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Macronutrient effect on animal models of periodontal disease: a systematic review

Efecto de los macronutrientes en modelos animales de enfermedad periodontal: una revisión sistemática

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ABSTRACT

Objectives: Periodontal disease is one of the most common chronic oral diseases worldwide and represents a major public health problem in many countries. Periodontal diseases include chronic periodontitis and aggressive periodontitis that are usually due to the inflammation of gingiva caused by bacterial infections. Nutrition could exert a pivotal role due to its involvement in a number of inflammatory condition and diseases. Up to now, only some researchers have investigated the role of nutrients on the development and progression of periodontal disease, but the results obtained are often difficult to compare because of the heterogeneity of the studies.

Methods: This paper systemically reviews the literature available on databases up to February 2018 on the relationship between macronutrients and the development and progression of periodontal diseases (periodontitis and gingivitis) in animals, with particular attention to the possible mechanisms involved in these pathologies.

Results: A total of 5,484 publications were found in the Pubmed database. Title and abstract screening left 88 potential articles according to the selection criteria. No duplicated articles were found. Full-text screening and reading led to a final number of 32 articles.

Conclusions: Among all the analyzed macronutrients, those that have any effect on oxidative stress or immune system seem to be important for the prevention of periodontal disease or periodontal disease improvement. On the one hand, there is evidence in favor of a positive role of n-3 fatty acid proportion in diet due to its antioxidant and immunomodulatory effects. On the other hand, saturated fat-rich or hypercaloric diets increase oxidative stress or promote inflammation, so they must be avoided.

Keywords: periodontitis; diet; lipid; carbohydrates; protein.

RESUMEN

Objetivos: La enfermedad periodontal es una de las enfermedades orales crónicas más comunes en todo el mundo y representa un importante problema de salud pública en muchos países. Las enfermedades periodontales incluyen periodontitis crónica y periodontitis agresiva que generalmente se debe a la inflamación de la encía causada por infecciones bacterianas. La nutrición podría ejercer un papel fundamental ya que participa en diferentes afecciones y enfermedades inflamatorias. Hasta ahora, solo algunos investigadores han investigado el papel de los nutrientes en el desarrollo y la progresión de la enfermedad periodontal, pero los resultados obtenidos a menudo son difíciles de comparar debido a la heterogeneidad de los estudios.

Métodos: Este documento revisa sistemáticamente la literatura disponible en bases de datos hasta Febrero de 2018 sobre la relación entre macronutrientes y el desarrollo y progresión de enfermedades periodontales (periodontitis y gingivitis) en animales, con particular atención a los posibles mecanismos implicados en estas patologías. Resultados: Se encontraron un total de 5.484 publicaciones en la base de

datos Pubmed. El análisis del título y resumen permitió seleccionar 88 artículos potenciales de acuerdo con los criterios de selección. No se encontraron artículos duplicados. La selección y lectura del texto complejo condujo a una cantidad final de 32 artículos.

Conclusiones: Entre todos los macronutrientes analizados, aquellos que tienen algún efecto sobre el estrés oxidativo o el sistema inmune parecen ser importantes para la prevención de la enfermedad periodontal o la mejoría de la enfermedad periodontal. Por un lado, hay evidencia a favor de un papel positivo de la proporción de ácidos grasos n-3 en la dieta debido a sus efectos antioxidantes e inmunomoduladores. Por otro lado, las dietas ricas en grasas saturadas o hipercalóricas aumentan el estrés oxidativo o promueven la inflamación, por lo que deben evitarse.

Palabras clave: periodontitis; dieta; lípidos; carbohidratos; proteínas.

INTRODUCTION

Periodontal disease is one of the most common chronic oral diseases worldwide and represents a major public health problem in many countries, considering also that poor oral health conditions have a negative impact on individual life quality[1]. Periodontal diseases include gingivitis, chronic periodontitis and aggressive periodontitis, and are usually due to the inflammation of gingiva caused by bacterial infections[2]. Common clinical manifestations in periodontitis consist of the deepening of periodontal pockets and loss of attachment, gradually leading to tooth loosening and finally to tooth loss[3]. In general, periodontitis can be efficaciously treated by scaling, root surface debridement and periodontal surgery, even if not all patients respond positively to this form of therapy[3]. Concerning the onset and the progression of periodontal breakdown, genetics[4], alcohol[5], smoking[6], stress[7], and the presence of some periodontal pathogens such as Porphyromonas gingivalis and Aggregatibacter actinomycetemcomitans[8] are the main risk factors.

In this context, nutrition could exert a pivotal role since its involvement in a number of inflammatory condition and diseases, including cardiovascular diseases, type 2 diabetes mellitus, rheumatoid arthritis and inflammatory bowel disease, all of which are undoubtedly associated with periodontitis[3]. The importance of diet on human health and well-being has been widely recognized worldwide: a good diet contributes not only to good general health but also to good oral health. Several studies have shown that an unbalanced diet and low food volume are associated to poor occlusion, tooth loss, and other oral pathological conditions [9],[10] . Subjects with fewer teeth, like the elderly, have the common habit of avoiding raw fruits and vegetables, thus reducing their intake of vitamins, minerals and dietary fiber, all nutrients that help to prevent many chronic pathologies, such as cardiovascular disease, cancer and other systemic conditions. At the same time, a balanced diet, characterized by a reduced intake of refined carbohydrates and a high consumption of whole grains, fruits, vegetables and dietary sources of calcium, not only improves global health and decreases chronic disease risk, but also impacts the host's immune response, contributing to the maintenance of host resistance, and affects the integrity of the hard and soft tissues of the oral cavity[11]. For these reasons, it is of crucial importance to deeply investigate the association between nutrition and periodontal disease. Up to now, only some researchers have investigated the role of nutrients on the development and progression of periodontal disease, but the results obtained are often difficult to compare because of the heterogeneity of the studies[3],[12]–[14] .

This paper systemically reviews the literature available on databases up to May 2016 on the relationship between macronutrients and the development and progression of periodontitis in animals, with particular attention to the possible mechanisms involved in these pathologies.

METHODS

Selection criteria

Inclusion and exclusion criteria for the selection of papers for review were established prior to search in databases. According to these, all studies investigating the effect of dietary interventions in relation to the different macronutrients on animal periodontal health, even when they use models of any special physiological condition or illness (pregnancy, menopause, diabetes mellitus and others models) were included. Those studies that did not allow distinguishing the macronutrient responsible for the reported effects due to extensive diet modifications were excluded.

Information source and search terms

The electronic database of National Library of Medicine, Washington, DC (MEDLINE: PubMed) was used to select appropriate papers. We first derived two themes that were then combined by using the Boolean operator ''AND''. Each theme was created by using the operator ''OR'' to search for terms appearing as either explorer text words or Medical Subjects Headings (Mesh), when they existed and it was not contained within other also used. The selected periodontal disease related terms were: periodontal, periodontitis, gingivitis and it was also included the following outcome measurement related to periodontal disease: «Alveolar Bone Loss»[Mesh] OR «Periodontal Diseases»[Mesh] OR «Periodontal Attachment Loss»[Mesh] OR «Periodontal Index»[Mesh] OR «Gingival Hemorrhage»[Mesh] OR «per-

iodontal disease» OR periodontitis OR «alveolar bone loss» OR «alveolar bone resorption» OR «tooth attachment» OR «tooth mobility» OR «gingivitis» OR «clinical attachment level» OR «periodontal attachment level» OR «attachment loss» OR «periodontal pocket» OR «pocket depth» OR «probing depth» OR «bleeding on probing» OR «gingival bleeding» OR «Gingival Hemorrhage» OR «gingival index» OR «bleeding index» OR «periodontal index». In addition to this, a second theme related to nutrition or diet was created, Search terms, were the next: «Food»[Mesh] OR «Diet»[Mesh] OR «Eating»[Mesh] OR «Nutrition Surveys»[Mesh] OR «Nutrition Assessment»[Mesh] OR «Nutrition Therapy»[Mesh] OR «Nutrition Processes»[Mesh] OR «Nutritional Status»[Mesh] OR nutrition* OR nutrition OR nutrient* OR nutrient OR food OR dietary OR diet* OR intake OR intakes OR Consumption* OR Consumption OR Ingestion OR Eating.

Search strategy

The search strategy aimed to find both published studies in the English language from the inception of the database until February 2016. A comprehensive literature search was run independently by two of review authors. At first, papers were screened by title and abstract. Screening procedures were adjusted for higher sensitivity (with restrictive search items omitted). Secondly, full text-papers were retrieved and selected based on the eligibility criteria, and duplicated studies were excluded. Titles without abstract of which the title suggested that they were related to the objectives of this review were selected to screen the full text. Review authors' disagreements or inconsistencies concerning inclusion of publications or extraction of data were discussed to eventually achieve mutual consensus. Inconveniently, our methodological approach did not allow obtain articles nor available on line.

Data collection process, data items and Summary measures

The quantitative data extracted from papers included specific details about the interventions, study methods, and significant results of for the review question and specific objectives. Data from eligible studies were independently evaluated. When they were available, outcome measurement means or mean differences between groups, and its standard deviation or error of the mean were included in result description. In the same sense, significance levels considered or *P*-values also were indicated, but only if significant differences were found.

RESULTS AND DISCUSSION

A total of 5,484 publications were found in the Pubmed database. Title and abstract screening left 88 potential articles according to the selection criteria. No duplicated articles were found. Full-text screening and reading led to a final number of 32 articles. Selected studies are presented in different sections below depending on the increased macronutrient in experimental groups.

Carbohydrates

The term carbohydrate includes both those compounds that are digestible and provide energy and those that are not classified as dietary fiber. However, because of the limited number of animal studies in relation to the latter and the complexity when selecting studies as many of them consider these compounds as prebiotics, we finally decided to focus on the first group. In relation to these compounds, two studies performed on rats (*Rattus norvegicus*)[15] and on Mongolian gerbils (*Meriones unguiculatus*)[16] were considered (Table 1).

Table 1: Studies on carbohydrate effects on periodontal disease.

Reference	Animal age/weight	Experimental treatments (duration)	Main results/ conclusions
Galvão *et al*. (2003)[15]	24 female Wistar rats 60 d	Sucrose-rich (53% sucrose + 30% dry milk) *vs.* regular soft diet[a] (30 d); in combination or not with ligature placement (30 d)	Ligature placement led to histological characteristics related to periodontitis in all cases
Moskow *et al*. (1969)[16]	40 female Mongolian gerbils 6 m	High-carbohydrate(66% corn-starch + 32% skin milk) *vs.* standard diet[a] ,either pelleted & powdered form (duration not provided)	High-carbohydrate diet led to lower gingival lesions regardless of its form

[a]*ad libitum* diet. Abbreviations:d: days, m: months, *vs.*: *versus,* w: weeks.

Results from the study in rats, namely in female adult Wistar rats[15] have shown that when this type of experimental diets (i.e. sugar-rich diets) has been combined with ligatures, their effect was not so clear or important. In this study, silk ligatures were placed around animal molars to induce experimental periodontal disease. The animals were fed on soft sucrose-rich (53%) or a standard diet. Additionally, other two groups were fed the same diet but did not receive ligatures. After 30 days, a histological study revealed that ligature placement was able to promote a chronic inflammatory process in rat periodontium regardless of the adopted diet, but animals without ligatures did not show any sign of periodontal destruction. However, at a histological level no differences were noted in relation to diet.

In addition to research in rats, Moskow *et al.*[16] studied Mongolian gerbils*(Meriones unguiculatus*) that were fed a high-carbohydrate, in this case constituted by 66% cornstarch and 32% skimmed milk, or a standard diet in two forms, powdered and pelleted. After some time (period not reported), animals on the high-carbohydrate diet showed lower scores (14.83±1.813 *versus [vs.]* 37.35±2.04) of gingival lesions (defined by the degree of separation of the gingiva and the tissue loss in the interdental and groove papillary areas). Notwithstanding, osseous lesions, that were scored according to the degree of alveolar resorption, were similar between both groups. On the other hand, diet had no effect. Therefore, in this experimental model the cornstarch-rich diet affected positively only the gum, but not the bone. Interestingly, animals fed on standard chow showed higher weight gains which can explain why this diet showed some negative effects compared with the experimental diet.

According to these studies results, it seems that carbohydrate intake or more specifically a high carbohydrate diet could have less importance than other risk factors and some situations could hide it. The absence of significant differences between dietary groups for many of the parameters assessed, particularly in relation to the alveolar bone, could be due to the fact that experimental diets have been combined with other interventions, which could be more important than the diet itself, as occurred with placement of ligatures. Likewise, the short duration of one of the experiments[15] (about one month) also might explain why the alveolar bone did not appear affected by diet despite the fact that it seemed to exacerbate the inflammation process to some degree. Moreover, in the other experiment the high-carbohydrate diet even seemed to have some beneficial effect at gum level. To compare both studies presented here, it should be considered that in the rat study the diet mainly had a higher amount of simple sugar (sucrose), but in the case of gerbils, cornstarch represented an important percentage. This would indicate the importance of glycemic index of the diet. Moreover, differences among standard or control diets also could be important.

Lipids

Among all the reports selected in this review, those focused on the effects of lipids with periodontal disease are the most numerous. This implies that there is much information which explores the role of these dietary components in this pathology. To facilitate understanding of this issue, the differences between methodology and experimental design were evaluated and it was finally decided to develop this theme in different sections according to the dietary treatment. Among the various dietary treatments, it was possible to differentiate studies where dietary fat content increased and others where only the fatty acid (FA) profile was modified. In turn, two groups of experiments were clearly recognized among the first. One group includes comparisons between standard diets and those rich in saturated fat, with addition of cholesterol (Ch) or both combined. Because saturated fat also contains high amount of Ch, all these articles were included together in the same section. In another experimental group diets with different FA profile were compared. These usually implied small increments of some polyunsaturated FAs (PUFA) types by supplementation. Moreover, there was no normolipid control group in most of them. For this reason, it was decided to include the latest studies with those comparing normolipid diets with different FA profiles. Therefore, studies were finally classified as those comparing high-fat diets rich in saturated fat (enriched or not with Ch) or only Ch with standard diets, and studies comparing diets with different fatty acid profile regardless whether they were hyperlipid or not.

High-fat diets rich in saturated fat

Almost all research shows that increasing the fat content of diets tends to induce obesity, metabolic syndrome or even type 2 diabetes mellitus. For this reason factors related to periodontal health may be linked to these conditions and any dietary pattern leading to their development could exert some of these effects. In most cases experimental diets used lard as fat source, used alone or combined with some vegetable oils. Control or standard diets are composed in most cases of commercial chow that presents different compositions depending on the manufacturer. Moreover, hyperlidid diets tended to maintain protein concentration per total kcal. Thus, changes in fat content were usually associated with changes in carbohydrate content in different degrees. In some studies, a diet known as the coffee diet was used which represents a typical western dietary pattern. However, this produces an increment of sucrose compared to standard diets, a modification that by itself could have

effect on periodontal health. Because a clear distinction of the modification of fat content is difficult, these studies were excluded.

Regarding model features, most of them have been based on rodents, both rats[17]-[22]and mice (*Mus musculus*)[23]-[29], and rabbits (*Oryctolagus cuniculus*)[30],[31] (Table 2). Among them, there are studies where periodontal disease was experimentally induced[18],[25],[31] but others are without intervention at periodontal level[17],[22]-[25],[30]. Concerning studies where interventions at periodontal level were not carried out, most of them used male C57BL/6J mice as a model[23]-[25]. One of them evaluated changes in periodontium morphology induced by a diet rich in saturated fat (where total fat represented 62% of total Kcal) using multiple techniques including a quantitative evaluation of alveolar bone architecture, a micro-computerized tomography (Micro-CT) of mandibles and a histological evaluation. Most of the measurement obtained by micro-CT, at trabecular and cortical level, were lower than in similar mice with normal-weight (P<0.05). These included a lower trabecular bone volume, cortical bone growth, cortical bone density. Moreover, histological analyses revealed a disruption of the periodontal ligament fibers, accompanied by inflammatory cell infiltration on the surface of the alveolar bone.

Table 2: Studies on high-fat diets rich in saturated fat &/or cholesterol (Ch) effect on periodontal disease.

Reference	Animal age/weight	Experimental treatments (duration)	Main results/ conclusions
Fujita & Maki (2015)[23]	42 male C57BL/6 J mice; 7 w	Commercial high-fat (62%Kcal) *vs.* standard diet[a] (4, 8 or 12 w)	High-fat diet led to lower trabecular bone volume, cortical bone growth, and cortical bone density as well as to histological alterations at periodontal ligament fibers.
Shikama *et al.* (2015)[24]	Male C57BL/6J mice 12 w	Commercial high-fat (24%wt) *vs.* normal diet[a] (4 w)	High-fat diet led to higher surface expression of CD36 in gingival fibroblasts
Branchereau*et al.*(2016)[25]	Male C57Bl/6J mic 5 w	High-fat (72%Kcal corn oil + lard)[b]*vs.* normal-chow diet[a] (3 m)	ABL was more accentuated in diabetic sensitive followed by Intermediate, diabetic resistant & control mice.
Huang *et al.* (2016)[17]	14 SD rat 8 w	High-fat (35%wt) *vs.* standard chow diet[a]	Periodontal expression of TLR2 & TLR4, gingival protein levels of MyD88 & TRAF6, activity of NF-*k*B signals & mRNA levels of TNF-α & IL-1*β* were higher in high-fat diet fed animals. These were accompanied by higher serum levels of AGEs.
Yoneda et al. (2017)[22]	18 male Wistar rats 8 w	High-fat *vs.* standard chow diet[a] (12 w)	The high-fat diet led to decreased alveolar BMD determined by micro-CT and increased gingival level of 8-OHdG.
Bullon *et al.* (2009)[32]	48 male New Zealand white rabbits 2.5 kg	Diet[a] enriched with saturated fat (+ 3.5%wt lard &1.5%wt Ch) *vs.* standard chow (50 d); following by a standard diet[a] supplemented or not with CoQ_{10}, squalene, HT (0 or 30 d)	HT treatment reduced endothelial activation & squalene additionally decreased fibrosis
Macri *et al.* (2014)[18]	Female Wistar rats 44±2 d	High-fat (15% wt)[c]*vs.* control diet[a] (9 w); in combination or not with ligatures placement in 1 side (last 7 w)	Experimental diet increased total Ch & non-HDL levels. Dietary-treated rats with ligatures showed the highest ABL

Reference	Animal age/weight	Experimental treatments (duration)	Main results/ conclusions
Li *et al.* (2015)[26]	28 male C57BL/6 mice 6 w	High-fat *vs.* regular diet[a] (16 w); in combination with *Aac*-LPS or PBS injections (vehicle) (last 4 w)	High fat diet markedly increased LPS-induced ABL, TRAP-cells, & inflammatory infiltration. This was associated with a synergic effect on cytokines that promoted bone resorption.
Muluke *et al.* (2016)[27]	60 male C57BL/6 mice 4 w	High-fat diet [a] (20%) rich in palmitic acid *vs.* normolipid (10%) diet[a] (16 w); in combination with *Pg* oral infection *or* placebo (last 6 w)	Palmitic acid-enriched diet exacerbated *Pg*-induced ABL. This was accompanied by higher levels of TNF-*α*& lower levels of bone remodeling markers (OC, CTX, & P1NP)
Chen *et al.* (2014)[31]	36 male New Zealand white rabbits 6 m	Standard *vs.* high-fat diet[a] (5%wt lard + 15%wt yolk + 1%wt Ch) (14 w); in combination or not with *Pg* or *Aac*-soaked ligatures placement (last 8 w)	High-fat diet led to increased ABL in rabbit inoculated with bacteria, but there were no differences between bacteria.
Blasco-Baque *et al.* (2012)[28]	129 C57BL6/J Wild type & CD14 knock-out female mice 4 w	High-fat (72% E, corn oil &lard)[b]*vs.* normal chow diet[d]; in combination with subcutaneously implants releasing E_2 or placebo, after ovariectomization (4 w)	Fat-enriched diet increased ABL, prevalence of periodontopathogens[e], & inflammation. These effects were reduced by E2-treated & in CD14 knock-out mice
Azuma *et al.* (2011)[19]	41 male rats 8 w	High-fat diet (32.6% Kcal cocoa butter & Ch)[d] either with & without exercise, *vs.* regular diet[d] (4 or 8 w)	High-fat diet increased ROM serum levels & gingival 8-OHdG & decreased GSH/GSSG ratio. Exercise prevents these changes
Zhou *et al.* (2011)[29]	80 C57BL/6J mice 4 w	High-fat (60% Kcal, lard as main source) *vs.* standard diet with or without moderate exercise after obesity development (5 w); in combination with *Pg*-soaked orsterile ligatures placement (last 1 w)	Moderate daily exercise with standard diet restores the higher levels of TNF-*α*, MCP-1 & IL-1*β*, & lower levels of IL-6 & IL-12p70 associated to obese mice
Sanbe *et al.* (2007)[20]	24 male Wistar rats 8 w	Ch-enriched (1%Ch & 0.5% cholic acid) supplemented with 0.1 or 2 g/l of vitamin C *vs.* regular diets[d](12 w)	High-Ch diet decreased alveolar bone density & increased TRAP–positive osteoclasts & upregulated 8-OHdG expression
Tomofuji *et al.* (2005)[21]	32 male Wistar rats 8 w	Ch-rich (1%Ch & 0.5% cholic acid) or standard diet[d](8 w); in combination with LPS & proteases[f] or pyrogen-free water treatment (last 4 w)	High-Ch diet increased bone resorption & cell-proliferative activity of the junctional epithelium induced by LPS & proteases

[a] *ad libitum* diet, [b]carbohydrate-free diet (less than 1%wt) [c]higher content of MUFA (4.05%wt), saturated fat (8.5%wt) and Ch (1.42%wt) respect than control,[d] not available data about diet or water accessibility,[e]*Fusobacterium nucleatum* and *Prevotella intermedia*.[f] *Escherichia coli* LPS & proteases from Streptomyces griseus introduced daily by micropipette into the palatal gingival sulcus Abbreviations: 8-OHdG: 8-Hydroxydeoxyguanosine, *Aac: Aggregatibacter actinomycetemcomitans,* ABL: ABL, Alveolar bone loss, AGE: advanced glycation end product, CoQ_{10}: coenzime Q_{10}, CTX: terminal collagen type 1 cross-linked C-telopeptide, , FA: fatty acid, GSH: reduced glutathione, GSSG: oxidized glutathione ratio, HDL: high density lipoprotein, HT: hidroxityrosol, IL: inteleukin, LPS: lipopolysaccharide, m: months, MCP-1: monocyte chemotactic protein-1,MyD88: Myeloid differentiation primary response gene 88,NF-*k*B : nuclear factor-*k*B, OC: osteocalcin,P1NP:: Procollagen type I propeptides, PBS: Phosphate buffer saline,*Pg: Porphyromonas gingivalis,* ROM: Reactive oxygen metabolites, SD: Sprague-Dawley, TLR: toll-like receptor, TNF-*α*: Tumor necrosis factor-*α* ,TRAF6: TNF receptor associated factor 6, TNF receptor associated factor 6 , TRAP: Tartrate-resistant acid phosphatase,*vs.: versus,*w: weeks, wt: weight.

Disruption of the fibers was also apparent on the bone surface at 19-weeks of age, accompanied by pronounced vasodilatation. In turn, in the control group, the cortical lamellar bone was well-organized, and periodontal ligament fibers were oriented obliquely at all ages. In addition, a higher osteoclast number on the bone surface of the periodontal ligament spaces was noted at 15-weeks of age ($P<0.05$)[23]. In another study, a high-fat commercial diet (24% weight) was also administrated for a month, but only the percentage of CD36 gingival fibroblasts (GFs) expressing CD36 in cell surface was measured. CD36 or fatty acid translocase is a membrane protein implicated in inflammatory reactions triggered by oxidized low density lipoprotein (LDL). This study provided two interesting results. On the one hand this protein is expressed in GFs surface. On the other hand, experimental diet cells led to a higher surface expression ($P<0.05$) which would indicate that in animals fed with high fat diets rich in saturated fat, GFs are more susceptible to release inflammatory mediators in response to oxidized fat[24]. In the future, it would be interesting to know if this occurs in other cell types present in periodontal tissue.

More recently, a similar experiment with diets with a slightly higher content of fat (72% of total Kcal) consisting in a combination of corn oil and lard, reported results in the same sense, although only alveolar bone loss (ABL) were evaluated in relation to bone ($P<0.05$). Additionally, obese mice were categorized according to their glucose-tolerance as diabetic resistant (glycaemic index<5500mg/dLx-min^{-1}), intermediate resistant (glycaemic index=7000-8000 mg/dLx min-1) and diabetic sensitive (glycaemic index->8500mg/dLx min-1) in this case. After comparing these groups, it was observed that ABL was higher in diabetic sensitive, followed by intermediate, diabetic resistant and finally, normal mice ($P<0.001$). Also, differences were reported in expression of cytokines genes with the exception of interleukin (IL)-6. In more detail, tumor necrosis factor-α (TNF-α) expression was different among all experimental groups ($P<0.0001$ for differences between diabetic sensitive and the other groups, $P<0.001$ for differences between intermediate and the remaining groups and $P<0.01$ for differences between diabetic resistant respect and normal mice). Plasminogen activator inhibitor (PAI-1) expression was higher in diabetic sensitive followed by intermediate mice ($P<0.0001$) but there was no difference between diabetic resistant and control groups. Lastly, IL-1β gene expression was higher in diabetic sensitive mice than in the other groups ($P<0.05$ for differences with control group and $P<0.01$ for differences with the other obese groups), but there were no differences among the other groups. These results highlight the importance of postprandial glycaemia peak effects, which is reinforced by the fact that many models produced by these high-fat diets develop type 2 diabetes mellitus or have components of insulin resistance.

Interestingly, researchers also profiled periodontal microbiota. Of note, Tannerella and Prevotella (two major periodontal pathogens) were not detected in diabetic resistant mice compared to intermediate and diabetic sensitive mice at the genus and species level. Furthermore, their periodontal microbiota was characterized by a lower diversity from phylum to species ($P<0.01$).

From a functional standpoint, a Picrust-based analysis of periodontal microbiome suggested that two major pathways appear to be potentially modulated in relation to the diabetic phenotype: retinoic acid-inducible gene 1 (RIG-I)-like receptor and Prenyltransferase pathway. RIG-I-like receptor signaling t was significantly downregulated in diabetic resistant and diabetic sensitive mice compared with the other ones ($P<0.05$). Prenyltransferase pathway was less downregulated from non-obese, followed by diabetic resistant, intermediate and diabetic sensitive mice ($P<0.01$). Of note, the proportion of sequences linked to the prenyltransferase pathway was significantly and negatively correlated with ABL ($P=0.003$), TNF-α periodontal expression ($P=0.005$) and glycaemic index ($P=0.006$). Finally, a Principal Component Analysis showed a complete separation of all groups, with a slight intersection between diabetic sensitive and intermediate groups confirming the association between all the cardio-metabolic parameters analyzed (including ABL) and metabolic adaptation diet[25].

Two further studies also carried out similar interventions, but in this case in rats. One study evaluated the consequences of a commercial high-fat diet feeding for four weeks in Spargue-Dawley rats with the aim of generating a prediabetes state compared to those maintained on a standard diet. As was expected distance between cementum-enamel junction and alveolar bone crest (CEJ–ABC) was lower in the experimental group (0.3107 –0.04504 vs. 0.2671 – 0.03740 mm^2, $P<0.05$). Again, histological analysis showed that a high fat diet led to a greater infiltration of inflammatory cells. Other histological alterations reported also included apical migration of the junctional epithelium and congestion of blood vessels. Furthermore, immunohistochemical staining for F4/80 showed that there was more monocyte/macrophage invasion into the periodontal tissues, especially in gingival tissue. These changes were accompanied by higher gingival expression of IL-1b gene (7.164–0.9757–fold, $P<0.005$) and protein levels of TNF-α (1.420–0.1709–fold, $P<0.005$). These changes were correlated with higher toll-like receptor (TLR)-2 and TLR-4 expression (7.428-3.732-fold, $P<0.01$ and 12.83–8.494-fold, $P<0.05$, respectively)

and protein levels (1.25-0.05-fold and 1.378–0.1125-fold, respectively)[17]. In addition, the gingival amounts of several proteins participating in their downstream signaling cascade also were higher. These included MyD88 (3.067-0.1528-fold, P=0.0018), the TNF receptor associated factor 6 (TRAF6) (3.167-0.2082-fold, P=0.0031) the activated nuclear factor kB (NF-kB) (namely the phosphorylated form of p65 subunit) (1.6-0.1-fold, P=0.0091) although total levels remained unaffected. Similarly NF-kB p-p65 fluorescence intensity was higher. At a systemic level more advanced glycation end products (AGEs) were detected (94.26-15.43 pg/mL vs. 67.98-3.155 pg/mL, P=0.03)[17]. Likewise, in Wistar rats, obesity induced by the consumption of a hyperlipidic diet for 17 weeks also led to more sites with spontaneous periodontitis compared to those fed a standard diet (20 vs. 8 sites, P<0.01)[33]. Likewise, in Wistar rats, obesity induced by consumption of a hyperlipidic diet for 17 weeks also led to more sites with spontaneous periodontitis respect than those fed a standard diet (20 vs. 8 sites, P<0.01)[18]. In other experiment in male Fischer rats with obesity induced by a 12-weeks diet, this condition led to lower bone mass density (BMD) at alveolar bone compared to the control group that was associated to higher gingival levels of 8-hydroxydeoxyguanosine (8-OHdG)[22].

Findings in rodents are supported by an experiment in male rabbits[30].The consumption of a diet with added saturated fat (lard) and Ch (1%) for 50 days led to an atherosclerotic state that was accompanied with a higher score of fibrosis (1.7±0.5vs.0.2± 0.2) and endothelial activation (1.5±0.5 vs.0) and lower cellularity (0.7±0.4 vs.2.0±0) in gingiva compared with health controls (P<0.05)[30]. Unfortunately, changes in alveolar bone were not measured.

Regarding studies bases on models where periodontitis was experimentally-induced, four used female Wistar rats[18], male C57BL/6 mice[27],[28] or rabbits[32]. Regarding experimental induction methodology all were different. These included the placement of silk ligatures around some teeth[18], injections of bacterial lipopolysaccharide (LPS)25 and oral inoculation of bacteria [27],[31]. In female adult Wistar rats, a diet rich in saturated fat enriched with Ch for 9 weeks combined with ligature placement for the last 7 weeks led to more ABL that those fed on a standard diet (1.59±0.12 vs.1.36±0.10 mm of (CEJ-ABC), P<0.0001). This was directly related to diet-induced differences in serum levels of total Ch and non- high density lipoprotein (HDL)-Ch[18]. In another model of periodontitis induced by gingival injections of bacterial LPS from A. actinomycetemcomitans, in this case in male C57BL/6 mice, high-fat diet (% of total Kcal) feeding increased the LPS-induced osteoclastogenesis (i.e. TRAP-positive cells) (P<0.01), and alveolar bone resorption (bone volume fraction) (P<0.01), although in animals treated with the vehicle both values were higher in high-fat diet fed mice than in those fed regular chow (P<0.01). This was accompanied by a higher LPS-induced leukocyte infiltration and stimulation of IL-6 (0.39-2.61vs. 0.10-1.98, P<0.05), macrophage colony stimulant factor (M-CSF) (1.57-5.52 vs. 0.40-1.85, P<0.05), monocyte chemoattracant protein (MCP)-1 (0.58-4.58 vs. 0.03-0.37, P<0.05), the receptor activator for NF-κB ligand (RANKL) (1.19-7.23 vs. 0.55-3.66, P<0.05). Pathologic analysis of tissue inflammation intensity showed that while LPS injection or high fat diet feeding alone increased inflammatory scores, the combination of LPS injection and high fat diet feeding led to a further increase in inflammatory scores (P<0.01)[26]. Another study in male mice used a diet enriched with the saturated FA (SFA) palmitic acid inducing periodontal disease by P. gingivalis inoculation in this case. When groups fed with a diet rich in SFA were compared with normal chow, differences between animals inoculated with P. gingivalis and those receiving only placebo were found for high-fat diet fed animal (increased by 20% in P. gingivalis treated mice, P<0.05). In addition lower levels of type 1 procollagen N-terminal propeptide (P1NP) and osteocalcin (OC) measured in serum were found (P<0.001), although P. gingivalis inoculation led to lower levels of both these remodelation markers in all animals (P<0.001)[27].

In the experiment conducted in male rabbits fed a saturated fat-rich diet with 1%Ch for 14 weeks, the animals were also orally inoculated with P. gingivalis or A. actinomycetemcomitans for the final 6 weeks. The experimental diet consisted of a high fat diet containing 1% Ch, 5% lard, and 15% yolk, leading to higher ABL regardless of the bacteria used (P<0.05). Additionally, these groups also presented higher levels of triacylglicerides (TG) and Ch than their counterparts fed same diets without bacteria inoculation. In the control diet case, LDL-Ch levels were higher too[31].

Furthermore, there are several studies including other factors that could increase periodontitis risk or modulate diet effect, combined or not with experimental disease induction. These were estrogen levels and physical activity. The former, was a study using ovariectomized female mice. In this, fat-enriched diets increased the prevalence of periodontal pathogenic microbiota like Fusobacterium nucleatum and/or Prevotella intermedia (11±44.0% vs. 2±7.4%, P=0.01), gingival inflammation and ABL (P<0.01), compared with those receiving a normal diet. Meanwhile, treatment with 17β-estradiol released by subcutaneously-implanted pellets prevented these effects[28]. Additionally, the same comparisons were made between CD14 knock-out mice which resisted high-fat diet-induced periodontal defects[28], extolling the importance of this receptor on inflammation.

Another two experiments have been developed in order to include physical exercise in addition to obesogenic diets (32.6% and respectively)[19],[29]. Obese rats, not subjected to exercise training showed higher 8-OHdG levels and lower reduced glutathione (GSH) to oxidized glutathione (GSSG) ratios in gingival tissue than their counterparts receiving a regular diet after eight weeks of treatment. These differences were accompanied by higher serum reactive oxidative metabolites (ROM) levels in the experimental group, which were noted even before four weeks. Exercise, instead, reduced serum ROM and 8-OHdG levels at eight weeks respect to no-training animals despite they had the same diets[19]. In the other study, the interaction between exercise and high-fat diets was analyzed in mice with or without experimental periodontitis induced by P. gingivalis-soaked silk ligatures placement. P. gingivalis-infected obese mice showed a 34% increase in ABL compared with the standard-chow fed group (P=0.001), whereas mice exhibited a 27% and 42% ABL after obesity induction when they daily trained depending on whether they continued taking the high-fat or the standard diet, compared to obese mice without training. However, in this case, dietary treatments or exercise had no effect on ABL if periodontitis was not induced. In addition, higher titers of P. gingivalis were observed in plaque samples from obese mice compared with non-obese mice on day 10 after periodontitis induction (P=0.036). The titers were decreased in exercised mice (P=0.050), but only when they changed their diet. At the systemic level, obese mice exhibited higher levels of TNF-α, MCP-1, and IL-1β, and lower levels of IL-6 and IL-12 subunit p70 in the sera, when compared with non-obese mice or with obese mice with regular diet during training period[29].

Investigations with diets specifically based only on higher amounts of Ch have been carried out mainly in rats[20],[21],[34]. In one study, male Wistar rats only received dietary treatment (additional 2% Ch) for eight weeks[21]. As in previous experiments with high-fat diets, higher Ch content alone induced a decreased alveolar bone density. This was shown to be due to an increase in the number of tartrate-resistant acid phosphatase (TRAP)–positive osteoclasts that were accompanied by higher 8-OHdG levels in the periodontal tissues. As in previous cases, the effect of this type of diet was also tested on an experimentally-induced model of periodontal disease, again in male Wistar rats. In this case, animals received a diet (enriched with 1%Ch and 0.5% cholic acid) for four weeks simultaneously with and during the four previous weeks. Periodontal intervention consisted in application of bacterial LPS and proteases into gingival sulcus. At the endpoint, the production of IL-1β and TNF-α by GFs and periodontal ligament fibroblasts (PLFs) associated to experimental periodontitis was enhanced by Ch-rich diet (6.8±2.2 vs. 3.0±0.5 IL-1β-positive cells/ 0.1mm2sections and 6±1.6 vs. 1.8±0.9 positive cells/0.1mm2 for GFs; and 7.4±1.9 vs. 4.9±0.6 IL-1β-positive cells/0.1mm2 sections and 7.4±1.0 vs. 4.5±0.6 TNF-α-positive cells/0.1mm2 for PLFs, P<0.05). However increases were not significant in GFs without diet compared to controls without periodontitis. High dietary Ch also increased cells/0.1mm×0.1mm area for GFs; and 7.4±1.0 vs. 4.5±0.6 positive cells/ 0.1mm×0.1mm area for PLFs; P<0.05). However increases were not significant in GFs without diet compared to controls without periodontitis. High dietary Ch also increased mitochondrial 8-OHdG in periodontal tissues compared to control (3.2±1.0 vs. 1.2±0.7 positive cells/ 0.1mm×0.1mm area, P<0.05) and combined treatments compared to only periodontitis induced group (7.0±2.2 vs. 3.5±1.1 positive cells/0.1mm×0.1mm area, P<0.05). Instead, the number of fibroblasts remained unaffected[21]. If we take into account other report[20] the same dietary treatment led to a proliferation of the junctional epithelium with increased bone resorption, as well as to increase of the cell-proliferative activity of the junctional epithelium induced by LPS and protease (7.2±1.3 vs. 5.1±1.2 proliferating cell nuclear antigen-positive cells/0.1mmx0.1mm area). Additionally, augmentation of total Ch in blood and decrease in HDL were confirmed.

Overall, it seems that fat-rich diets are positively associated with periodontal disease. However a high consumption of fat is usually linked to hypercaloric diets and this energy excess could be the true cause of observations collected. Broadly, it seems that excessive intake of this type of nutrients has negative effects on periodontal health, especially if they are saturated fat or Ch. In addition to induction of atherosclerosis or obesity by these diets, higher ABL or inflammation degree were observed in periodontium in different experiments and animal models. Moreover, when measured, markers of inflammation or oxidative stress were elevated in periodontal tissues and in many cases also at a systemic level. Additional experiments also suggest that estrogen deficient animals or without daily training are more susceptible to the actions of these diets. These factors could possibly improve tolerance against oxidative stress and inflammation processes, both of which seem to be the two major threats to periodontal diseases, promoted by high-fat diets rich in saturated fat and Ch.

Diets with different FA profiles or FA type supplements

Most research included in this group was focused on PUFA, particularly on n-3 PUFA, although other FA types have also been studied (Table 3). Results were different depending on dietary treatment schedule and duration. With the exception of a study on rats[35], periodontitis was ex-

perimentally induced in most of them. However, different methods were used, such as LPS injection[36,37], ligatures placement[38], application of bacterial products or bacteria inoculation to gingival sulcus[27,39-41]. Additionally, animals received dietary treatments before periodontitis induction in most of these investigations. Almost all revised studies were performed on rats[34-40],but also on mice[26,41] and mainly focusing on n-3 PUFA to n-6 PUFA ratio in the diet. Most of them obtained their conclusions by comparing the consumption of FAs from natural sources; fish oil for n-3 PUFA, and corn or safflower (Carthamus tinctorius) oil for n-6 PUFA. The remaining experiments, instead, analyzed the effect of n-3 PUFA represented by a combination of docosahexanoic acid (DHA) and eicosapentanoic acid (EPA)[36]. P. gingivalis-infected female Sprague-Dawley rats treated with fish oil in their diet had significantly less alveolar bone resorption respect to those fed on a corn oil based diet after 12 weeks[39]. In another report[40] with a similar experiment, it was noted that some rats exhibited decreased IL-1β, TNF-α and enhanced interferon-γ, catalase and superoxide dismutase mRNAs levels compared to rats fed a corn oil diet (P<0.05).

Moreover, analyses of alveolar bone resorption in rats related to gene expression profiles demonstrated significant positive correlations with IL-1β (r=0.553, P=0.0093; and r=0.690, P=0.0005), IL-6 (r=0.690, P=0.0005) and ciclooxigenase-2 and negative correlations with catalase (r=-0.628 P=0.0023 for strain A7A1-28; and r=-0.550, P=0.0098 for strain 381), and superoxide dismutase (r=-0.714 P=0.0003 for strain A7A1-28; and r=-0.551, P=0.0096 for strain 381). Similarly, female BALB/c mice fed tuna oil inoculated with P. gingivalis or with the combination of F. nucleatum and P. gingivalis exhibited 72% and 54% less ABL respectively compared with the sunola oil (used as control) treated group (P<0.05). n-3 PUFA levels were also higher in oral soft tissues of mice fed tuna oil[41]. In this study, infected animals were further subdivided into two groups that received P. gingivalis alone or as a combination of F. nucleatum and P. gingivalis (148±12 vs. 310±12 mg/100g of tissue in P. gingivalis-infected; and 116±12 vs. 216±12 mg/100g of tissue in bacterial combination infected for γ-linolenic acid; and 19±1 vs. 2±1 mg/100g of tissue in P. gingivalis-infected; and 2±1 and 16±1 in bacterial combination-infected for EPA; and 621±16 vs. 336±16mg/100g of tissue in P. gingivalis-infected; 536±16 vs. 333±16mg/100g of tissue in bacterial combination-infected for DHA) (P<0.001) [36]. When periodontitis was induced by LPS injections in male Sprague-Dawley rats, n-3 PUFA supplements after injections also led to lower TNF-α levels in gingival tissue (231.11±26.01 vs. 409.84±110.02pg/mg of tissue protein, P<0.005) and a moderate inflammation compared to positive controls (only LPS-treated) that showed severe inflammation. In turn, if supplements were given in a prophylactic way, rats showed only a slight degree of inflammation, which was accompanied by a lowering of both TNF-α (291.75±24.36 vs. 418.24±40.52pg/mg of tissue protein, P<0.005) and IL-β (181.59±27.93 vs. 409.84±110.02pg/mg of tissue protein, P<0.005)[37]. On the other hand, a study (included in the previous section) in a mouse model of periodontal disease also compared the effects of SFAs represented by palmitic acid and the monounsaturated FA (MUFA), oleic acid. Here, animals were fed on high-fat diets rich in one of these fatty acids. Only animals receiving palmitic acid and inoculated with P. gingivalis showed differences in CEJ-ABC compared with those fed on same diet treated with placebo (an increase of 20%, P<0.05). Interestingly, there were no differences among animals fed different diets not challenged with bacteria. Moreover, it was reported that palmitic acid-rich diet fed animals exhibited lower serum levels of measured bone metabolism markers that involved P1NP and OC than animals fed on other diets (P<0.001), although P. gingivalis inoculation led to lower levels of them in all groups (P<0.001). In turn, TNF-α levels were higher with palmitic acid (P<0.001) and increased in all groups inoculated with P. gingivalis (P<0.01). The inverse relationship between serum levels of TNF-α and bone metabolism markers was further confirmed by correlation analysis (r=–0.55, P=0.002 for OC; r=–0.6, P=0.0005 for P1NP; r=–0.61, P=0.0004 for CTX). It is important to highlight that, as was mentioned, a group fed on a normal chow was also included in this study, but no differences with oleic acid-rich diet fed animals was found for CEJ-ABC[27]. This finding suggests important beneficial effects of MUFA-rich diets.

Another two reports included investigations on FA proportions present on diet, but without experimental induction of periodontal disease[35,36]. Positive effects of fish oil were also confirmed in cats with chronic feline gingivitis/stomatitis, a painful inflammatory disease. Animals were fed diets with chicken fat and fish oil as FAs sources after tooth extraction, including all premolars and molars. In one diet, part of the fish oil was replaced by safflower oil, resulting in two diets with n-6 to n-3 PUFA ratios of 10:1 and 40:1, respectively. The diet with the higher amount of n-3 PUFA decreased levels of prostaglandin D2 (-0.9×10^{-6}vs. 1.2×10^{-6} g/ml), prostaglandin E2 (-3.1×10^{-8}vs. 4.2×10^{-8} g/ml) and leukotriene (LT)B4 (-1×10^{-5} vs. 0.8×10^{-5}g/ml) in plasma after 4 weeks of treatment (P<0.05), but there were no differences on the degree of inflammation or wound healing among groups[43]. On the other hand, when n-3 PUFA was used as a dietary supplement in male Sprague-Dawley rats, it was ineffective in preventing ABL associated to periodontitis, which in this case was provoked by means of LPS injec-

tions. Furthermore, groups treated with n-3 PUFA revealed significantly higher IL-1β (P<0.05) and OC levels (P<0.01) than the LPS alone treated group. However, neither group exhibited differences in serum C-reactive protein levels, which were assessed as well[36]. The last study where periodontitis was not experimentally induced focused also on monounsaturated FAs effects. Wistar rats were fed three different diets, which contained virgin olive oil, sunflower oil, or fish oil as fat sources, to check their lifespan effects; thus they were sacrificed when they were 6 and 24 months old. At endpoint, sunflower oil fed rats showed the highest age-related ABL, followed by those fed on fish oil (280.1±21.7 vs. 167.2±59.4 vs. 82.8±30.8 μm, for sunflower, fish and virgin olive oil fed animals, respectively, P<0.05).

Table 3: Diets with different fatty acid (FA) profiles or supplements with a particular FA type.

Reference	Animal age/weight	Experimental treatments (duration)	Main results/ conclusions
Kesavalu et al. (2006)[39]	95 female SD rats 8–9 w	AIN93 diet [a]enriched with Fish oil (17% menhaden + 3% corn oil) or corn oil (5%)(20 w); in combination or not with *Pg* strain 381 or A7A1-28 infection (last 12 w)	Fish oil-treated rats showed less ABL
Kesavalu et al. (2007)[40]	82 female SD rats 8–9 w	AIN93 diet [a] enriched with Fish oil (17% menhaden + 3% corn oil) or corn oil (5%) (20 w), in combination or not with *Pg* strain 381 or A7A1-28 infection (last 12 w)	Fish oil-rich diet decreased IL-1*β*, TNF-*α*& enhanced IFN-*γ*, CAT & SOD mRNAs
Bendyk *et al.* (2009)[41]	70 female BAL-B/c mice 6–8 w	Diet with tuna oil (10%) or sunola oil (57 d) in combination with orally inoculation with *Pg*, with a mixture of *Pg & Fn*, or untreated (last 43 d); in combination or not with oral inoculation of *Pg* or a mixture of *Pg & Fn* (last 43 d)	Tuna oil-treated & inoculated mice showed lower ABL than control group
Muluke *et al.* (2016)[27]	60 male C57BL/6 mice 4 w	High-fat diet [a](20%) rich in palmitic or oleic acid *vs.* normolipid (10%) diet[a] (16 w); in combination with *Pg* oral infection *vs.* placebo (last 6 w)	ABL was greater in animals fed on a palmitic acid-enriched diet accompanied by higher levels of TNF-*α*& lower levels of bone remodeling markers (OC, CTX, & P1NP)
Vardar-Sengül *et al.* (2006)[36]	39 male SD rats adults	Daily-gavaged (40 mg/kg) n-3 PUFA (40% EPA+ 60% DHA) *vs.* saline supplements in combination with *E. coli* LPS or saline injections (15 d)	n-3 PUFA-treated group revealed higher IL-1β & OC levels than the only LPS-treated group
Araghizadeh*et al.* (2014)[37]	40 male SD rats 12 w	Daily-gavaged (60 mg/kg) n-3 PUFA (20 mg DHA + 180 mg EPA) supplements (15 d after or 15 before) *vs.* saline serum ; in combination with LPS-injections (3 d)	n-3 PUFA-treated rats showed lower TNF-α levels & inflammation degrees than only LPS-treated. In prophylactic-treated rats also IL-1β levels were lower
Bullon *et al.* (2014) [35]	72 male Wistar rats 80-90g	Diets[b] with virgin olive, sunflower or fish oil as dietary fat sources (4%wt) (6 or 24 m)	ABL was higher in sunflower oil group, while other fats seem to maintain an adequate mitochondrial turnover avoiding ETC alterations
Balci Yuce *et al.* (2016)[38]	44 male Wistar rats 390-450g	Diet with conjugated linoleic acid enriched-milk *vs.* standard diets in combination with ligature-inducedperidontitis and/or streptozoin injections (4 w)	Conjugated linoleic acid enriched-milk decreased ABL and $TRAP^+$ cells and increaed osteoblastic activity respect than those fed standard diets under all conditions.

[a]*ad libitum* diet,[b] pair fed diet. Abbreviations: 8-OhdG: 8-Hydroxydeoxyguanosine, ABL: Alveolar bone loss; AIN93: American Institute of Nutrition diet for rodents,[42] CAT: catalase, C-TT1C: terminal telopeptide of type I collagen, CTX collagen type 1 cross-linked C-telopeptide, d: days, DHA: docosahexanoic acid, *E. coli: Escherichia coli*, EPA: eicosapentanoic acid, ETC: mitochondrial electronic transport chain, *Fn: Fusobacterium nucleatum*, GPX: glutathione peroxidase, IFN-*γ*: Iterferon-*γ,* IL: Inteleukin, LPS: lipolysaccharide, OC: osteocalcin, P1NP: procollagen type I propeptides, PUFA: Polyunsaturated fatty acids, SD: Sprague-Dawley, SOD: superoxide dismutase, TNF-*α*: tumor necrosis factor-*α*, w: week, wt: weight.

Findings concerning bone resorption markers (RANKL and osteoprotegerin (OPG)) could explain these differences at least in part, RANKL expression at gums was the highest for virgin olive oil fed animals at 6 months (P<0.05), and decreased in old animals (P<0.05). Nevertheless, its circulating levels changed in the same way for all dietary treatments (P<0.05). Regarding OPG, the lowest mRNA levels were found for fish oil fed animals at six months, that increased in twenty four month old animals (P<0.05). On the other hand, animals fed on virgin olive oil showed the lowest concentration of plasma levels at six months (P<0.05), but both virgin olive oil and fish oil fed rats reported higher concentrations at twenty four than at 6 months (P<0.05). At the histological level, only the sunflower group showed a high degree of fibrosis and a moderate degree of inflammation at 24 months; although both sunflower and fish oil group showed a reduction of cellularity. Similarly, at twenty four months, the highest levels of lipid peroxidation at the gums was observed in sunflower oil fed animals (0.16±0.02nmol/mg for sunflower oil group, 0.09±0.01nmol/mg for olive oil group, and 0.06±0.01 nmol/mg for fish oil group; P<0.05), but age differences were only found for virgin olive and fish oil fed animals (0.09±0.01 vs. 0.13±0.01nmol/mg and 0.06±0.01 vs. 0.14±0.03nmol/mg; P<0.05). Additionally, a wide gene expression analysis was performed on gingival tissue for genes implicated in several processes like inflammation (Il1b, Il8, Il6 and TNF), apoptosis (Bad, Bax and Bcl-2), mitochondrial biogenesis (Tfam, Ppargc1 and Sirt1), mitochondrial autophagy, and antioxidant defense (Sod1, Sod2 and Nrf2); as well as for electron transport chain complex I constituents (mt-Nd1, mt-Nd4 and Ndufs1). Results for all suggest that positive effects of MUFAs (in the form of virgin olive oil) or n-3 PUFA (in the form of fish oil) could be due to the ability of these FAs to allow mitochondrial maintaining turnover through biogenesis or autophagy mechanism, which seem to induce the corresponding antioxidant systems to counteract age-related oxidative stress, and do not inhibit mitochondrial electron transport chain[35].

According to the result of these studies, it seems that n3-PUFA have a positive effect on periodontal health. This is particularly noticeable when dietary content of n-3 PUFA is higher than that of n-6 PUFA, since differences are observed even if periodontitis was not induced. This is expected because of findings from many of these studies in relation to other parameters. Again, the degree of pro-inflammatory signals and oxidative stress markers at gingival or systemic levels were related to harmful effects. Despite this evidence, when n-3 PUFA were used as supplement their action was not enough to prevent ABL. On the other hand, MUFAs might offer similar or even better advantages than n-3 PUFA if effects of life-long feeding on diets containing olive oil, sunflower oil, or fish oil on rat ABL are taken into account[35]. Moreover, it has been suggested that this is due to some mechanism related to mitochondria.

More recently, the effects of a set of fatty acids present in dairy products and meat conjugated linoleic acids was tested in Wistar rats consuming enriched milks for 4 weeks. This dietary intervention decreased ABL in groups with ligature-induced periodontitis, which also was combined with streptozoin treatment to induce diabetes mellitus. Such effect was associated with decreased osteoclastogenesis determined by TRAP-positive cell number and osteoblastic activity, but only in animals with diabetes. In addition, it increased Bax levels, a phenomenon also associated to diabetes mellitus induction[38].

Proteins

According to the studies collected, research on the effect of dietary proteins on periodontal disease has been performed mainly in rats[44]-[48],but Marsh rice rats (Oryzomys palustris)[49] and Mongolian gerbils[50] have also been used (Table 4). Generally, these studies have been based on dietary interventions with different amounts of proteins[44]-[47],[50]. As in interventions with varying lipid components, modifications of protein quantities are accompanied by changes in carbohydrate amounts in many cases[44],[46],[47],[50].

Results from some of these studies would indicate negative effects on alveolar bone for diets with very high protein contents[44],[50]. These include an investigation in female Sprague-Dawley rats[44] and another in Mongolian gerbils[47]. The study tested the effects of feeding on high- and low-protein diets by casein (62%vs. 10% casein) in rats, which were subdivided into two groups depending on whether they received drinking water supplemented with Fluor or not. After 3 months, a significant increase in ABL occurred in rats receiving the casein-rich diet compared with the other dietary group. ABL scores on the two protein levels respectively were 7.69±0.85 and 4.76±0.55, for fluoride-treated rats and 9.11±0.82 and 5.77±0.90 for the animals drinking distilled water at the same level of protein intake. Furthermore, calculus deposition was affected in the same manner. Curiously, changes in carbohydrates in these diets implied mainly higher variations in sucrose (13 vs. 65%) that could have had some negative effects, but consequences of hyperproteic diets seemed more important[44]. Similarly, in Mongolian gerbils fed a high-protein diet (also consisting of 62% casein), most animals showed significant osseous lesions in maxilla and mandible (P=0.02), although these revealed little or no evidence of calcareous deposits[50]. However, a high performance bias existed because of weight and hair loss and even some deaths observed only in animals fed a high-protein diet.

Table 4: Studies on proteins, peptides and aminoacids (Aa) effects on periodontal disease.

Reference	Animal; age/ weight	Experimental treatments (duration)	Main results/ conclusions
Zipkin et al. (1970)[44]	144 female SD rats; Age not provided	Isocaloric diets[a] containing 62%, 25% or10 % casein supplemented or not with F (as NaF) in drinking water (90 d)	Low-protein diets increased ABL & Calculus deposition
Moskow et al. (1973)[50]	24 female Mongolian gerbil 6 m	High-protein diet[a] (62% Casein + 13% Sucrose) *vs*. standard ground chow (100 d)	Most high-protein diet-fed animals showed high osseous lesion scores
Seto et al. (2007)[45]	70 male Fischer rats 8 w	Ligature placement on one side *vs*. sham-ligation (65 or 110 d) combined with feeding on powder diets[b] containing 0, 0.2 or 1.0% milk basic protein (last 45 or 90 d) In combination with ligatures placement in 1 side or sham-ligation (65 or 110 d)	High-dose milk basic protein diets recovered ligature-induced alveolar bone resorption as well as increased osteoid thickness of alveolar bone
Johnson &Thliveri (1989)[46]	54 male SD rats 3 m	Low-protein(8%) *vs*. standard (24%) diet[a](6 m)	Low-protein diet alleviated ABL in the absence of periodontal inflammation noted in hyperglycemic diabetic rats
Mavropoulos et al. (2005)[47]	44 female SD rats 6 m	Isocaloric diets[b]containing 15% or 2.5% casein (16 w)	Protein undernutrition & ovariectomy negatively influenced alveolar bone, but to a lesser extent than the proximal tibia
Shaw (1966)[49]	16 rice rats at weaning	Diets[a] containing 7Aa[c]or diet 700 (with casein as protein source) (84 d)	Diet containing casein reduced alveolar bone resorption & destruction of the soft tissues
Breivik et al. (2005)[48]	19 male Wistar rats 310–330 g	Gly-supplemented *vs*. non-supplemented water[a] (37 d); in combination with ligatures placement (last 34 d)	Gly supplementation reduced periodontal bone loss, which was negatively correlated with increased serum Gly

[a]*ad libitum* diet, [b]pair fed, [c]mixture XXIII by Rose, Oesterling, and Womack.[51] Abbreviations:Aa: amino acids, ABL: alveolar bone loss, BMD: bone mass density, d: days, F: fluor, Gly: glycine, IL-10: Interleukin-10; m: months, Na: sodium, SD: Sprague-Dawley, *vs.: versus*, w: weeks

Additionally, as with previous nutrients, interactions with certain pathologies or physiological situations have also been taken into account, specifically diabetes mellitus[46] and estrogen deficiencies [47].

However, these studies compared the effects of diet with protein content according to animal needs with those with contents close to standard values, finding that a low-protein diet also has detrimental effects. In one report, authors induced diabetes mellitus with streptozotocin to male Sprague-Dawley rats that received a treatment with protamine-zinc insulin to maintain or not normal blood glucose levels. Euglycemic and hyperglicemic diabetic as well as non-diabetic rats, in turn, were fed a low-protein (8%) or a standard protein diet (24%). After 6 months, alveolar bone height after the experimental period was greater in hyperglycemic diabetic rats fed a standard diet than in those fed a low-protein one (318.1±3.2 vs. 282.6±3.5µm, P<0.05) or non-diabetic animals fed a standard diet (318.1±3.2 vs. 268.5±1.5µm, P<0.001). Thus, hyperglycemic diabetic rats have significant ABL in the absence of periodontal inflammation (P<0.001) and this bone loss can be alleviated by diet (P<0.05). Nonetheless, there was no evidence of gingival or periodontal inflammation or osteoclastic bone resorption at the alveolar crest in any animal studied [46]. Problems in interpretation of these findings could come from differences between the two diets. When composition is evaluated, it can be observed that, although the energy provided was very similar, experimental diet was richer in carbohydrates and lipids, but fiber was absent. In the second study, old Sprague-Dawley rats received diets with different amounts of casein (2.5% or 15%) but with limited access. In this case the two diets were isocaloric by means of cornstarch content variation. In addition, half the rats on each diet were ovarectomized whereas the other half was sham-operated. After 17 weeks of treatment, histomorphometry and densitometry evaluations were made on the mandible, and also on proximal tibia. In sham-operated animals, low-protein intake led to a 17.3% reduction of bone volume per trabecular volume in the mandible and 84.6% in the tibia (P<0.001) as well as to a thinner trabeculae in mandible (P<0.05), whereas ovarectomy led to a reduction of trabecular number (P<0.05)[47]. The effect of a protein deficient diet is expected due to the importance of proteins for tissue integrity and immune maintenance. Nevertheless, the mechanism by which high-protein diets could exert negative effects is less clear. Possible changes in physical properties or nutrient deficiencies derived from modifications should be con-

sidered in this sense. Therefore, more human studies are needed to finally confirm another danger of high-protein diets.

On the other hand, studies from the qualitative point of view of these macronutrients have been few and very divergent[45],[48],[49], making it difficult and risky to establish some affirmations from their results. The possible role of protein source and certain amino acids (Aa) has been considered in some of the chosen studies. Despite casein being an established source of Aa for most rodent lab diets, the three remaining studies focused on the importance of protein quality by means of changes in its dietary source or certain Aa supplementation. In one model, in Fischer rats with ligature-induced periodontitis, the consumption of high-dose milk basic protein (1.0%) clearly recovered ligature-induced alveolar bone resorption on days 45 (432.8±47.8 vs. 527.2±77μm, P<0.05) and 90 of treatment (407.3±32.9 vs.527.1±62.3μm, P<0.05), whereas low-dose milk basic protein (0.2%) did not show such a clear effect compared to control diet from initiation of treatment. Histological examination clarified that the osteoid thickness of the alveolar bone was dose-dependently increased by milk basic protein treatment for 90 days[45]. In rice rats, substitution of casein for an Aa mixture based on the ratios of 19 essential and non-essential Aa had a negative effect on soft tissue lesions compared to number of areas (16.2±2.4 vs. 26.2±0.9, P<0.01) and extent of areas (16.3±4.9vs. 50.8±5.3, P<0.01) and alveolar bone resorption measured by number of areas (25±1.4 vs. 27.8±0.1, P<0.05) and extent of areas (36.0±7.0 vs. 66.2±4.7, P<0.01)[49]. The last investigated survey reported beneficial effects of glycine through supplementation in drinking water. Male Wistar rats with periodontitis due to ligature placement showed less periodontal bone loss when supplements were taken (0.75±0.11 vs.0.97±0.20 mm, P<0.007), which also presented higher serum levels of glycine (1866±410vs.603±96 μmol/l, P<0.0001). For all animals, there was a significant negative correlation between their serum glycine levels and bone loss (r=0.47, P<0.05). In serum also levels of other Aa were measured and a positive correlation was observed between glycine and Serine (r=0.72, P<0.01) whose levels were higher in the treated group as well (358±44 vs. 224±42 μmol/l, P<0.0001), but there were no significant results compared to them [48]. Therefore, because some interventions in relation to this aspect have resulted as positive for periodontal health, further investigations on this aspect could prove to be interesting.

CONCLUSIONS

The role of the different macronutrients in periodontal disease has been tested in animals using multiple models and experimental designs. Among them research on dietary has been notably higher. The use of models where periodontitis and other pathologies are induced allows further insight in possible therapeutic and/or preventive effects, as well as interaction with certain physiological situations. However, general interpretation of these studies is complex due to the different modifications that diets can be subjected to, variability in periodontal disease induction methodologies and species used. Moreover, the fact that changes in a particular macronutrient content in the diet can affect the amount of others is an additional difficulty to establish any conclusion. Anyhow, it seems that macronutrients with effect on antioxidant status or immune system are particularly important in relation to periodontal disease management and prevention. In this sense, there is evidence for a positive role of n-3 PUFA oin diets. In particular, its proportion in the diet compared to n-6 PUFA content seems to be a key dietary feature. On the other hand, nutrients or diets that increase oxidative stress and promote inflammation as occurrs with saturated fat and hypercaloric diets must be avoided to prevent periodontal disease. In the future, more studies should be carried out to clarify the role of MUFA and the different carbohydrate types or glycemic index of the diets.

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