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RESEARCH ARTICLE

Impact of obesity and aging on crestal alveolar bone height in mice

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ABSTRACT

Obesity and aging are associated with periodontitis, which represents a chronic inflammatory disease of the tooth-supporting tissues, *i.e.* the periodontium. However, if both risk factors also have a negative impact on crestal alveolar bone in a clinically healthy periodontium, has yet to be elucidated and was analyzed in this *in-vivo* study.

Eight C57BL/6 mice were fed a normal diet during the entire study. Half of these mice were sacrificed at week 19 (group 1: younger lean mice), whereas the other half of the animals were sacrificed at week 25 (group 2: older lean mice). In addition, four mice were fed a high-fat diet until their sacrifice at week 19 (group 3: younger obese mice). Mandibles and maxillae were scanned by micro-computed tomography and, subsequently, the distance between the cementoenamel junction and alveolar bone crest (CEJ-ABC) at all molars was determined. Levels of interleukin-6, cyclooxygenase-2, visfatin and adiponectin in gingival samples were quantified by real-time PCR. For statistical analyses, the Mann–Whitney-U test was applied ($p < 0.05$).

As compared to lean mice, obese animals presented a significantly increased CEJ-ABC distance, *i.e.* reduced alveolar bone crest height, at week 19. The alveolar bone loss was mainly found at the first molars of the mandibles. In animals fed a normal diet, the alveolar bone crest height in the mandibles and maxillae was significantly lower in the older mice as compared to the younger animals. Furthermore, gingival cyclooxygenase-2 and visfatin expressions were higher in the obese versus lean mice and in the older versus younger mice.

This *in-vivo* investigation shows that obesity and older age can result in reduced alveolar bone crest height and suggests that they represent risk factors even in a clinically healthy periodontium.

1. Introduction

Periodontitis is a chronic inflammatory disease, which is characterized by alveolar bone and attachment loss. This complex disease is caused by microorganisms of the subgingival biofilm in combination with other risk factors, such as smoking, a genetic/epigenetic predisposition and a number of systemic diseases including diabetes mellitus, rheumatoid arthritis and osteoporosis (Knight et al., 2016; Hajishengallis and Korostoff, 2017). Overweight and obesity have also been shown to be linked with periodontal diseases in different ethnic populations and age groups (Chaffee and Weston, 2010; Moura-Grec et al., 2014; Martens et al., 2017). In an experimental periodontitis rat model, Perlstein and Bissada already demonstrated in 1977 that obesity infers an increased risk of periodontitis (Perlstein and Bissada, 1977). In addition, more recent longitudinal studies have provided further evidence for a causal relationship between obesity and periodontal diseases (Nascimento et al., 2015).

The current predominant view regarding the association between periodontitis and obesity is that obesity increases the risk for periodontitis but not *vice versa*. However, the mechanisms whereby obesity could aggravate periodontal inflammation and destruction, are as yet only partially understood. It is conceivable that obesity compromises the immune system so that no efficient host response to the periodontal microorganisms can be mounted, resulting in bacterial tissue invasion, inflammation, collagen degradation and bone resorption (Amar et al., 2007;

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Table 1
Composition of the normal and high-fat diet.

	U	Normal diet	High-fat diet
Crude nutrients	%		
Dry matter	%	96.1	97.1
Crude protein (Nx6.35)	%	18.1	24.1
Crude fat	%	5.1	34.0
Crude fibre	%	6.0	6.0
Crude ash	%	6.2	6.1
N free extracts	%	62.0	27.0
Starch	%	40.7	2.2
Sugar	%	8.6	22.4
Dextrines	%	8.0	–
Energy, ME — Atwater[a]	MJ/kg	15.0	21.4
kJ% fat		13	60
kJ% protein		20	19
kJ% carbohydrates		67	21
Minerals	%		
Calcium	%	1.09	1.05
Phosphorus	%	0.70	0.61
Sodium	%	0.20	0.20
Magnesium	%	0.21	0.17
Pottasium	%	0.96	1.00
Fatty acids	%		
C 8:0	%	–	–
C 10:0	%	–	–
C 12:0	%	–	0.03
C 14:0	%	0.02	1.03
C 16:0	%	0.55	8.06
C 16:1	%	0.03	0.78
C 17:0	%	–	0.38
C 18:0	%	0.24	5.61
C 18:1	%	1.32	12.13
C 18:2	%	2.65	2.37
C 18:3	%	0.33	0.33
C 20:0	%	0.03	0.04
C 20:1	%	–	0.01
C 20:4	%	–	0.07
C 20:5	%	–	–
C 22:6	%	–	–
Cholesterol	mg/kg	–	290[b]
Amino acids	%		
Lysine	%	1.48	1.98
Methionine	%	0.64	0.83
Cystine	%	0.33	0.46
Met + Cys	%	0.97	1.28
Threonine	%	0.80	1.07
Tryptophan	%	0.23	0.31
Arginine	%	0.65	0.88
Histidine	%	0.57	0.76
Valine	%	1.22	1.64
Isoleucine	%	0.94	1.25
Leucine	%	1.76	2.36
Phenylalanine	%	0.96	1.29
Phe + Tyr	%	1.92	2.57
Glycine	%	0.37	0.50
Glutamic acid	%	4.04	5.41
Aspartic acid	%	1.34	1.79
Proline	%	2.07	2.76
Alanine	%	0.59	0.79
Serine	%	1.07	1.43
Vitamins	per kg		
Vitamin A	IU/kg	15,000	15,000
Vitamin D3	IU/kg	1500	1500
Vitamin E	mg/kg	150	150
Vitamin K (as menadione)	mg/kg	20	20
Vitamin C	mg/kg	30	30
Thiamin (B_1)	mg/kg	16	16
Riboflavin (B_2)	mg/kg	16	16
Pyridoxine (B_6)	mg/kg	18	18
Cobalamin (B_{12})	mg/kg	30	30
Nicotinic acid	mg/kg	45	45
Pantothenic acid	mg/kg	55	55
Folic acid	mg/kg	19	19
Biotin	mg/kg	305	310
Choline-chloride	mg/kg	1040	2300
Inositol	mg/kg	80	80
Trace elements	per kg		
Iron	mg/kg	166	139
Manganese	mg/kg	98	82
Zinc	mg/kg	64	56
Copper	mg/kg	14	12
Iodine	mg/kg	1.16	0.97
Selenium	mg/kg	0.14	0.13
Cobalt	mg/kg	0.14	0.13

[a] ME calculated with the Atwater factors.
[b] Original content.

Maciel et al., 2016). Furthermore, obesity is characterized by a chronic low-grade inflammatory state. This metabolically triggered inflammation (metainflammation) has been linked to many obesity-related diseases, such as cardiovascular diseases, diabetes mellitus and cancer (Marseglia et al., 2014). The adipose tissue produces and secrets a great number of bioactive molecules, such as leptin, visfatin and adiponectin, which are collectively called adipokines and also detectable in gingival tissues and crevicular fluid (Deschner et al., 2014; Jung and Choi, 2014; Kanoriya et al., 2017). Although adipokines have a role in the regulation of insulin sensitivity and energy expenditure, they can also modulate inflammatory and wound healing processes, thereby possibly enhance periodontal inflammation (Deschner et al., 2014; Jung and Choi, 2014). However, additional pathomechanisms may underlie the obesity-periodontitis link and should be the focus of further studies.

Whereas the contribution of obesity to periodontitis-induced alveolar bone loss is more or less widely accepted, it is still unclear if the negative impact of obesity on periodontal bone can also be observed under clinically healthy periodontal conditions. If so, this could significantly affect the determination of tooth prognosis and treatment decisions in obese patients whose number are rising worldwide (Smith and Smith, 2016).

Another factor that is associated with the loss of periodontal tissues is aging (Ebersole et al., 2016). However, if aging alone, *i.e.* in the absence of a chronic periodontal inflammation, results in alveolar bone loss is highly controversial (Burt, 1994; Huttner et al., 2009). Like obesity, aging is associated with a state of continuous low-grade inflammation (inflammaging) (Franceschi et al., 2000). This chronic inflammatory condition could again function as predisposing factor for the aforementioned systemic diseases as well as periodontitis. Other hypotheses suggest that the higher prevalence of periodontitis in older individuals is the result of a longer exposure to periodontal bacteria, age-related alterations in the immune response or chronic systemic diseases (Huttner et al., 2009; Hajishengallis, 2014; Ebersole et al., 2016).

As already mentioned, obesity and aging are associated with periodontitis. However, if obesity and aging also have a negative impact on crestal alveolar bone in a clinically healthy periodontium, has yet to be elucidated and was analyzed in this *in-vivo* study.

2. Material and methods

2.1. Animal model

Twelve male C57BL/6 mice were purchased from Charles River (Charles River, Germany), housed in individually ventilated cages at 23 ± 1 °C and 40–65% humidity with a 12-h dark–light cycle, and provided with food and water *ad libitum*. All procedures performed in the study were in accordance with the ethical standards of the University of Bonn and approved by local authorities (Landesamt für Natur, Umwelt und Verbraucherschutz Nordrhein-Westfalen;