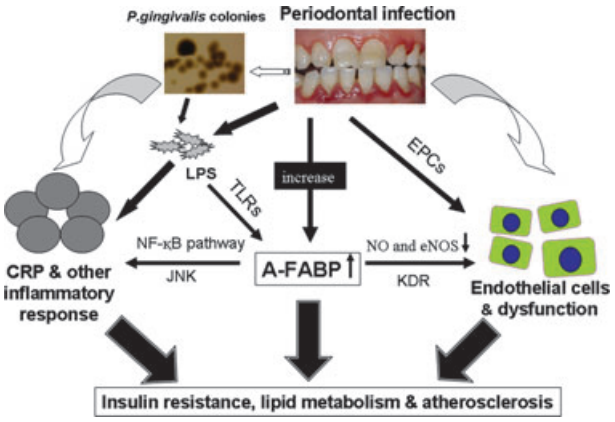


*Fig. 1*. The potential link between periodontal infection and serum levels of adipocyte fatty acid-binding protein (A-FABP), as well as the possible regulatory mechanisms involved, which may be related to diabetes mellitus and cardiovascular disease. CRP, C-reactive protein; eNOS, nitric oxide synthase; EPCs, endothelial progenitor cells; JNK, c-Jun N-terminal kinase; KDR, kinase-insert-domain-containing receptor; NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells); NO, nitric oxide; LPS, lipopolysaccharide; *P. gingivalis*, *Porphyromonas gingivalis*; TLRs, toll-like receptors.

association of periodontal inflammation with atherosclerosis and metabolic responses. Further longitudinal study with a large sample size is warranted to confirm the findings and elaborate the relevant clinical implications.

## Acknowledgements

The authors are grateful to the administrative assistants, nurses, dental-surgery assistants and staff hygienists from the Cardiology Division, Department of Medicine, Queen Mary Hospital and the Prince Philip Dental Hospital, for their great contributions to the study. This clinical study was registered at http://www.hkclinicaltrials.com (no. HKCTR-458). This study was supported by the Hong Kong Research Grants Council (HKU766909M and HKU768411M), The University of Hong Kong (CRCG Funds 200507176137, 200607176038 and 200707176095 and 200907176052) and the Sun Chieh Yeh Heart Foundation. The authors declare that they have no conflicts of interest.

## References

1. Makowski L, Hotamisligil GS. Fatty acid binding proteins–the evolutionary crossroads of inflammatory and metabolic responses. *J Nutr* 2004;**134**:2464S–2468S.
2. Boord JB, Fazio S, Linton MF. Cytoplasmic fatty acid-binding proteins: emerging roles in metabolism and atherosclerosis. *Curr Opin Lipidol* 2002;**13**:141–147.
3. Yoo HJ, Kim S, Park MS, *et al*. Serum adipocyte fatty acid-binding protein is associated independently with vascular inflammation: analysis with (18)F-fluorodeoxyglucose positron emission tomography. *J Clin Endocrinol Metab* 2011;**96**:E488–E492.
4. Yeung DC, Xu A, Cheung CW, *et al*. Serum adipocyte fatty acid-binding protein levels were independently associated with carotid atherosclerosis. *Arterioscler Thromb Vasc Biol* 2007;**27**:1796–1802.
5. Tso AW, Lam TK, Xu A, *et al*. Serum adipocyte fatty acid-binding protein associated with ischemic stroke and early death. *Neurology* 2011;**76**:1968–1975.
6. Makowski L, Boord JB, Maeda K, *et al*. Lack of macrophage fatty-acid-binding protein aP2 protects mice deficient in apolipoprotein E against atherosclerosis. *Nat Med* 2001;**7**:699–705.
7. Perrella MA, Pellacani A, Layne MD, *et al*. Absence of adipocyte fatty acid binding protein prevents the development of accelerated atherosclerosis in hypercholesterolemic mice. *Faseb J* 2001;**15**:1774–1776.
8. Boord JB, Maeda K, Makowski L, *et al*. Adipocyte fatty acid-binding protein, aP2, alters late atherosclerotic lesion formation in severe hypercholesterolemia. *Arterioscler Thromb Vasc Biol* 2002;**22**:1686–1691.
9. Xu A, Wang Y, Xu JY, *et al*. Adipocyte fatty acid-binding protein is a plasma biomarker closely associated with obesity and metabolic syndrome. *Clin Chem* 2006;**52**:405–413.
10. Engl J, Ciardi C, Tatarczyk T, *et al*. A-FABP–a biomarker associated with the metabolic syndrome and/or an indicator of weight change? *Obesity (Silver Spring)* 2008;**16**:1838–1842.
11. Xu A, Tso AW, Cheung BM, *et al*. Circulating adipocyte-fatty acid binding protein levels predict the development of the metabolic syndrome: a 5-year prospective study. *Circulation* 2007;**115**:1537–1543.
12. Tso AW, Xu A, Sham PC, *et al*. Serum adipocyte fatty acid binding protein as a new biomarker predicting the development of type 2 diabetes: a 10-year prospective study in a Chinese cohort. *Diabetes Care* 2007;**30**:2667–2672.
13. Yeung DC, Xu A, Tso AW, *et al*. Circulating levels of adipocyte and epidermal fatty acid-binding proteins in relation to nephropathy staging and macrovascular complications in type 2 diabetic patients. *Diabetes Care* 2009;**32**:132–134.
14. Williams RC, Offenbacher S. Periodontal medicine: the emergence of a new branch of periodontology. *Periodontol 2000* 2000;**23**:9–12.
15. Tonetti MS, D'Aiuto F, Nibali L, *et al*. Treatment of periodontitis and endothelial function. *N Engl J Med* 2007;**356**:911–920.
16. Tonetti MS. Periodontitis and risk for atherosclerosis: an update on intervention trials. *J Clin Periodontol* 2009;**36** (Suppl 10):15–19.
17. Piconi S, Trabattoni D, Luraghi C, *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.
18. Higashi Y, Goto C, Jitsuiki D, *et al*. Periodontal infection is associated with endothelial dysfunction in healthy subjects and hypertensive patients. *Hypertension* 2008;**51**:446–453.
19. Fadini GP, de Kreutzenberg SV, Coracina A, *et al*. Circulating CD34+ cells, metabolic syndrome, and cardiovascular risk. *Eur Heart J* 2006;**27**:2247–2255.
20. Li X, Tse HF, Yiu KH, *et al*. Increased levels of circulating endothelial progenitor cells in subjects with moderate to severe chronic periodontitis. *J Clin Periodontol* 2009;**36**:933–939.
21. Li X, Tse HF, Yiu KH, *et al*. Effect of periodontal treatment on circulating CD34+ cells and vascular endothelial function: a randomized controlled trial. *J Clin Periodontol* 2011;**38**:148–156.
22. Li X, Tse HF, Jin LJ. Novel endothelial biomarkers: implications for periodontal

disease and CVD. *J Dent Res* 2011; **90**:1062–1069.
23. Altman DG, Bland JM. Treatment allocation by minimisation. *BMJ* 2005; **330**:843.
24. Hewitt CE, Torgerson DJ. Is restricted randomisation necessary? *BMJ* 2006;**332**: 1506–1508.
25. Choi KM, Kim TN, Yoo HJ, *et al*. Effect of exercise training on A-FABP, lipocalin-2 and RBP4 levels in obese women. *Clin Endocrinol (Oxf)* 2009; **70**:569–574.
26. Kazemi MR, McDonald CM, Shigenaga JK, Grunfeld C, Feingold KR. Adipocyte fatty acid-binding protein expression and lipid accumulation are increased during activation of murine macrophages by toll-like receptor agonists. *Arterioscler Thromb Vasc Biol* 2005;**25**:1220–1224.
27. Parahitiyawa NB, Jin LJ, Leung WK, Yam WC, Samaranayake LP. Microbiology of odontogenic bacteremia: beyond endocarditis. *Clin Microbiol Rev* 2009; **22**:46–64.
28. Carroll GC, Sebor RJ. Dental flossing and its relationship to transient bacteremia. *J Periodontol* 1980;**51**:691–692.
29. Fine DH, Korik I, Furgang D, *et al*. Assessing pre-procedural subgingival irrigation and rinsing with an antiseptic mouthrinse to reduce bacteremia. *J Am Dent Assoc* 1996;**127**:641–642, 645–646.
30. Geerts SO, Nys M, De MP, *et al*. Systemic release of endotoxins induced by gentle mastication: association with periodontitis severity. *J Periodontol* 2002; **73**:73–78.
31. Amar S, Gokce N, Morgan S, Loukideli M, Van Dyke TE, Vita JA. Periodontal disease is associated with brachial artery endothelial dysfunction and systemic inflammation. *Arterioscler Thromb Vasc Biol* 2003;**23**:1245–1249.
32. Peichev M, Naiyer AJ, Pereira D, *et al*. Expression of VEGFR-2 and AC133 by circulating human CD34(+) cells identifies a population of functional endothelial precursors. *Blood* 2000;**95**:952–958.
33. Pelosi E, Valtieri M, Coppola S, *et al*. Identification of the hemangioblast in postnatal life. *Blood* 2002;**100**:3203–3208.
34. Elmasri H, Karaaslan C, Teper Y, *et al*. Fatty acid binding protein 4 is a target of VEGF and a regulator of cell proliferation in endothelial cells. *Faseb J* 2009;**23**:3865–3873.
35. Lee MY, Li H, Xiao Y, Zhou Z, Xu A, Vanhoutte PM. Chronic administration of BMS309403 improves endo -thelial function in apolipoprotein E-deficient mice and in cultured human endothelial cells. *Br J Pharmacol* 2011; **162**:1564–1576.
36. Aragones G, Ferre R, Lazaro I, *et al*. Fatty acid-binding protein 4 is associated with endothelial dysfunction in patients with type 2 diabetes. *Atherosclerosis* 2010;**213**:329–331.
37. Hotamisligil GS, Johnson RS, Distel RJ, Ellis R, Papaioannou VE, Spiegelman BM. Uncoupling of obesity from insulin resistance through a targeted mutation in aP2, the adipocyte fatty acid binding protein. *Science* 1996;**274**:1377–1379.
38. Uysal KT, Scheja L, Wiesbrock SM, Bonner-Weir S, Hotamisligil GS. Improved glucose and lipid metabolism in genetically obese mice lacking aP2. *Endocrinology* 2000;**141**:3388–3396.
39. Scheja L, Makowski L, Uysal KT, *et al*. Altered insulin secretion associated with reduced lipolytic efficiency in aP2-/- mice. *Diabetes* 1999;**48**:1987–1994.
40. Furuhashi M, Tuncman G, Gorgun CZ, *et al*. Treatment of diabetes and atherosclerosis by inhibiting fatty-acid-binding protein aP2. *Nature* 2007;**447**:959–965.
41. Wellen KE, Hotamisligil GS. Inflammation, stress, and diabetes. *J Clin Invest* 2005;**115**:1111–1119.
42. Hui X, Li H, Zhou Z, *et al*. Adipocyte fatty acid-binding protein modulates inflammatory responses in macrophages through a positive feedback loop involving c-Jun NH2-terminal kinases and activator protein-1. *J Biol Chem* 2011;**285**: 10273–10280.
43. Terra X, Quintero Y, Auguet T, *et al*. FABP 4 is associated with inflammatory markers and metabolic syndrome in morbidly obese women. *Eur J Endocrinol* 2011;**164**:539–547.