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ORIGINAL ARTICLE



Dental side effects of long-term obstructive sleep apnea therapy: a 10-year follow-up study

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Abstract

Objectives Patients with obstructive sleep apnea (OSA) are usually treated with either mandibular advancement device (MAD) or continuous positive airway pressure (CPAP) therapy. The objective of this study is to evaluate changes in dental occlusion associated with long-term MAD and CPAP therapy.

Materials and methods Data from 14 OSA patients using MAD and 17 OSA patients using CPAP therapy were evaluated at baseline, 2-year and 10-year follow-up. Changes in dental occlusion were analyzed from dental plaster casts with a digital sliding caliper.

Results At 2-year follow-up, MAD therapy resulted in significant dental changes when compared with baseline values. In MAD therapy, overjet and overbite decreased with 1.1 ± 1.8 mm and 1.1 ± 1.2 mm respectively. With CPAP therapy overjet and overbite decreased significantly with 0.2 ± 0.5 mm and 0.3 ± 0.5 mm, respectively. Both groups also showed significant changes in molar occlusion. After a 10-year follow-up, significant and more pronounced changes were seen in overjet and overbite. In MAD therapy, overjet and overbite decreased with 3.5 ± 1.5 mm and 2.9 ± 1.5 mm respectively when compared with baseline values. In CPAP therapy, overjet and overbite decreased with 0.7 ± 1.5 mm and 0.8 ± 1.4 mm respectively when compared with baseline values.

Conclusions This study demonstrates that MAD and CPAP therapy result in significant changes in dental occlusion. These changes appear progressive and more pronounced with MAD compared to CPAP therapy.

Clinical relevance Long-term OSA treatment results in significant dental side effects that may progress over time. Informed consent is fundamental before starting MAD treatment and individualized long-term follow-up is of eminent importance.

Keywords Obstructive sleep apnea \cdot Mandibular advancement device \cdot Continuous positive airway pressure \cdot Treatment outcome \cdot Dental side effects

Boudewijn Stegenga deceased.

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Obstructive sleep apnea (OSA) is an increasing problem in today's society. OSA is a sleep related breathing disorder, characterized by repetitive obstructions of the upper airway during sleep [1]. It is associated with excessive daytime sleepiness and an increased risk of cardiovascular disease, but also with endothelial dysfunction and neurocognitive deficits [2].

During sleep, the muscle tone of the upper airway decreases which may result in a collapse. A complete obstruction of the upper airway is labeled as an apnea and a partial obstruction for more than 10 s as an hypopnea [3]. OSA can be diagnosed in case of 5 or more apneas/hypopneas per hour of sleep and is quantified by the apnea–hypopnea index (AHI), as determined by polysomnography. Based on the AHI OSA may be classified as mild (AHI 5–15), moderate (AHI 15–30), or severe (AHI > 30) [4].

Treatment of OSA depends mainly on the severity of the disease and symptoms as well as the patient's preference. In addition, anatomical characteristics and health status are important factors that should be contemplated when selecting the appropriate treatment modality. In mild to moderate OSA, mandibular advancement devices (MAD) are frequently used, whereas in moderate to severe OSA continuous positive airway pressure (CPAP) is usually applied as primary intervention. Other treatment modalities include lifestyle changes and upper airway surgery [3, 5, 6].

A MAD acts by advancing the mandible in a more forward and downward position, thereby reducing upper airway collapsibility. It improves upper airway patency by pulling the tongue base, epiglottis and soft palate forward, and by stimulating upper airway musculature [7]. MADs are especially effective in patients with mild to moderate OSA [8].

CPAP acts by means of continuous positive airway pressure, which is applied through a nasal, mouth, or facemask. As a result of this continuous positive airway pressure, the airway is restored and obstructive breathing events are reduced. CPAP is very effective in patients with moderate to severe OSA, but can be complicated by suboptimal acceptance and compliance in a substantial degree of patients [6, 9].

With both therapies mild and transient side effects occur. The MAD may cause tooth pain, myofascial pain, temporomandibular joint pain, excessive salivation, a dry mouth, or gum irritation [10–12]. Long-term MAD use results in dental changes, including a decrease in overbite and overjet. Secondly, the anterior–posterior distance and the number of occlusal contact points decreases with MAD therapy. Besides, craniofacial changes and temporomandibular dysfunction are associated with MAD treatment. Craniofacial changes result in an increased lower and total anterior facial height and downward rotation of the mandible. Temporomandibular dysfunction results in temporomandibular joint pain, temporomandibular joint sounds, and myofascial pain [13, 14]. No differences in terms of bite changes have been observed between younger persons and elderly [15].

Mild and transient side effects with CPAP therapy include nasal congestion, rhinorrhea, eye irritation, and a sense of suffocation [16]. Long-term CPAP use may also result in dental changes, such as a decrease in overbite and overjet [17–19]. In addition, CPAP may result in a decrease in intermaxillay relationship, a retrusion with retroclination of the maxillary frontal teeth, and a setback of supramentale and chin position with a decrease of facial convexity [20]. These changes are less pronounced when compared to MAD [19].

Previous studies have evaluated the progression of dental changes with MAD treatment over time [21]. However, to date, studies comparing dental side effects following 10 years of MAD and CPAP therapy are lacking. It is unknown to what extent MAD and CPAP cause dental side effects after a decade of treatment and whether these changes are progressive over time. This study aims to evaluate changes in dental occlusion, which are associated with long-term MAD and CPAP therapy.

Materials and methods

Patient selection

Participants were recruited from a randomized controlled trial, which was previously conducted and reported in two separate publications (ISRCTN18174167) [5, 6]. Patients were recruited between September 2002 and August 2005 through the Department of Home Mechanical Ventilation of the University Medical Center Groningen, the Netherlands. This study was approved by the Groningen University Medical Center's ethics committee (METc2002/032). A written informed consent was obtained from each patient before enrollment.

Study design

For this cross-sectional study 103 patients were selected. At baseline patients were randomly allocated to MAD or CPAP therapy (Supplement).

Interventions

At baseline, all patients in the MAD group received the TAP appliance (Thornton Adjustable Positioner type-1, Airway Management Inc., Dallas, TX, USA). This duo block MAD fixes the patients mandible in a forward position by a screw mechanism incorporated in the frontal area of the appliance. At baseline, the appliance was set at approximately 50% of the range from central relation to the patient's maximum protrusion. Patients were instructed to advance their appliance until

symptoms abated or until further protrusion of the mandible resulted in discomfort. After an 8-week follow-up period, a checkup was arranged to further adjust the appliance if necessary. When OSA symptomatology was improved or when further protrusion was not tolerated by the patient, a second evaluation was performed (i.e., a polysomnographic study, physical examination and subjective questionnaire evaluation). The mandibular protrusion and vertical dimension of the oral appliance was kept constant during the further follow-up period.

After CPAP-titration, all patients received a similar CPAP device (Breas® PV10, Mölnlycke, Sweden). After a 2-week adaptation period, patients returned for a follow-up visit to resolve any difficulties. After an 8-week follow-up period, a checkup was arranged to further adjust therapy if necessary. When OSA symptomatology was improved or when the patient did not tolerate further pressure adjustments, a second evaluation was performed (i.e., a polysomnography, physical examination and subjective questionnaire evaluation). If polysomnography indicated, an AHI \geq 5, CPAP was adjusted if possible and a third polysomnography was performed.

Successful treatment was defined as an AHI < 5 or an AHI reduction of > 50% from the baseline value to a value < 20 in a patient without symptoms while using therapy. If treatment was not successful or if patients were non-adherent, the alternative (either MAD or CPAP therapy) was offered. Patients

were excluded when they switched therapy or received upper airway surgery during follow-up. Furthermore, minimum therapeutic use for inclusion in this study was > 5 nights per week for > 5 h per night [5]. The therapeutic use was discussed with the patient at each checkup. All patients included for analyses in the present study met this compliance criterion.

During follow-up, patients were subjected to a regular yearly checkup and were encouraged to contact our clinic when problems were faced concerning their OSA treatment. Besides, patients were asked about their opinion on their teeth and possible dental side effects. No specific questionnaire was used.

Study model analysis

At baseline, 2-year and at the 10-year follow-up, dental plaster models were obtained from all patients to determine changes in dental occlusion. The intermaxillary relationship between the arches was recorded with vinyl polysiloxan registration material Exabite II NDS[™] (GC America Inc., Alsip, Il, USA) in maximal occlusion (Table 1).

Measurements were obtained clinically (mandibular protrusion) and on the dental plaster casts by means of a 0.01mm resolution digital sliding caliper. All measurements were performed twice by the same observer who was blinded for

Table 1	Baseline characteristics,	therapeutic use,	, and cast analysis	of patients who	completed the	10-year follow-up
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Variable	Baseline		2-year follow-u	р	10-year follow-up		
	MAD	CPAP	MAD	СРАР	MAD	СРАР	
Male/female ratio	12/2	17/0					
Age (years)	61 ± 8	59 ± 10					
Follow-up (years)			2.3 ± 0.2	2.4 ± 0.3	10.0 ± 0.6	10.3 ± 0.6	
Apnea hypoapnea Index (AHI/h)	31.7 ± 20.6	49.2 ± 26.1	$2.7 \pm 3.2*$	$0.47 \pm 1.1^{*}$	9.9 ± 10.3*	3.4 ± 5.4*	
Body mass index (kg/m2)	32.4 ± 6.6	33.2 ± 3.6	32.1 ± 6.6	33.9 ± 4.2	31.3 ± 5.9	32.5 ± 4.8	
Neck circumference (cm)	43.6 ± 3.7	44.6 ± 3.2	42.9 ± 3.7	44.6 ± 2.6	43.8 ± 3.8	44.4 ± 2.8	
Therapy frequency nights/week			6.9 ± 0.4	7.0 ± 0.0	6.6 ± 1.0	7.0 ± 0.0	
Therapy frequency hours/night			7.1 ± 0.7	6.5 ± 0.9	7.8 ± 0.9*	$6.8 \pm 0.9*$	
Number of teeth upper arch	13.0 ± 1.5	13.7 ± 1.3			12.5 ± 2.1	13.3 ± 1.4	
Number of teeth lower arch	13.3 ± 1.4	13.1 ± 1.0			13.1 ± 1.3	12.6 ± 1.8	
Overjet (mm)	4.8 ± 2.3	4.2 ± 2.3	3.7 ± 3.0	3.9 ± 2.3	$1.3 \pm 3.1*$	3.5 ± 2.1*	
Delta overjet (mm) 0 to 2-year and 0 to 10-year follow-up			-1.1 ± 1.8	-0.2 ± 0.5	$-3.5 \pm 1.5*$	$-0.7 \pm 1.5*$	
Overbite (mm)	4.2 ± 2.4	3.9 ± 2.0	3.0 ± 2.7	3.6 ± 1.9	1.3 ± 2.2	$\textbf{2.9} \pm \textbf{2.4}$	
Delta overbite (mm) 0 to 2-year and 0 to 10-year follow-up			- 1.1 ± 1.2*	$-0.3 \pm 0.5*$	- 2.9 ± 1.5*	$-0.8 \pm 1.4*$	
Percentage of maximal protrusion (%)			84.3 ± 22.5				

MAD, mandibular advancement device; *CPAP*, continuous positive airway pressure; *1*, independent samples *T* test *2*, one-way ANOVA. Values are means \pm standard deviations. Therapy frequency is determined as a self-reported questionnaire. Bold values significant differences calculated, compared to baseline (p < 0.05)

*Significant differences calculated, MAD compared to CPAP (p < 0.05)

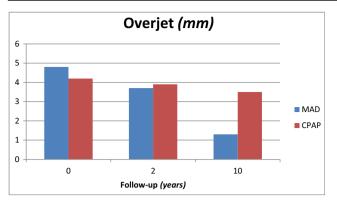


Fig. 1 Overjet of MAD (mandibular advancement device) and CPAP (continuous positive airway pressure) treatment at baseline 2-year and 10-year follow-up

the patient's treatment. For continuous variables, the mean of both measurements was used for further analysis.

Anterior overjet and overbite were measured at the maxillary central incisors. Overjet was measured as the horizontal distance, of the mesial end of the incisal edge of the upper central incisor to the labial plane of the lower central incisor. The overbite was measured as the vertical distance, of the incisal edge of the lower central incisor to the incisal edge of the labial plane of the upper central incisor. Delta overjet and overbite were calculated as the difference between pre-treatment and 2- or 10-year follow-up models (Figs. 1, 2, 3 an 4). Negative values were related to a reduction of overjet and overbite and positive values to an increase.

Angle classification was identified at the left and right cuspids and first molars of the maxilla and mandible. When cuspids or first molars were missing or damaged, outcomes were listed as indefinable. Class I was identified as neutro-occlusion, class II as disto-occlusion, and class III as mesio-occlusion. Differences in Angle classification between pretreatment and 2- or 10-year follow-up models were listed as a mesial or distal shift in occlusion.

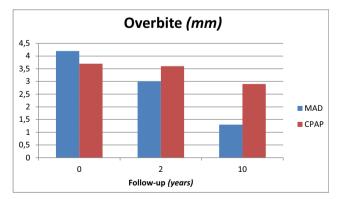


Fig. 2 Overbite of MAD (mandibular advancement device) and CPAP (continuous positive airway pressure) treatment at baseline 2-year and 10-year follow-up

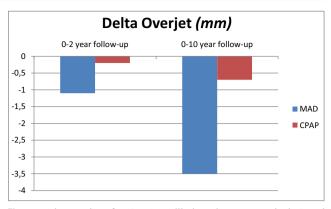


Fig. 3 Delta overjet of MAD (mandibular advancement device) and CPAP (continuous positive airway pressure) treatment

Statistical analysis

Statistical analyses were performed using the Statistical Package for the Social Sciences (version 24, SPSS Inc., Chicago, IL, USA). Means and standard deviations were reported. For comparing outcomes between continuous variables between baseline 2- and 10-year follow-up, ANOVA analyses were performed. For comparing categorical variables, Chi-square tests were performed. In all cases a significance level $\alpha = 0.05$ was used.

Results

At the 2-year follow-up, 29 MAD and 37 CPAP patients were analyzed and included in the study by Doff et al. [6]. At 10year follow-up, 18 MAD patients and 24 CPAP patients were still using their therapy. Eventually, 14 out of 18 MAD and 17 out of 24 CPAP patients were willing to return for evaluation and could subsequently be included in this 10-year follow-up study. The mean age of the patients at baseline was 50.2 years

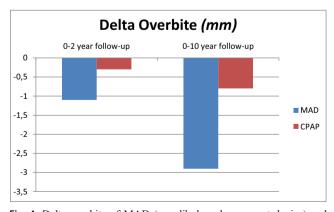


Fig. 4 Delta overbite of MAD (mandibular advancement device) and CPAP (continuous positive airway pressure) treatment

 $(\pm\,8.1)$ in the MAD group and 49.4 years $(\pm\,10.3)$ in the CPAP group.

At the 10-year follow-up, 12 out of 14 (85.7) MAD patients were defined as successful (AHI < 5 or AHI < 20 with > 50% reduction in AHI). One patient switched to CPAP therapy, yielding an AHI of 1.3. The other patient was diagnosed with central sleep apnea syndrome. At the 10-year follow-up, 16 out of 17 (94.1%) CPAP patients were defined as successfully treated. The mean AHI in both groups dropped significantly between the baseline and the 10-year follow-up. The AHI dropped from 31.7 ± 20.6 to 9.9 ± 19.3 in the MAD group and from 49.2 ± 26.1 to 3.4 ± 5.4 in the CPAP group.

When evaluating therapeutic use of the appliances, no significant differences were found at the 2-year follow-up. Both devices were used on average 7 days a week and 7 h a night. At 10-year follow-up a significant difference (p < 0.05) was found between the hours/night use of the appliances. The MAD was used 7.8 ± 0.9 h/night and the CPAP was used 6.8 ± 0.9 h/night. The mean mandibular protrusion of the mandibular protrusion.

Study model analysis

When evaluating the number of present teeth, no significant differences were found between the 0 and 10-year follow-up in each group or between the MAD and CPAP group. However, the delta overbite and delta overjet did differ significantly between the 2 and 10-year follow-up in each treatment group and between the treatment groups. When evaluating the delta overbite, there was a tendency towards the occurrence of an open bite, especially in the MAD treatment group. The overbite in the MAD group reduced on average 2.9 ± 1.5 mm in 10 years. In the CPAP group, the reduction in overbite was 0.8 ± 1.4 mm in 10 years. These values differed significantly (p < 0.05). When evaluating the delta overjet, there was a tendency towards a reduction of the overjet to a mesio-occlusion. The overjet in the MAD group reduced on

Table 2 Cuspid occlusion at baseline and follow-up, MAD group

average 3.5 ± 1.5 mm in 10 years. In the CPAP group, the reduction was 0.7 ± 1.5 mm in 10 years. These values also differed significantly (p < 0.05).

In the MAD group, the Angle classification of the teeth in the cuspid region at the 10-year follow-up could be determined in 12 patients on the left side and in 8 patients on the right side. In 12 out of 12 on the left side and 6 out of 8 on the right side, the Angle classification did change in the MAD group. In the CPAP group, the Angle classification of the teeth in the cuspid region at the 1-year follow-up could be determined in 14 patients on the left side and 11 patients on the right side. In 8 out of 14 on the left side and 6 out of 11 on the right side, the Angle classification did change in the CPAP group. The proportion of Angle class III cases increased in both treatment groups over time (Tables 2, 3, 4, and 5).

In the MAD group, the Angle classification of the teeth in (pre)molar region at the 10-year follow-up could be determined in 8 patients on the left side and 8 patients on the right side. In 6 out of 8 on the left side and 7 out of 8 on the right side, the Angle classification did change in the MAD group. In the CPAP group, the Angle classification of the teeth in (pre)molar region at the 10-year follow-up could be determined in 10 patients on the left side and 7 patients on the right side. In 5 out of 10 on the left side and 4 out of 7 on the right side, the Angle classification did change in the CPAP group. The proportion of Angle class III cases increased over time, especially in the MAD group (Tables 4 and 5).

Discussion

This study compared the dental side effects of two major OSA therapies after a decade of treatment. To date, no studies have been published regarding the development of dental side effects comparing MAD and CPAP therapy over a 10-year treatment period. From earlier performed research, it is known that CPAP, but especially, MAD therapy may result in dental side effects [17–19, 22]. However, it is unknown whether these

Angle classification	Mandibular advancement device (MAD) group										
	Right-side cuspid occlusion					Left-side cuspid occlusion					
	Baseline $n = 14$	• •	10-year follow-up $n = 14$	Unchanged $n = 5$	Baseline $n = 14$	• •	10-year follow-up $n = 14$	Unchanged $n = 1$			
Class I	1	1	1	0	4	1	3	0			
Class II	8	9	4	2	7	8	6	0			
Class III	0	1	3	0	0	0	3	0			
Indefinable	5	3	6	3	3	5	2	1			

Angle classification	Continuous positive airway pressure (CPAP) group										
	Right-side cuspid occlusion					Left-side cuspid occlusion					
	Baseline $n = 17$, 1	<i>y</i> 1	0		<i>v</i> 1	10-year follow-up $n = 17$	Unchanged $n = 7$			
Class I	3	3	3	2	5	3	6	2			
Class II	6	6	8	3	10	10	6	4			
Class III	0	0	0	0	0	0	2	0			
Indefinable	8	8	6	5	2	4	3	1			

 Table 3
 Cuspid occlusion at baseline and follow-up, CPAP group

side effects are progressive over time with both treatments. Because MAD and CPAP therapy are considered a lifelong requisite, attention must be paid to their dental side effects. In this study, the cast models of MAD and CPAP patients were evaluated at baseline, 2-year and 10-year follow-up. It was observed that both MAD and CPAP therapy resulted in significant dental changes with long-term use. However, the dental changes in the CPAP group were less pronounced when compared with the MAD group. In addition, the dental changes in both treatment groups were progressive over time.

Inter-individual treatment variations are likely to occur in a 10-year follow-up study. In this study, the margin of interindividual error was maintained as small as possible by treating all patients in this cohort by the same clinician. Furthermore, all measurements were done by a skilled and trained professional blinded for the treatment assignment.

One of the inclusion criteria was to use the appliance at least 5 days a week and 5 h a night. This results in a treatment group of highly compliant patients. The average treatment use in this study was 7 days per week for the MAD and CPAP group, 8 h per night for the MAD group and 7 h per night for the CPAP group. It could be suggested that highly compliant patients, such as the patients in this treatment group, also display more side effects. It must however be noted that patients on average did not grade the dental side effects as negative or disturbing. Patients whom are less compliant are possibly less susceptible to dental changes but are more likely to experience continued OSA symptomatology due to a suboptimal therapeutic effect.

When evaluating the delta overbite and overjet, it was notable that over time, there was a reduction of both with a tendency towards an Angle class III, sometimes resulting in an end-to-end frontal occlusion [17, 18, 23, 24]. This difference was especially prominent in the MAD treatment group. The reduction in overbite and overjet in the MAD group probably resulted from the pressure, transferred by the appliance. The maxillary incisors were subjected to a palatal directed force and the mandibular incisors were subjected to a labial directed force. Because of these forces, there was a tendency towards an anterior open bite. However, in the CPAP group, there was also a significant reduction of the overbite and overjet [20]. This reduction may occur as a result of pressure of the (nasal) mask on the frontal part of the maxilla, which may result in a more palatal inclination of the maxillary incisors. Previous research regarding natural movement of teeth and change of overbite and overjet over time showed that, in general population, there are changes; however, they are insignificant [25]. This suggests that, in this study, the changes in both groups are a result of the therapy. Also, a posterior open bite was observed in many of the patients in both the

Angle classification	Mandibular advancement device (MAD) group										
	Right-side (pre)molar occlusion					Left-side (pre)molar occlusion					
	Baseline $n = 14$	<i>v</i> 1	10-year follow-up $n = 14$	U	Baseline $n = 14$	5 1	10-year follow-up $n = 14$	Unchanged $n = 4$			
Class I	0	2	2	0	1	1	1	0			
Class II	9	9	2	1	8	9	4	2			
Class III	0	1	4	0	0	1	3	0			
Indefinable	5	2	6	2	5	3	6	2			

 Table 4
 Molar occlusion at baseline and follow-up, MAD group

Angle classification	Continuous positive airway pressure (CPAP) group										
	Right side (pre)molar occlusion					Left side (pre)molar occlusion					
	Baseline $n = 17$	<i>v</i> 1	10-year follow-up $n = 17$	0		5 1	10-year follow-up $n = 17$	Unchanged $n = 8$			
Class I	1	2	0	0	2	3	1	1			
Class II	7	6	7	3	6	8	7	4			
Class III	1	1	0	0	0	0	2	0			
Indefinable	8	8	10	7	9	6	7	3			

 Table 5
 Molar occlusion at baseline and follow-up, CPAP group

CPAP and MAD group. Quantification of changes in posterior support was however difficult since in many patients a determination of the number of posterior contact points was not reliable at every time-point. For this reason, statistics in these parameters were not included in the analyses of the present study. When evaluating the difference in delta overbite and overjet, it was notable that in some patients these changes are larger than in other patients. This could possibly be explained by the position of the mandible. Previous research regarding dental changes in MAD treatment showed that the higher the percentage of mandibular protrusion the larger the changes [7, 26]. Besides, it is hypothesized that changes will be larger in patients, who have fewer teeth and reduced periodontal and dental health. However, from this study, this cannot be concluded because the periodontal health was not scored other than the fact whether a patient was suitable or not for inclusion.

Between the baseline to 2-year and the 2-year to 10-year follow-up, the changes in dental occlusion appear to decrease in magnitude. The alteration in overjet and overbite therefore seems to decrease over the years. However, these changes are still significant. The stabilization of the dental side effects can be explained by the fact that most patients in this study did not alter the protrusion of their appliance after the 2-year follow-up. Besides, in a general population, dental changes such as a reduction of overjet and overbite are also observed [25].

The total number of teeth per jaw was stable over the 10year follow-up. It was expected that patients would lose more teeth as a result of tooth decay or periodontal problems, especially in a group where much more force is applied to the teeth as a result of CPAP and especially MAD therapy [27]. However, there was no significant decrease in the number of teeth per jaw measured after a 10-year treatment period. This could possibly be explained by a higher dental self-care in OSA patients because they are constantly faced with their dental health and repeatedly explained that is vital in OSA treatment.

It can be concluded that there are dental side effects associated with long-term MAD therapy. To keep these side effects as small as possible, attention must be paid to an adequate selection of suitable patients. First of all, attention must be paid to dental and periodontal health. Secondly, attention must be paid to the number of healthy teeth in each jaw (c.q. at least 8 teeth). Thereby, possible dental side effects will be kept to a minimum. If changes are unfavorable or too prominent, an alternative intervention should be considered. Conversely, it should also be noted that CPAP therapy may also result in changes in dental occlusion [25]. However, these changes are less pronounced when compared to MAD therapy on the long term. Attention must be paid to the clinical relevance of these changes. It can be concluded that patients are satisfied with their treatment if they complete 10-year follow-up, so the observed dental side effects are unlikely to be a complicating factor in daily life. Besides, factors such as periodontal health and vertical skeletal characteristics were not taken into account and could possibly be responsible for the observed differences.

It can be concluded that MAD and CPAP therapy in this RCT both resulted in significant dental changes with longterm use. It must be kept in mind that OSA usually requires lifelong treatment. Therefore it is important to pay attention to the possible dental side effects and to discuss this with the patient before initiation of therapy and at every checkup visit if these changes are observed.

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Compliance with ethical standards

Conflict of interest Aarnoud Hoekema is a medical advisor for Airway Management Inc., Somnomed and Zephyr Sleep Technologies. All other authors do not have any conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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