CARDIOMETABOLIC DISORDERS AND ENDOTOXEMIA IN THE CONTEXT OF PERIODONTITIS - THE IMPORTANCE OF METABOLOMICS. A NARRATIVE REVIEW.

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Abstract
Periodontitis patients are known to have increased circulating lipopolysaccharide activity and metabolic disturbances, which may be either the cause or effect of endotoxemia.
This review approaches endotoxemia as a possible molecular mediator between periodontitis and increased risk of cardiometabolic disorders. Observations that bacteria disseminate into circulation after toothbrushing and periodontal procedures and assumptions that endotoxin may disseminate through inflamed periodontium and bleeding gums support the idea of endotoxemia in periodontitis. However, the evidence that periodontitis-associated dysbiosis contributes to endotoxemia is not as strong as in the case of gut microbiome dysbiosis.
Although the causality is insufficiently demonstrated thus far, many studies have associated changes of the gut microbiome composition, function, and specific bacterial metabolites with cardiometabolic diseases, whereas research on the oral microbiome has severely fallen behind.

Keyword:*periodontitis, cardiometabolic disorders, endotoxemia, metabolomics*

1. Introduction

Periodontitis patients are known to have increased circulating lipopolysaccharide activity and metabolic disturbances, which may be either the cause or effect of endotoxemia. [1]
Lipopolysaccharide is an important virulence factor of gram-negative bacteria. It is often referred to as endotoxin, which is used synonymously with lipopolysaccharide, although there are a few endotoxins that are not lipopolysaccharides.[2]
Several virulence factors or characteristics contribute to the ability of a microbe to cause disease. It plays a major role in the pathogenesis of periodontitis, where an abundant number of gram-negative species is a typical determinant of the periodontal microbiota. [3]
Chronic endotoxemia is involved in the pathogenesis of many inflammation-driven conditions, especially cardiometabolic disorders, including atherosclerotic cardiovascular
diseases, obesity, liver diseases, diabetes, and metabolic syndrome, and thus it is regarded as a risk factor. [4] Observations that bacteria disseminate into circulation after toothbrushing and periodontal. Procedures [5,6] and assumptions that endotoxin may disseminate through inflamed periodontium and bleeding gums support the bacteremia is not an absolute necessity for the translocation of endotoxins.

This review approaches endotoxemia as a possible molecular mediator between periodontitis and increased risk of cardiometabolic disorders.[7]
We describe the structure-function relationship of lipopolysaccharide, the local and systemic inflammatory and immunological responses caused by lipopolysaccharide, current knowledge on endotoxemia in periodontitis, factors affecting the levels of endotoxemia, and its relation to cardiometabolic disorders.

2. Endotoxemia

Metabolic endotoxemia was first defined as high-fat diet–induced approximately twofold increase in plasma lipopolysaccharide levels due to increased proportions of lipopolysaccharide-containing microbes in the gut microbiota. [4] This endotoxemia is associated with low-grade inflammation.[4]
Different arms of the contact activation pathway lead to increased coagulation, thrombosis, fibrinolysis, hemorrhage, vasoactivity, and inflammation.[7]
Inflammation is an essential component of host defense, but an unresolved chronic low-grade inflammatory state may lead to a wide range of chronic conditions.[8,9]
Inflammation may derive from endotoxemia, which is a risk factor for chronic cardiometabolic disorders, such as obesity, nonalcoholic fatty liver disease, metabolic syndrome, insulin resistance, type 2 diabetes, dyslipidemia, and cardiovascular diseases [3,9, 10-14] Inflammatory markers associated with periodontitis and cardiometabolic disorders largely overlap. In addition to inducing systemic inflammation, bacteria and lipopolysaccharide may have direct effects on the vessel walls and atherosclerotic lesions.
Deoxyribonucleic acid of periodontal pathogens (eg, *P. gingivalis*) and live bacteria has been detected in atherosclerotic plaques, [15-17] and experimental studies have shown that lipopolysaccharide is proatherogenic in vitro and in vivo.
In atherosclerotic lesions, macrophages and endothelial cells in particular express toll-like receptor 2 and toll-like receptor 4 receptors, and toll-like receptor 4 expression in macrophages is upregulated by oxidized low-density lipoprotein.[18,19]
The vascular inflammation activates coagulation and may lead to thrombosis.[20]
P. gingivalis lipopolysaccharide facilitates monocyte adhesion to the endothelium in vitro through upregulation of intercellular cell adhesion molecule 1 and vascular cell adhesion molecule. [21]
Lipopolysaccharide may also promote foam cell formation, as macrophages challenged with *A. actinomycetemcomitans* lipopolysaccharide showed enhanced secretion of tumor necrosis factor alpha and IL-1β and induction of foam cell formation and accumulation of low-density lipoprotein.[22] In a mouse model, injection of lipopolysaccharide accelerated the formation of instable atherosclerotic plaques.[23]

2.1. Sources of systemic lipopolysaccharide

Oral pathogens or lipopolysaccharide molecules expressed on their surfaces may enter the lymph and the bloodstream through inflamed gingival tissues. Additionally, some of the species associated with periodontitis, such as *P. gingivalis*, are able to invade and replicate in epithelial cells.[24] However, the gut microbiota is considered the main source of the lipopolysaccharide in metabolic endotoxemia.
However, oral bacteria tolerating the acidic environment in the stomach may proliferate also in the gastrointestinal tract, which

seems to be the case with *P. Gingivalis* affecting functionality of colon.[25, 26]

2.2 | Lipoproteins and neutralization

In the circulation, lipopolysaccharide can be recovered in bacterial cell walls, bacterial outer membrane vesicles, bound to bacterial or host proteins, or in blood cells, but the predominant fraction is carried after disaggregation with plasma lipoproteins.[27]

Only 20%-25% of lipopolysaccharide activity has been reported to exist unbound to lipoproteins.[27-29]

The triglyceride-saturated lipoprotein-lipopolysaccharide complex is eliminated by hepatocytes, preventing lipopolysaccharide-induced toxicity, [30] or phagocytosed by
macrophages. [31]

The inflammatory state, the lipoprotein profile, and concentrations of lipopolysaccharide-binding proteins of the subject determinate the metabolic fate of lipopolysaccharide. [7]

The proportion of lipopolysaccharide bound to very low-density lipoproteins was positively correlated with the number of deepened periodontal pockets, number of mobile teeth, and C-reactive protein, whereas lipopolysaccharide bound to either high-density lipoprotein subfraction 2 or 3 was correlated negatively with these clinical parameters. [32]

Therefore, endotoxemia may depend on inflammatory status, lipoprotein profiles, and concentrations of specific lipopolysaccharide-transferring proteins. [33,34]

Bactericidal/permeability-increasing
protein inhibits bacterial growth and prevents leukocyte
activation by binding to lipopolysaccharide and forming complex directly with the bacterial outer membrane. [35]

Interestingly, serum lipopolysaccharide-binding protein and soluble cluster of differentiation [36] are not correlated with lipopolysaccharide or lipopolysaccharide neutralizing capacity.

Serum IgG antibodies to *A. actinomycetemcomitans* predicted high lipopolysaccharide neutralizing capacity with equal responses between different serotypes (from A to E). [37]

2.3 | Metabolomics

Endotoxemia is tightly connected to lipoprotein metabolism, since lipoproteins are the main carriers of lipopolysaccharide and responsible for neutralization of its biological activity.

A major manifestation of aberrant metabolic pathway utilization during inflammatory diseases is the process of lipoprotein remodeling. [38]

The association with dyslipidemic lipoprotein phenotype with high serum triglyceride and cholesterol concentrations and low high-density lipoprotein cholesterol has been observed repeatedly, [3,12,37]

In a meta-analysis, periodontitis patients had significantly higher low-density lipoprotein cholesterol and triglyceride and lower high-density lipoprotein cholesterol concentrations compared with controls, whereas the effect on total cholesterol concentrations had more variation. [39]

The activated macrophages further promote the oxidation of low-density lipoprotein and its uptake to the cells by releasing reactive oxygen species and oxidative enzymes. [40]

These proatherogenic phenomena were also shown using isolated low-density lipoprotein
preparations from periodontitis patients, where the extent of affected tissue (periodontal pocket depth, suppuration, bleeding on probing) was directly associated with the enhanced cholesterol uptake and production of cytokines by macrophages ex vivo. [41]

These results emphasize the importance of lipoprotein metabolism in the connection between periodontitis and atherosclerosis

through lipoprotein remodeling and lipopolysaccharide carriage.

In the metabolomic study, endotoxemia was inversely associated with high-density lipoprotein particle size, whose concentration is associated directly with endotoxemia. [42]

Either due to endotoxemia or independently of it, a combination of decreased concentrations and increased dysfunction of high-density lipoprotein forms a vicious proatherogenic circle during infection.

2.4 | Endotoxemia in periodontitis

Owing to the presence of a large number of gram-negative bacterial species in subgingival microbiota, patients with periodontitis suffer from endotoxemia and have antibodies against lipopolysaccharide deriving from periodontal pathogens. [41-43]

In accordance with the hypothesis, only the quantity of gram-negative oral species, especially the classical periodontal pathogens, contributed to salivary lipopolysaccharide activity. [44]

Therefore, saliva lipopolysaccharide can be considered as a merged biomarker of gram-negative subgingival species. Similarly, saliva lipopolysaccharide is associated with a high cumulative risk score of periodontitis, number of teeth, and alveolar bone loss, [44, 45,46] whereas the association between the cumulative risk score and serum lipopolysaccharide is weaker. [46]

The cumulative risk score is a salivary biomarker for the risk of periodontitis composed

of three measurements connected to the periodontal inflammatory process: bacteria, inflammation, and tissue destruction. [47]

Several lipopolysaccharide-transferring proteins have been detected in periodontitis. Periodontitis is associated with soluble cluster of differentiation 14 levels, which also decreased due to periodontal treatment and predicted the severity of periodontal destruction. [48,49] Endotoxin levels were higher in patients with localized aggressive periodontitis than in healthy subjects, and the levels correlated with gingival crevicular fluid inflammatory markers and clinical signs of periodontitis. [50]

Serum phospholipid transfer protein activity decreases after periodontal treatment,[41] whereas its salivary activity does not correlate with any periodontal parameters even though phospholipid transfer protein can be detected in saliva. [51]

Serum lipopolysaccharide-binding protein concentrations were higher in patients with aggressive periodontitis than in healthy controls. [52]

Lipopolysaccharide-binding protein concentrations decreased after periodontal treatment in liver cirrhosis patients along with decreasing lipopolysaccharide levels, whereas these levels

increased during the 30-day follow-up in cirrhosis patients not receiving periodontal therapy. [53]

Very low-density lipoprotein (and low-density lipoprotein) preparations isolated from periodontitis patients induce macrophages to produce cytokines and to convert into foam cells, connecting periodontitis with atherogenic processes. [32,43]

Endotoxemia may persist despite successful periodontal treatment, and only small alterations

can be seen in the lipopolysaccharide distribution among the lipoprotein classes.[29]

Dental extraction causes bacteremia, which is rapidly cleared, and endotoxemia, which is quickly detoxified. [54,55] Interestingly, the edentulous subjects have both high lipopolysaccharide activity and high C-reactive protein, clearly showing that there are also other sources of lipopolysaccharide than the oral cavity.

At the same time, edentulous subjects have low antibody levels against periodontal

bacteria that derive from dysbiotic oral microbiota. [56]

3. Endotoxemia and Cardiometabolic Disorders

3.1. Factors associating with endotoxemia

In a population-based study conducted in Finland (FINRISK-97), lipopolysaccharide activity was measured by the *Limulus* amebocyte lysate assay from serum samples of 6782 participants. [11] The study examined possible associations between the measured lipopolysaccharide activity with demographic factors and cardiometabolic disorders as well as related factors. The associations of lipopolysaccharide activity with demographic factors, physical activity measures, liver function, and cardiometabolic disorders.

3.1.1. *Demographics*

Only a modest positive association was observed with age, whereas no association was detected with gender, current smoking, education years, or fasting time before sampling.

Number of participants in the groups divided according to the number of missing teeth are 0-1

missing teeth, 1440; 2-4 teeth, 1270; 5-8 teeth, 883; 9-31 teeth, 1979; edentulous, 1099.

Mean and 95% confidence interval of logarithmically (ln) transformed lipopolysaccharide activity, C-reactive protein, and immunoglobulin G (IgG)-class antibodies to *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis* (combined) [57,58] are shown.

The association was strongest with the physical activity during the leisure time, although heavy exercise may increase intestinal permeability and endotoxemia. [59]

3.1.2.| *Genetics*

Although endotoxemia has been a hot topic in research for two decades, the role of genetics in the responsiveness to lipopolysaccharide or circulating levels of endotoxemia have been seldom investigated in humans.

Common missense mutations in the toll-like receptor 4 receptor are associated with a phenotype hyporesponsive to inhaled lipopolysaccharide, [60] and an intergenic cluster located in 7p11.2 is associated with lipopolysaccharide-induced febrile response.[61]

However, neither of these genetic variations were among those associating with endotoxemia on a genome-wide level in over 11 000 Finnish subjects.[62]

Thus, the single-nucleotide polymorphisms were mainly located at genes that affect the contact activation of the coagulation cascade and lipoprotein metabolism, and, indeed, the composed genetic risk score had a strong association with venous thromboembolism. Activation of the kallikrein-kininogen pathway enhances the production of the vasodilator
bradykinin.

It has been hypothesized that bradykinin release in the intestinal tract could decrease gut barrier function and promote translocation of endotoxins from the intestinal lumen to the circulation.[63]

3.1.3. *Nutrition*

A high-fat diet has been associated with increased intestinal permeability and metabolic endotoxemia.[64]

Lipopolysaccharide is able to cross the gastrointestinal mucosa, and it has a high affinity to chylomicrons.[65] Therefore, lipopolysaccharide has been suggested to be the molecular link between a high-fat diet, the microbiota, and inflammation.[4]

According to several interventions among healthy individuals, high-fat and/or high-carbohydrate and/or energy-rich meals lead to endotoxemia.[7]

The postprandial increase of circulating endotoxin may be stronger in subjects with metabolic disorders, such as impaired glucose intolerance or type 2 diabetes.
When patients with type 1 diabetes and nondiabetic controls were given high-caloric,
fat-containing meals for 1 day, they had only a modest effect on serum lipopolysaccharide
activity, although profound changes in chylomicron and high-density lipoprotein metabolism as well as serum cytokine levels were observed.[66,67]
Dietary patterns are crucial in shaping the gut microbiota, and they contribute to gut permeability and thereby endotoxemia.[68]
Saturated fatty acids, especially lauric acid (C12:1), are toll-like receptor 4 agonists,[69] leading to a similar inflammatory response as lipopolysaccharide.
An unhealthy diet is a known risk factor for caries and periodontal disease,[70] but the oral microbiota seems to be more resilient to dietary effects compared with that of the large intestine.[71]

3.2. Outcomes

On the one hand, the outcome of chronic endotoxemia and continuous low-grade
inflammation may be cardiometabolic disorders, which are commonly connected to each other. On the other hand,these disorders promote intestinal permeability and systemic inflammation, accelerating the process of deteriorating systemic health.

3.2.1. *Liver diseases*

The healthy liver expresses low levels of toll-like receptor 4, whereas lipopolysaccharide and toll-like receptor 4 signaling have been proposed to play a role in the pathogenesis of alcoholic liver disease, nonalcoholic fatty liver disease, and nonalcoholic steatohepatitis.
[72] In a prospective population-based cohort, high serum lipopolysaccharide activity predicted incident advanced liver disease.[73]
Although lipopolysaccharide may induce liver diseases, its source has been seldom linked to the oral cavity. *P. gingivalis* has been detected more frequently in the oral samples of nonalcoholic fatty liver disease patients than in control subjects.[74]
In mouse studies, gram-negative bacteria, such as *A. actinomycetemcomitans*, infected the liver and caused proatherogenic alterations and dyslipidemia.[75]
The pathogen administration also led to induction of *A. Actinomycetemcomitans* antibodies, serum amyloid A, and lipopolysaccharide in the circulation.[75]
In vitro, *P. gingivalis* lipopolysaccharide induces lipid accumulation and inflammation in hepatic cells.[76]

3.2.2. *Obesity and metabolic syndrome*

A high-fat/ high-energy diet was shown to induce modest elevations of endotoxemia (1.5-fold) in lean mice, and this elevation was accompanied with increased fat deposition, systemic inflammation, and insulin resistance.[4]
The adipose tissue was considered as the source of low-grade inflammation frequently observed in obese individuals; but after the previously mentioned mouse studies, the increased gut permeability has been implicated in systemic inflammation.
Recurrent infection of mice by *A. Actinomycetemcomitans* has been shown to result in marked changes in the fatty acid composition of both inguinal and epididymal adipose tissue; these alterations had a strong correlation with serum lipopolysaccharide activity.[77]
Metabolic syndrome is defined based on the presence of five criteria, which include an elevated waist circumference, triglyceride level, fasting glucose blood concentration, and blood pressure and reduced high-density lipoprotein cholesterol concentration.

Endotoxemia is associated with obesity and with separate components and the presence of metabolic syndrome.[4,11,12]

3.2.3. *Diabetes*

Subjects with diabetes are more susceptible to fungal and bacterial infections compared with the general population. It is also a well-recognized fact that elevated glucose levels in blood increase the risk of acute and chronic infections.

High blood glucose variability, which is usually associated with poor glycemic control, correlates with the use of antibiotics in individuals with diabetes.

Since diabetes is commonly associated with dysregulation of the immune system, the patients are more susceptible to infections caused by opportunist pathogens; for example, *Staphylococcus aureus* (skin infections), *E. coli* (urinary infections), *Pseudomonas aeruginosa* (pneumonia), and *P. gingivalis* (periodontitis).

Compared with nondiabetic subjects, individuals with type 1 or type 2 diabetes have generally two to four times higher risk for hospitalization due to bacterial infections.[11]

High serum endotoxin activity could be one of the explanations for the persistent low-grade inflammation, especially in subjects with existing diabetes-related omplications.[12,14,78,79]

Metabolic endotoxemia is one of the risk factors for diabetes and its associated complications.[11,80,81,82]

3.2.4. *Cardiovascular diseases*

Endotoxemia is associated with cardiovascular diseases, such as myocardial infarction (MI), incident coronary artery disease events, and stroke.[3,10,13,80]

The hazards for incident cardiovascular disease events were greater when a subject with high endotoxemia had at the same time low high-density lipoprotein cholesterol, or high C-reactive protein or interleukin-6.[3]

In several studies, circulating concentrations of lipopolysaccharide-transferring proteins have also been used as a proxy of endotoxemia. In another study, however, lipopolysaccharide-binding protein or soluble cluster of differentiation [36] were not correlated with lipopolysaccharide activity or serum lipopolysaccharide-neutralizing capacity in patients with ischemic stroke or their matched controls.[37]

Genetic polymorphisms contributing to serum lipopolysaccharide activity were associated with the risk of "*ischemic stroke*," "*any stroke*," whereas the genetic risk score of lipopolysaccharide activity presented associations with deep vein thrombosis, pulmonary embolism, and venous thromboembolism.[62] Even a causal association of lipopolysaccharide in stroke was suggested in this study, which is the first one exploring the genetic background of endotoxemia. [62]

3.3. Animal studies

The influence of *P. gingivalis* on the gut microbiome and colon functions has been further studied in mouse models. Orally administered *P. gingivalis* caused increased levels of plasma endotoxin and insulin and reduced mRNA expression of the tight junction protein zonula occludens-1 in the small intestine of C57BL/6N mice.

In addition, microbiome analysis showed that the amount of Bacteroidales was significantly increased compared with sham-treated mice.[83]

As *P. gingivalis* was not among the bacterial species detected in the blood of the *P. gingivalis* administered mice, it was speculated that *P. gingivalis* contributed to the development of endotoxemia by affecting the composition of gut microbiota, leading to increased intestine permeability.

The same phenomenon was also seen in another study with C57BL/6N mice, where

even a single administration of *P. Gingivalis* caused disturbance in the gut microbiota with an increased level of Bacteroidetes and decrease of Firmicutes.[84]

Regardless of the source of systemic lipopolysaccharide, lipopolysaccharide-associated systemic inflammationmay additionally cause long-term metabolic and epigenetic rewiring in hematopoietic stem cells, leading to sustained enhancement of inflammatory myelopoiesis that may aggravate both cardiometabolic disorders and periodontitis.[85]

However, evidence is accumulating that dysbiosis is a disease affecting the whole body, from head to toe.

4 Conclusions

Chronic endotoxemia is involved in the pathogenesis of many inflammation-driven conditions, especially cardiometabolic disorders, including atherosclerotic cardiovascular

diseases, obesity, liver diseases, diabetes, and metabolic syndrome, and thus it is regarded as a risk factor. Observations that bacteria disseminate into circulation after toothbrushing and periodontal. The evidence that periodontitis-associated dysbiosis contributes to endotoxemia is not as strong as in the case of gut microbiome dysbiosis.

Although the causality is insufficiently demonstrated thus far, many studies have associated changes of the gut microbiome composition, function, and specific bacterial metabolites with cardiometabolic diseases, whereas research on the oral microbiome has severely fallen behind.

References;

1. Pussinen PJ., Kopra E, Pietiäinen M,| Lehto| M, Svetislav Z, Paju, Salminen AS Periodontitis and cardiometabolic disorders: The role of lipopolysaccharide and endotoxemia, *Periodontology 2000.* 2022;89:19–40. DOI: 10.1111/prd.124
2. Rietschel ET, Kirikae T, Schade FU, et al. Bacterial endotoxin: molecular relationships of structure to activity and function. *FASEB J*. 1994;8:217-225.
3. Pussinen PJ, Tuomisto K, Jousilahti P, Havulinna AS, Sundvall J, Salomaa V. Endotoxemia, immune response to periodontal pathogens, and systemic inflammation associate with incident cardiovascular disease events. *Arterioscler Thromb Vasc Biol*.2007;27:1433-1439.
4. Cani PD, Amar J, Iglesias MA, et al. Metabolic endotoxemia initiates obesity and insulin resistance. *Diabetes*. 2007;56:1761-1772.
5. Tomás I, Diz P, Tobías A, Scully C, Donos N. Periodontal health status and bacteraemia from daily oral activities: systematic review/ meta-analysis. *J Clin Periodontol*. 2012;39:213-228.
6. Forner L, Larsen T, Kilian M, Holmstrup P. Incidence of bacteremia after chewing, tooth brushing and scaling in individuals with periodontal inflammation. *J Clin Periodontol*. 2006;33:401-407.
7. Kallio E. Lipopolysaccharide: a link between periodontitis and cardiometabolic disorders. Dissertations of the University of Helsinki 46/2014. PhD thesis. http://urn.fi/URN:ISBN:978-951-51- 0459- 5
8. Hotamisligil GS. Inflammation and metabolic disorders. *Nature*. 2006;444:860-867.
9. Libby P. Inflammation in atherosclerosis. *Nature*. 2002;420:868-874.
10. Wiedermann CJ, Kiechl S, Dunzendorfer S, et al. Association of endotoxemia with carotid atherosclerosis and cardiovascular disease: prospective results from the Bruneck study. *J Am Coll Cardiol*. 1999;34:1975-1981.
11. Pussinen PJ, Havulinna AS, Lehto M, Sundvall J, Salomaa V. Endotoxemia is associated with an increased risk of incident diabetes. *Diabetes Care*. 2011;34:392-397.
12. Lassenius MI, Pietiläinen KH, Kaartinen K, et al. Bacterial endotoxin activity in human serum is associated with dyslipidemia, insulin resistance, obesity, and chronic inflammation. *Diabetes Care*. 2011;34:1809-1815.

13. Klimiec E, Pasinska P, Kowalska K, Pera J, Slowik A, Dziedzic T. The association between plasma endotoxin, endotoxin pathway proteins and outcome after ischemic stroke. *Atherosclerosis*. 2018;269:138-143.
14. Simonsen JR, Järvinen A, Harjutsalo V, Forsblom C, Groop PH, Lehto M. The association between bacterial infections and the risk of coronary heart disease in type 1 diabetes. *J Intern Med*. 2020;288:711-724.
15. Figuero E, Sánchez-Beltrán M, Cuesta-Frechoso S, et al. Detection of periodontal bacteria in atheromatous plaque by nested polymerase chain reaction. *J Periodontol*. 2011;82:1469-1477.
16. Gaetti-Jardim E, Marcelino SL, Feitosa ACR, Romito GA, Avila-Campos MJ. Quantitative detection of periodontopathic bacteria in atherosclerotic plaques from coronary arteries. *J Med Microbiol*. 2009;58(Pt 12):1568-1575.
17. Kozarov EV, Dorn BR, Shelburne CE, Dunn WA Jr, Progulske-Fox A. Human atherosclerotic plaque contains viable invasive *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis*. *Arterioscler Thromb Vasc Biol*. 2005;25:e17-18.
18. Edfeldt K, Swedenborg J, Hansson GK, Yan ZQ. Expression of toll-like receptors in human atherosclerotic lesions: a possible pathway for plaque activation. *Circulation*. 2002;105:1158-1161.
19. Xu XH, Shah PK, Faure E, et al. Toll-like receptor-4 is expressed by macrophages in murine and human lipid-rich atherosclerotic plaques and upregulated by oxidized LDL. *Circulation*. 2001;104:3103-3108.
20. Esmon CT. The impact of the inflammatory response on coagulation. *Thromb Res*. 2004;114:321-327.
21. Nakamura N, Yoshida M, Umeda M, et al. Extended exposure of lipopolysaccharide fraction from *Porphyromonas gingivalis* facilitates mononuclear cell adhesion to vascular endothelium via toll-like receptor-2 dependent mechanism. *Atherosclerosis*. 2008;196:59-67.
22. Lakio L, Lehto M, Tuomainen AM, et al. Pro-atherogenic properties of lipopolysaccharide from the periodontal pathogen *Actinobacillus actinomycetemcomitans*. *J Endotoxin Res*. 2006;12:57-64.
23. Jaw JE, Tsuruta M, Oh Y, et al. Lung exposure to lipopolysaccharide causes atherosclerotic plaque destabilisation. *Eur Respir J*. 2016;48:205-215.
24. Kinane DF, Galicia JC, Gorr SU, Stathopoulou PG, Benakanakere M. *P. gingivalis* interactions with epithelial cells. *Front Biosci*. 2008;13:966-984.
25. Schmidt TS, Hayward MR, Coelho LP, et al. Extensive transmission of microbes along the gastrointestinal tract. *Elife*. 2019;8:e42693.
26. Olsen I, Yamazaki K. Can oral bacteria affect the microbiome of the gut? *J Oral Microbiol*. 2019;11:1586422.
27. Munford RS. Endotoxemia—menace, marker, or mistake? *J LeukocBiol*. 2016;100:687-698.
28. Harris HW, Johnson JA, Wigmore SJ. Endogenous lipoproteins impact the response to endotoxin in humans. *Crit Care Med*. 2002;30:23-31.
29. Kallio KA, Buhlin K, Jauhiainen M, et al. Lipopolysaccharide associates with pro-atherogenic lipoproteins in periodontitis patients. *Innate Immun*. 2008;14:247-253.
30. Barcia AM, Harris HW. Triglyceride-rich lipoproteins as agents of innate immunity. *Clin Infect Dis*. 2005;41(Suppl 7):S498-503.
31. Brown MS, Goldstein JL. Lipoprotein metabolism in the macrophage: implications for cholesterol deposition in atherosclerosis. *Annu Rev Biochem*. 1983;52:223-261.
32. Kallio KA, Hyvärinen K, Kovanen PT, Jauhiainen M, Pussinen PJ. Very low density lipoproteins derived from periodontitis patients facilitate macrophage activation via lipopolysaccharide function. *Metabolism*. 2013;62:661-668.
33. Kitchens RL, Thompson PA. Impact of sepsis-induced changes in plasma on LPS interactions with monocytes and plasma lipoproteins: roles of soluble CD14, LBP, and acute phase lipoproteins. *J Endotoxin Res*. 2003;9:113-118.
34. Gnauck A, Lentle RG, Kruger MC. Chasing a ghost?—Issues with the determination of circulating levels of endotoxin in human blood. *Crit Rev Clin Lab Sci*. 2016;53:197-215.

35. Balakrishnan A, Marathe SA, Joglekar M, Chakravortty D. Bactericidal/permeability increasing protein: a multifaceted protein with functions beyond LPS neutralization. *Innate Immun*. 2013;19:339-347.
36. Fisher JF, Mobashery S. Constructing and deconstructing the bacterial cell wall. *Protein Sci*. 2020;29:629-646.
37. Leskelä J, Pietiäinen M, Safer A, et al. Serum lipopolysaccharide neutralizing capacity in ischemic stroke. *PLoS One*. 2020;15:e0228806.
38. Feingold KR, Grunfeld C. The effect of inflammation and infection on lipids and lipoproteins. In: Feingold KR, Anawalt B, Boyce A, Chrousos G, de Herder WW, Dhatariya K, Dungan K, Grossman A, Hershman JM, Hofland J, Kalra S, Kaltsas G, Koch C, Kopp P,
39. Korbonits M, Kovacs CS, Kuohung W, Laferrère B, McGee EA, McLachlan R, Morley JE, New M, Purnell J, Sahay R, Singer F, Stratakis CA, Trence DL, Wilson DP, eds. *Endotext* [Internet]. MDText.com, Inc.; 2000.
40. Nepomuceno R, Pigossi SC, Finoti LS, et al. Serum lipid levels in patients with periodontal disease: a meta-analysis and meta-regression. *J Clin Periodontol*. 2017;44:1192-1207.
41. Libby P, Hansson GK. From focal lipid storage to systemic inflammation: JACC review topic of the week. *J Am Coll Cardiol*. 2019;74:1594-1607.
42. Pussinen PJ, Jauhiainen M, Vilkuna-Rautiainen T, et al. Periodontitis decreases the antiatherogenic potency of high density lipoprotein. *J Lipid Res*. 2004;45:139-147.
43. Paju S, Pussinen PJ, Sinisalo J, et al. Clarithromycin reduces recurrent cardiovascular events in subjects without periodontitis. *Atherosclerosis*. 2006;188:412-419.
44. Pussinen PJ, Vilkuna-Rautiainen T, Alfthan G, et al. Severe periodontitis enhances macrophage activation via increased serum lipopolysaccharide. *Arterioscler Thromb Vasc Biol*. 2004;24:2174-2180.
45. Liljestrand JM, Paju S, Buhlin K, et al. Lipopolysaccharide, a possible molecular mediator between periodontitis and coronary artery disease. *J Clin Periodontol*. 2017;44:784-792.
46. Hyvärinen K, Mäntylä P, Buhlin K, et al. A common periodontal pathogen has an adverse association with both acute and stable coronary artery disease. *Atherosclerosis*. 2012;223:478-484.
47. Liukkonen J, Gürsoy UK, Könönen E, et al. Immunological and microbiological profiling of cumulative risk score for periodontitis. *Diagnostics (Basel)*. 2020;10:560.
48. Gursoy UK, Könönen E, Pussinen PJ, et al. Use of host and bacteria-derived salivary markers in detection of periodontitis: a cumulative approach. *Dis Markers*. 2011;30:299-305.
49. Hayashi J, Masaka T, Ishikawa I. Increased levels of soluble CD14 in sera of periodontitis patients. *Infect Immun*. 1999;67:417-420.
50. Nicu EA, Laine ML, Morré SA, Van der Velden U, Loos BG. Soluble CD14 in periodontitis. *Innate Immun*. 2009;15:121-128.
51. Shaddox LM, Wiedey J, Calderon NL, et al. Local inflammatory markers and systemic endotoxin in aggressive periodontitis. *J Dent Res*. 2011;90:1140-1144.
52. Liukkonen J, Gürsoy UK, Könönen E, et al. Salivary biomarkers in association with periodontal parameters and the periodontitis risk haplotype. *Innate Immun*. 2018;24:439-447.
53. Wohlfeil M, Scharf S, Siegelin Y, et al. Increased systemic elastase and C-reactive protein in aggressive periodontitis (CLOI-D- 00160R2). *Clin Oral Investig*. 2012;16:1199-1207.
54. Bajaj JS, Matin P, White MB, et al. Periodontal therapy favorably modulates the oral-gut- hepatic axis in cirrhosis. *Am J Physiol Gastrointest Liver Physiol*. 2018;315:G824-G837.
55. Lockhart PB, Brennan MT, Sasser HC, Fox PC, Paster BJ, Bahrani-Mougeot FK. Bacteremia associated with toothbrushing and dental extraction. *Circulation*. 2008;117:3118-3125.
56. Habbab KM, D'Aiuto F, Habbab MA, Porter SR. Molecular markersrelevant to myocardial injury following dental extraction in patients with or without coronary artery disease. *BDJ Open*. 2019;5:9.
57. Pussinen PJ, Könönen E, Paju S, et al. Periodontal pathogen carriage, rather than periodontitis, determines the serum antibody levels. *J Clin Periodontol*. 2011;38:405-411
58. Liljestrand JM, Havulinna AS, Paju S, Männistö S, Salomaa V, Pussinen PJ. Missing teeth predict incident cardiovascular events, diabetes, and death. *J Dent Res*. 2015;94:1055-1062.

59. Pussinen PJ, Jousilahti P, Alfthan G, Palosuo T, Asikainen S, Salomaa V. Antibodies to periodontal pathogens are associated with coronary heart disease. *Arterioscler Thromb Vasc Biol*. 2003;23:1250-1254.
60. Karhu E, Forsgård RA, Alanko L, et al. Exercise and gastrointestinal symptoms: running-induced changes in intestinal permeability and markers of gastrointestinal function in asymptomatic and symptomatic runners. *Eur J Appl Physiol*. 2017;117:2519-2526.
61. Schwartz DA. The role of TLR4 in endotoxin responsiveness in humans. *J Endotoxin Res*. 2001;7:389-393.
62. Ferguson JF, Meyer NJ, Qu L, et al. Integrative genomics identifies 7p11.2 as a novel locus for fever and clinical stress response in humans. *Hum Mol Genet*. 2015;24:1801-1812.
63. Leskelä J, Toppila I, Härma MA, et al. Genetic profile of endotoxemia reveals an association with thromboembolism and stroke. *J Am Heart Assoc*. 2021;10:e022482.
64. Lehto M, Groop P-H. The gut-kidneyaxis: putative interconnectionsbetween gastrointestinal and renal disorders. *Front Endocrinol*. 2018;9:553.
65. Neves AL, Coelho J, Couto L, Leite-Moreira A, Roncon-Albuquerque R Jr. Metabolic endotoxemia: a molecular link between obesity and cardiovascular risk. *J Mol Endocrinol*. 2013;51:R51-64.
66. Ghoshal S, Witta J, Zhong J, de Villiers W, Eckhardt E. Chylomicrons promote intestinal absorption of lipopolysaccharides. *J Lipid Res*. 2009;50:90-97.
67. Lassenius MI, Mäkinen VP, Fogarty CL, et al. Patients with type 1 diabetes show signs of vascular dysfunction in response to multiple high-fat meals. *Nutr Metab (Lond)*. 2014;11:28.
68. Fogarty CL, Nieminen JK, Peräneva L, et al. High-fatmeals induce systemic cytokine release without evidence of endotoxemia-mediated cytokine production from circulating monocytes or myeloid dendritic cells. *Acta Diabetol*. 2015;52:315-322.
69. Régnier M, Van Hul M, Knauf C, Cani PD. Gut microbiome, endocrine control of gut barrier function and metabolic diseases. *J Endocrinol*. 2021;248:R67-R82.
70. Rocha DM, Caldas AP, Oliveira LL, Bressan J, Hermsdorff HH. Saturated fatty acids trigger TLR4-mediated inflammatory response. *Atherosclerosis*. 2016;244:211-215.
71. Hujoel PP, Lingström P. Nutrition, dental caries and periodontal disease: a narrative review. *J Clin Periodontol*. 2017;44(Suppl 18):S79-S84.
72. Wade WG. Resilience of the oral microbiome. *Periodontol 2000*. 2021;86(1):113-122.
73. Soares JB, Pimentel-Nunes P, Roncon-Albuquerque R, Leite-Moreira A. The role of lipopolysaccharide/toll-like receptor 4 signaling in chronic liver diseases. *Hepatol Int*. 2010;4:659-672.
74. Männistö V, Färkkilä M, Pussinen P, et al. Serum lipopolysaccharides predict advanced liver disease in the general population. *JHEP Rep*. 2019;1:345-352.
75. Yoneda M, Naka S, Nakano K, et al. Involvement of a periodontal pathogen, *Porphyromonas gingivalis* on the pathogenesis of non-alcoholic fatty liver disease. *BMC Gastroenterol*. 2012;12:16.
76. Hyvärinen K, Tuomainen AM, Laitinen S, et al. Chlamydial and periodontal pathogens induce hepatic inflammation and fatty acid imbalance in apolipoprotein E-deficient mice. *Infect Immun*. 2009;77:3442-3449.
77. Ding LY, Liang LZ, Zhao YX, et al. *Porphyromonas gingivalis*–derived lipopolysaccharide causes excessive hepatic lipid accumulation via activating NF-κB and JNK signaling pathways. *Oral Dis*. 2019;25:1789-1797.
78. Hyvärinen K, Tuomainen AM, Laitinen S, et al. The effect of proatherogenic pathogens on adipose tissue transcriptome and fatty acid distribution in apolipoprotein E–deficient mice. *BMC Genom*. 2013;14:709.
79. Critchley JA, Carey IM, Harris T, DeWilde S, Hosking FJ, Cook DG. Glycemic control and risk of infections among people with type 1 or type 2 diabetes in a large primary care cohort study. *Diabetes Care*. 2018;41:2127-2135.
80. Simonsen JR, Järvinen A, Hietala K, et al. Bacterial infections as novel risk factors of severe diabetic retinopathy in individuals with type 1 diabetes. *Br J Ophthalmol*. 2021;. 105:1104-1110. doi:10.1136/bjoph thalm ol-2020- 316202

81. Kallio KAE, Hätönen KA, Lehto M, Salomaa V, Männistö S, Pussinen P. Endotoxemia, nutrition, and cardiometabolic disorders. *Acta Diabetol*. 2015;52:395-404.
82. Nymark M, Pussinen PJ, Tuomainen AM, Forsblom C, Groop P-H, Lehto M. Serum lipopolysaccharide activity is associated with the progression of the kidney disease in Finnish patients with type 1diabetes. *Diabetes Care*. 2009;32:1689-1693.
83. Nguyen ATM, Akhter R, Garde S, et al. The association of periodontal disease with the complications of diabetes mellitus. A systematic review. *Diabetes Res Clin Pract*. 2020;165:108244.
84. Arimatsu K, Yamada H, Nakajima MH, et al. Oral pathobiont induces systemic inflammation and metabolic changes associated with alteration of gut microbiota. *Sci Rep*. 2014;4:4828.
85. Nakajima M, Arimatsu K, Kato T, et al. Oral administration of *P. gingivalis* induces dysbiosis of gut microbiota and impaired barrier function leading to dissemination of enterobacteria to the liver. *PLoS One*. 2015;10:e0134234.
86. Hajishengallis G, Chavakis T. Local and systemic mechanisms linking periodontal disease and inflammatory comorbidities. *Nat Rev Immunol*. 2021;28:1-15.