

# IMPACT OF OCCLUSAL SCHEMES ON PROGNOSIS OF CONVENTIONAL COMPLETE DENTURES IN PATIENTS WITH SEVERELY RESORBED ALVEOLAR RIDGES

Gabriela Mayara Oliveira NASCIMENTO<sup>1</sup> , Wender Batista de SOUZA<sup>2</sup> , Germana de Villa CAMARGOS<sup>3</sup> , Maira Foresti Vieira MUNHOZ<sup>4</sup> , Eduardo Henrique da SILVA<sup>2</sup> , André Gustavo PALEARI<sup>5</sup> 

<sup>1</sup>Private Practice, Alfenas, Minas Gerais, Brazil.

<sup>2</sup>Masters Student at the Postgraduate Program in Integrated Dental Clinic, Dental School, Universidade Federal de Uberlândia, Uberlândia, MG, Brazil.

<sup>3</sup>Department of Removable Prosthodontics, Dental School, Universidade Federal de Uberlândia, Uberlândia, Minas Gerais, Brazil.

<sup>4</sup>Department of Removable Prosthodontics, Dental School, Universidade Federal de Alfenas, Alfenas, Minas Gerais, Brazil.

<sup>5</sup>Department of Prosthodontics, Dental School, Universidade de Franca, Franca, São Paulo, Brazil.

## Corresponding author:

Germana de Villa Camargos  
germana.camargos@ufu.br

**How to cite:** NASCIMENTO, G.M.O., et al. Impact of occlusal schemes on prognosis of conventional complete dentures in patients with severely resorbed alveolar ridges. *Bioscience Journal*. 2024, **40**, e40039. <https://doi.org/10.14393/BJ-v40n0a2024-72612>

## Abstract

Bilateral Balanced Occlusion (BBO) and Canine Guidance (CG) are occlusal schemes that have been successfully used in complete dentures (CDs). The objective of this study was to evaluate the effects of CG compared to BBO on satisfaction, oral health-related quality of life (OHRQoL) and masticatory performance in CDs wearers with severe resorbed alveolar ridge by means of a crossover trial. Sixteen edentulous patients with severe resorbed ridges were treated with new maxillary and mandibular CDs. After adaptation period, they were randomly divided into two groups according to a sequence of occlusal schemes: BBO for 30 days followed by CG for 30 days or, CG for 30 days followed by BBO for 30 days. Patient satisfaction with their dentures, OHRQoL and masticatory performance were assessed immediately 30 days after of using each occlusal scheme. The results of satisfaction and quality of life were using Wilcoxon test, and data regarding masticatory performance were analyzed through paired t-test ( $\alpha = .05$ ). The occlusal scheme used in complete dentures had no significant influence on general patient satisfaction ( $p = 0.06$ ), overall quality of life ( $p = 0.09$ ), or masticatory performance of patients ( $p = 0.182$ ). Both BBO and CG can be considered as suitable for oral rehabilitation with CDs in patients with severe resorbed alveolar ridges. However, CG may be considered as a scheme of choice by clinicians due to its greater simplicity and shorter execution time.

**Keywords:** Bone Resorption. Complete Denture. Dental Occlusion. Patient Satisfaction. Quality of life.

## 1. Introduction

Conventional complete dentures (CDs) are still an important treatment option to rehabilitate the oral functions of edentulous people, as a considerable part of this population cannot afford prosthetic treatment with dental implants (Leles et al. 2019). Despite the mucosal support, adequate outcomes related to aesthetics and biomechanics are perfectly achievable with CDs (Leles et al. 2019). However, the choice of occlusal schemes used in CDs seems to influence the clinical performance of those prostheses and patient satisfaction, which are critical factors for the success of this treatment (Borges et al. 2023).

Occlusal schemes for CDs can be broadly categorized into balanced occlusion, which includes lingualized occlusion and bilateral balanced occlusion (BBO), and nonbalanced occlusion, which includes canine-guided occlusion (CG) (Goldstein et al. 2021). While different occlusal schemes for CDs are available to select from, there is no consensus in the literature about the ideal one (Carlsson 2009; Lemos et al. 2018; Borges et al. 2023). Despite the lack of scientific evidence about the superiority of one occlusal scheme over another, BBO and CG schemes have been commonly adopted in clinical practice (Farias-Neto et al. 2010; Paleari et al. 2012) with a major preference given to the BBO concept due to its possible role in denture stability during eccentric movements (Moradpoor et al. 2020). On the other hand, CDs with CG mimic the occlusal pattern found in dentate patients (Paleari et al. 2012; Schierz and Reissmann, 2016; Pero et al. 2019), resulting in lower muscle activity in CDs wearers (Grubwieser et al. 1999). Furthermore, CG is straightforward to develop and makes CDs fabrication easier compared with BBO (Srinivasan et al. 2023).

Despite the practical differences between BBO and CG, previous studies did not find any significant differences between these two occlusal schemes on masticatory performance (Farias-Neto, 2010), satisfaction (Paleari et al. 2012) and oral health-related quality of life (Schierz and Reissmann 2016) when patients presented good residual bone ridges. In these situations, the remaining bone volume can provide adequate support and retention to CDs, which could have masked the real effect of occlusion on CDs success (Goldstein et al. 2021).

Nevertheless, the biggest problem facing patients wearing CDs is the progressive loss of alveolar bone, especially in mandible (Tallgren 1972). Severe ridge resorption interferes in CDs clinical performance (Goldstein et al. 2021; Borges et al. 2023). In this critical clinical situation, the type of occlusal scheme used in CDs might play an important role on dentures stability and retention, affecting patient's satisfaction, quality of life and masticatory function (Goldstein et al. 2021; Borges et al. 2023). Previous studies have shown that lingualized occlusion scheme promoted improved patient satisfaction (Kawai et al. 2017) and masticatory performance (Matsumaru 2010) compared to BBO in patients with severely resorbed mandibular ridges. However, there is still a lack of scientific evidence regarding the effect of BBO or CG on the prognosis of conventional CDs in patients with severely resorbed alveolar ridges (Goldstein et al. 2021; Borges et al. 2023). Therefore, this study aimed to evaluate the impact of two occlusal schemes used in CDs, BBO or CG, on satisfaction, oral health-related quality of life and masticatory performance of edentulous patients with severely resorbed alveolar ridges.

## **2. Material and Methods**

### **Study design**

This manuscript reports the results of a crossover trial comparing different occlusal schemes used in complete dentures for rehabilitation of edentulous patients with severely resorbed alveolar ridges. The ethical approval was obtained from the Institution' Ethics Committee (CAAE: 53466516.4.0000.5142) and the study was also registered in the Brazilian Registry of Clinical Trials ([ensaiosclinicos.gov.br](http://ensaiosclinicos.gov.br), identification code UTN: U1111-1184-6412).

It was assessed for eligibility 66 individuals who had been referred to the Prosthodontic Clinic of the Department of Restorative Dentistry, Dental School, Federal University of Alfenas, Brazil. The following inclusion criteria were applied: complete edentulism in the maxillary and mandibular arches for at least one year, receptiveness and mental agility, ability to return for recall appointments or follow-up visits, good overall health and residual edentulous ridges classified as severely resorbed. Residual ridges were considered severely resorbed when the mandibular bone height, measured on panoramic radiographs at its least vertical height, was less than 20 mm (Kawai et al. 2017). Patients with uncontrolled systemic diseases, temporomandibular disorders and a need for pre-prosthetic surgery were excluded.

### **Assessment of mandibular bone height from panoramic digital radiographs**

All panoramic digital radiographs were obtained using EAGLE X-ray apparatus (Dabi Atlante, Ribeirão Preto, SP, Brazil) with a magnification factor of 20% and saved in TIFF format. Subsequently, the radiographic

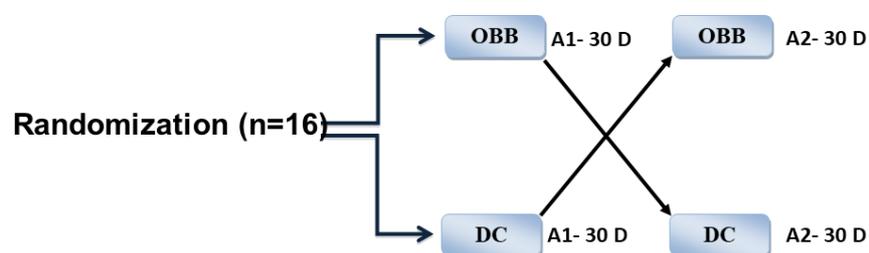
images were exported to Radioimp software (Radio memory®, Belo Horizonte, Minas Gerais, Brazil), where calibrations for magnification and resolution rates were performed before measurements. Then, mandibular bone height was measured on panoramic radiographs at the least vertical height of the mandible and, patients were categorized into four distinct classes according to criteria defined by the American Academy of Prosthodontics (McGarry et al. 1999). Patients with mandibular bone height less than 20 mm, classified as severely resorbed ridges (Kawai et al. 2017), were included in this study. All radiographic evaluations were performed by a single examiner. As a result of radiographic evaluation, sixteen individuals who met the inclusion criteria were invited to participate in this clinical trial and, written consent was obtained prior to enrollment.

### Fabrication of new complete dentures

Participants of this study received one set of new complete dentures fabricated according to the conventional method used in Alfenas Dental School, which involved the following steps: (1) Preliminary impression using aluminum stock trays and irreversible hydrocolloid (Jeltrate, Dentsply Ind. e Com. Ltda., Petrópolis, RJ, Brazil). Trays were previously individualized with warm utility wax trips (Wilson, Polidental Indústria e Comércio Ltda., Cotia, SP, Brazil) and shaped using tongue movements and manipulation of labial and buccal soft tissues. (2) Final impression using custom acrylic resin tray and elastomeric impression materials, following the selective pressure technique. (3) Occlusal plane orientation and maxillomandibular relationships were established using record bases. The position of maxillary rim was transferred to a semi-adjustable articulator using face-bow. The CDs received anatomic teeth with cuspal inclination of 33°, set according to the BBO concept. (4) Denture try-in performed after anterior teeth arrangement. (5) Second denture try-in conducted after arranging of posteriors teeth. The complete dentures were polymerized in water bath and subsequently reassembled in semi-adjustable articulator for occlusal adjustment. (6) Delivery of conventional CDs. The fabrication of new prostheses was performed by undergraduate students under the supervision of Dental Prosthesis Professors with more than 10 years of clinical experience. After the placement of new CDs, denture bases and occlusion were adjusted weekly, if necessary, during a period of 30 days to allow for functional adaptation prior to experimental procedures (Paleari et al. 2012; Leles et al. 2003).

### Randomization process and evaluation periods

Sixteen patients were enrolled and randomized using the lottery method with opaque envelopes into one of the occlusal schemes' sequences (BBO-CG or CG-BBO) (Figure 1). Eight participants initially received CG, while the other eight continued with BBO until the first outcome assessment.



**Figure 1.** The participant flow diagram of the randomized controlled trial.

In the BBO-CG group, a sham procedure was performed by adding composite resin (Z250, 3M, Sumaré, SP – Brazil) to the palatal surface of the upper canines at cervical region, to blind the sample, without altering centric and excursive contacts. After 30-days period, the first outcome assessment was conducted, and the occlusal scheme was changed to CG. Canine guidance establishment was performed by the same researcher using the method described by Paleari et al. (2012), which involved adding composite resin to the palatal surface of the upper canines. The resin should be extensive enough to promote canine

disocclusion without causing interference in centric occlusion. After 30-days period, the second outcome assessment was conducted (Paleari et al. 2012).

In the CG-BBO group, canine guidance was established initially. After 30-days period, the first outcome assessment was conducted. Afterwards, the composite resin was removed, and the bilateral balanced occlusion was restored according to sequence. Each occlusal scheme remained for 30 days, after that period, denture satisfaction, oral health-related quality of life and masticatory performance were evaluated.

### **Denture satisfaction**

Patient satisfaction with CDs was evaluated using a questionnaire adapted from Celebić; Knezović-Zlatarić (2003), which has been previously used in trials by Souza et al. (2012) and Paleari et al. (2012). The questionnaire considered eight items as follow: comfort of wearing mandibular and maxillary dentures, retention of maxillary and mandibular dentures, aesthetics, speech, chewing and general satisfaction. Possible answers for each question and their respective scores were as follows: (A) unsatisfactory (scored as "0"), (B) regular (scored as "1"), or (C) good (scored as "2"). For each evaluation, the sum of the scores ranged from 0 to 16, where lower values indicated dissatisfaction with dentures and higher values represent a higher level of satisfaction. The questionnaire also was administered by a researcher who had been blinded to all the other procedures involved in this trial.

### **Oral Health Related Quality of Life Assessment (OHRQoL)**

The Brazilian version of the OHIP-EDENT (de Souza et al. 2007; de Souza et al. 2010; de Souza et al. 2012) was used to evaluate OHRQoL in this study. This instrument contains four conceptual domains in the following areas: masticatory-related complaints, psychological discomfort and disability, social disability, and oral pain and discomfort. Responses were recorded using a 3-point Likert-type scale: 0 (never), 1 (sometimes), or 2 (almost always). The overall OHIP-EDENT score was calculated by summing the scores of each question, ranging from 0 to 38. Higher scores indicated poor OHRQoL. The same researcher, who was blinded to the other phases of the study, administered all questionnaires.

### **Masticatory performance assessment**

Masticatory performance was assessed using the sieve method (Wayler and Chauncey 1983; Demer et al. 1996; Schneider and Senger 2001; Kapur and Soman 2004; de Oliveira Junior et al. 2014). Participants were asked to chew 5 almonds deliberately for 20 chewing strokes. A single calibrated operator counted the chewing cycles and collected the comminuted particles in a container. After, each participant received 50 ml of water to rinse their mouth and remove remaining particles, which were also collected in container. The contents were then poured onto a sieve (sieve size 1-7 cm, dimensions 175 x 78 x 40 mm) adapted with a filter paper to separate liquid and chewed material. 500 ml of water were poured onto the sieve to remove saliva from the almonds and reduce clumping of particles.

The crushed almonds were dehydrated in an electric oven (Fanem, Guarulhos, SP - Brazil) at controlled temperature of 130°C for 40 minutes. The content was sieved using a 4-sieve series, under constant vibration for 60 seconds on a gypsum vibrator. The sieves used (Granutest, Telastem, Bom Retiro, SP, Brazil) had different hole sizes: 4.0 mm (ABNT 5), 2.8 mm (ABNT 7), 2.0 mm (ABNT 10) and 1.0 mm (ABNT 18). The crushed almonds, after dehydration, were weighed on a precision balance (Ind. e Com. Eletrônica Gehaka Ltda., São Paulo, SP – Brazil), and the values were recorded. Masticatory performance was calculated as the weight of comminuted material that passed through the 2.8 mm sieve. From these values, masticatory performance was calculated based on index proposed by Kapur, Soman (2004):  $MP = P1 \times 100/Pt$ . Where: MP represents masticatory performance (in percentage); P1 denotes the sum of material weights in sieves 3, 4 and background collector; Pt corresponds to the total material weight subjected to sieving. This evaluation was conducted by one researcher blinded to all other procedures of this study to mitigate potential biases.

## Statistical analysis

Statistical analysis was carried out using the IBM SPSS Statistics program (version 19.0). Between-group comparisons for denture satisfaction and OHRQoL data were performed using Wilcoxon test, while Student t-test was used for masticatory performance data. All analyses were performed with a significance level of 5%.

## 3. Results

### Subjects baseline characteristics

Table 1 shows the baseline characteristics of the study population. The patients' mean age was 67.5 years, ranging from 50 to 80 years, and all patients were female. The edentulism period varied from 1 to 50 years, with mean values of  $28.9 \pm 17.7$  and  $26.5 \pm 18.7$  years for the maxillary and mandibular arches, respectively. The mandibular complete dentures wearing time was  $22.6 \pm 19.3$  years. The lowest mandibular height found was 9.8 mm in the BBO-CG group and 14 mm in the CG-BBO group.

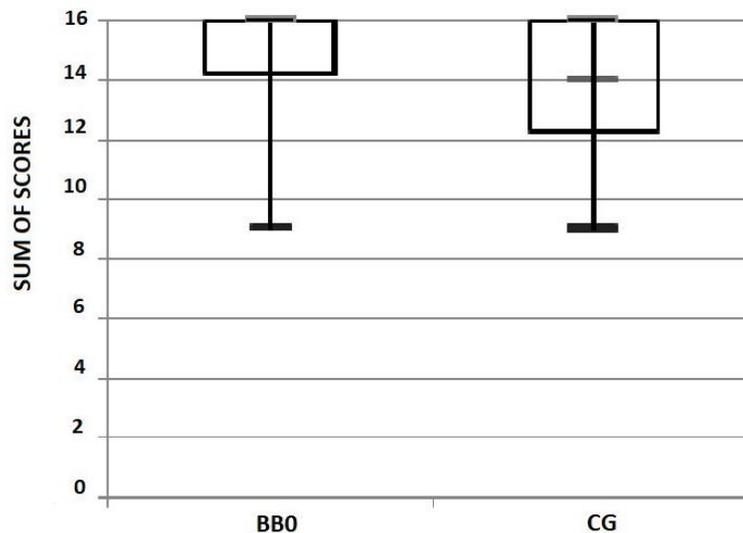
**Table 1.** Demographic characteristics of the study sample.

Characteristics	Group		All	All %
	BBO-CG	CG-BBO		
<b>Age (years), mean <math>\pm</math> SD<sup>a</sup></b>	69.3 $\pm$ 6.3	65.8 $\pm$ 9.4	67.5 $\pm$ 7.9	
<b>Gender, n (%)</b>				
Female	6	6	12	100%
Male	0	0	0	-
<b>Edentulous period (years), mean <math>\pm</math> SD<sup>a</sup></b>				
Maxilla	36.1 $\pm$ 16.8	21.6 $\pm$ 16.7	28.9 $\pm$ 17.7	
Mandible	36.1 $\pm$ 16.8	16.8 $\pm$ 16.2	26.5 $\pm$ 18.7	
<b>Prior use of denture (years), mean <math>\pm</math> SD<sup>a</sup></b>				
Maxilla	35.8 $\pm$ 16.5	20 $\pm$ 15.3	27.9 $\pm$ 17.3	
Mandible	35.8 $\pm$ 16.5	9.4 $\pm$ 11.4	22.6 $\pm$ 19.3	
<b>Professional activity, n (%)</b>				
Retiree	4	1	5	41.65%
Pensioner	0	0	0	-
Housewife	2	3	5	41.65%
Unemployed	0	0	0	-
Employee	0	2	2	16.7%
<b>Marital status (n)</b>				
Married	2	6	8	66.6%
Single	0	0	0	-
Divorced	2	0	2	16.7%
Widower	2	0	2	16.7%
Cohabiting	0	0	0	-
<b>ACP Classification, n (%)</b>				
Classe I: $\geq$ 21mm	0	0	0	-
Classe II: 16-20mm	3	3	6	50%
Classe III: 11-15mm	2	3	5	41.65%
Classe IV: $\leq$ 10mm	1	0	1	8.35%

<sup>a</sup>Standard deviation.

## Denture satisfaction

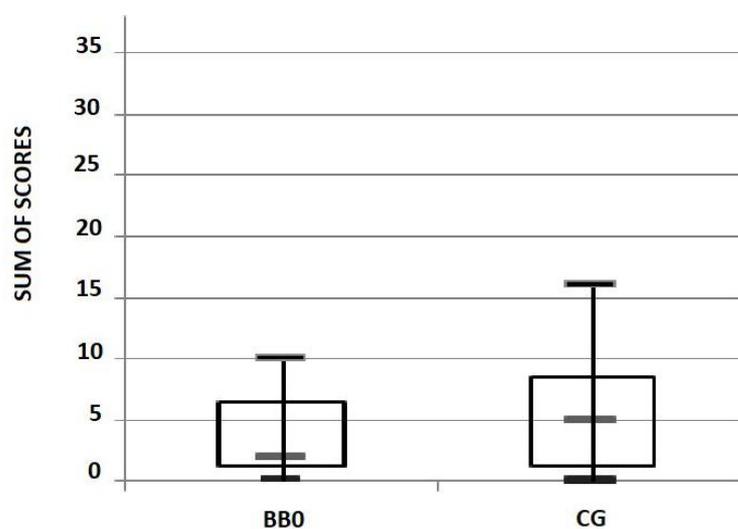
It was observed that there was no statistically significant difference in general satisfaction among wearers of CDs with BBO or CG (Wilcoxon's test,  $p = 0.06$ ) (Figure 2). The range of the data, represented by the maximum and minimum sum of the scores ranged from 9 to 16 for both groups. The median, which represents the center of the data distribution, was 16 for BBO and 14 for CG, indicating no significant difference.



**Figure 2.** Box-plot graph comparing the general satisfaction of CDs wearers with BBO or CG.

## Oral Health-related Quality of Life

The box-plot graph (Figure 3) shows the distribution of scores for the general quality of life of patients with severely resorbed ridges, considering the influence of the occlusion of the CDs. It was observed that the occlusion scheme did not significantly influence ( $p = 0.09$ ) the general quality of life of patients with resorbed ridges. It is also possible to observe a larger range of the data for the CG (0 to 16) compared to the BBO (0 to 10). The median was 2 for BBO and 5 for CG, indicating a more symmetric distribution of the data for CG and a distribution that tended to be more positively skewed for BBO. Thus, a higher concentration of responses for both groups may be observed for the "never" option, indicating a positive treatment effect on patients' perceived quality of life, regardless of the type of occlusion.



**Figure 3.** Box-plot chart comparing the general quality of life of CDs wearers with resorbed alveolar bone ridges.

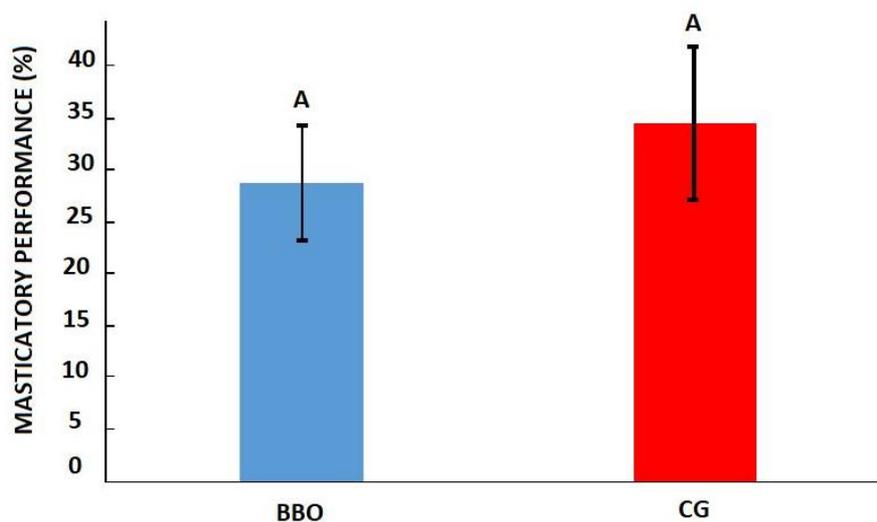
Table 2 shows the mean values of the scores for each of the four domains of the OHIP-Edent questionnaire in both groups evaluated, and the result of the comparison between the scores of each domain using Wilcoxon test. It was observed that the occlusal schemes also did not influence the quality of life of patients when considering the four domains: masticatory discomfort and disability (D1,  $p = 0.37$ ); psychological discomfort and disability (D2,  $p = 0.39$ ); social disability (D3,  $p = 1$ ) oral pain and discomfort (D4,  $p = 0.78$ ).

**Table 2.** Mean (Standard Deviation) of Oral Health-related Quality of Life.

Group	Domain 1	Domain 2	Domain 3	Domain 4
BBO	1.45 ± 1.9	0.73 ± 1	0 ± 0	1.55 ± 1.1
CG	2.09 ± 2.9	1.27 ± 1.3	0.09 ± 0.3	1.73 ± 1.5
p	0.37	0.39	1	0.78

### Masticatory Performance

Figure 4 shows the means and standard deviation of the masticatory performance. It was observed that there was no statistically significant difference in the masticatory performance of patients with resorbed alveolar ridges using BBO or CG CDs (Student's t-test,  $p = 0.18$ ). The masticatory performance was  $28.7 \pm 5.4\%$  for the BBO group and  $34.4 \pm 7.4$  for the CG group.



**Figure 4.** Masticatory performance of patients with resorbed alveolar ridge using CDs with BBO or CG occlusal schemes (mean and standard deviation%).

### 4. Discussion

The progressive resorption of the residual mandibular bone after tooth loss can lead to issues associated to retention and stability of CDs, and consequently, to complaints of pain and/or discomfort (Tallgren 1969; 1972; Allen and McMilan 2003; Huuonen et al. 2012), resulting in the failure of treatment with lower dentures. To improve the treatment outcomes with CDs in cases of severely resorbed alveolar bone ridges, factors critical to dentures stability (Schierz and Reissmann 2016; Pero et al. 2019), such as occlusal schemes (BBO or CG), such as occlusal schemes were evaluated in this study.

Few studies have evaluated the influence of occlusal schemes used in CDs on denture satisfaction, OHRQoL and masticatory performance of edentulous patients with severely resorbed alveolar ridges (Kimoto et al. 2006; Kawai et al. 2017; Pero et al. 2019). Previous studies reported that alternative occlusal schemes to BBO, such as lingualized occlusion (LO), result in higher satisfaction, quality of life (Kimoto et al. 2006; Kawai et al. 2017) and masticatory performance (Matsumaru 2010) in CDs wearers with severely

resorbed ridges. However, no randomized clinical trial has evaluated the efficacy of BBO compared to CG in patients with severely resorbed alveolar ridges (Borges et al. 2023).

Regarding denture satisfaction, the results showed no significant difference ( $p = 0.06$ ) between BBO and CG. Both occlusal schemes provided high levels of denture satisfaction, even in patients with severely resorbed ridges. Paleari et al. (2012) evaluated the influence of these occlusal schemes on denture satisfaction in patients with adequate bone volume, also found similar results, although more stability of the maxillary denture during chewing was associated to CG (Paleari et al. 2012). Thus, besides dentures functional quality and gender, other factors are more crucial in the patients' final judgment of treatment, such as their attitude towards dentures, the number of previous dentures, personality, expectations, and patient-dentist relationship, rather than the type of occlusal scheme used (Huumonen et al. 2012).

Patients' satisfaction with their CDs is a crucial factor for treatment success and is strongly associated with oral health-related quality of life (Hassel et al. 2007; Rehmann et al. 2008; Gjengedal et al. 2011; Srinivasan et al. 2023). In this study, similar to the results of denture satisfaction, there was no significant influence ( $p = 0.09$ ) of the type of occlusal scheme on OHRQoL. Schierz and Reissmann (2016) found that both occlusal concepts, BBO or CG, had similar effects on OHRQoL of patients with adequate bone volume. Patients with severely resorbed alveolar ridges may require longer adaptation period with their dentures, approximately six months, compared to patients with normal mandibular bone height (Kawai et al. 2017). It is speculated that after this adaptation period, differences in the comparison between occlusal schemes in terms of satisfaction and quality of life could more apparent (Marcello-Machado et al 2017). However, future studies using similar methodologies and longer evaluation periods are necessary to clarify this point.

Despite the similar levels of satisfaction and quality of life among BBO and CG in CDs wearers, BBO, defined by simultaneous occlusal contacts during centric and eccentric movements, has been considered superior due to its purported improvement in denture stability and retention, thereby enhancing masticatory performance (Zarb et al. 2003). However, in this study, CG the occlusal scheme was superior to BBO when masticatory performance was evaluated after a 30-days period, although the results were not statistically significant ( $p = 0.18$ ). Previous studies evaluating occlusal schemes alternative to BBO have reported significant advantages of lingualized occlusion (LO) occlusal scheme compared to BBO, both in objective (Matsumaru 2010) and subjective (Kawai et al. 2017) parameters related to mastication in patients with severely resorbed alveolar ridges after six months of follow-up. Similar to CG, LO reduces the incidence of oblique forces on dentures during function compared to BBO (Phoenix et al. 2010), resulting in greater stability of the mandibular dentures and consequently, less trauma and pressure that could accelerate the resorption of the alveolar bone ridge. For this reason, patients feel more comfortable while chewing (Pero et al. 2019), especially during the aging process, when oral changes can make chewing and swallowing more difficult (Streicher et al. 2018; Muniz et al. 2022).

Furthermore, additional advantages have been associated with CG, such as reduced muscle activity, which helps to prevent the development of temporomandibular disorders in CD wearers. Grubwieser et al. (1999), through electromyographic analyzes, found that patients with BBO complete dentures exhibited higher muscle activity, particularly in the masseter muscle during protrusion and lateroprotusion movements. In addition, Peroz et al. (2003) confirmed that dentures with CG were more stable during eccentric movements compared to BBO, resulting in improved retention and stability of mandibular dentures. Given that both occlusal schemes provide similar levels of satisfaction, quality of life and masticatory performance, CG may be considered the preferred choice by clinician due to its simplicity and shorter execution time.

The reabsorption of mandibular edentulous bone ridge has been extensively studied by various authors, resulting in several methods for classifying residual bone based on its height (Wical et al. 1974; Lekholm and Zarb 1985; Cawood and Howell 1988; McGarry et al. 1999). Each classification method focuses on different aspects of the mandible, leading to some discrepancies among them. The method proposed by ACP was chosen for this study due to its ease and clarity on panoramic radiographs, allowing for the classification of mandibular residual bone status based on the minimum vertical height of the mandible (Leles et al. 2003). Since this method is based on diagnostic findings that assist practitioners in determining appropriate treatments for their patients, the present study aligns this classification with the research of Kawai et al. (2017), who categorized mandibular bone resorption as severe if the lowest mandibular height

measures less than 20mm. Additionally, according to ACP guidelines, Class II (lower mandibular bone height between 16-20 mm) indicates ongoing physical degradation of the prosthesis support area, signifying marked resorption (McGarry et al. 1999).

The limitations of the study include the reduced sample size. However, crossover studies offer the significant advantage of enhancing statistical power, allowing hypotheses to be tested with fewer participants. In this study, the research design was employed to mitigate intersubject response variations to the same treatment and reduce the influence of confounding variables, as each participant serves as their own control, thereby enhancing statistical power (Ellis et al. 2007). Future studies with a larger sample sizes, encompassing male patients with severely resorbed ridges and larger evaluation periods, are necessary to elucidate the impact of occlusion on denture prognosis in this critical clinical situation.

## 5. Conclusions

The results of this study indicate that both BBO and CG can be considered as suitable occlusal schemes for oral rehabilitation with conventional complete dentures, even in patients with severe resorbed alveolar ridges. Given that both occlusal schemes provide similar levels of satisfaction, quality of life, and masticatory performance, CG may be considered as a scheme of choice by the clinician due to its greater simplicity and shorter execution time.

**Authors' Contributions:** NASCIMENTO, G.M.O.: performed the experiments, analyzed the data and wrote the manuscript; SOUZA, W.B.: analyzed the data and wrote the manuscript; CAMARGOS, G.V.: conceived/ designed the research and wrote the manuscript; MUNHOZ, M.F.V.: conceived and designed the research; SILVA, E.H.: analyzed the data; PALEARI, A.G.: conceived and designed the research, analyzed the data, wrote the manuscript. All authors have reviewed the manuscript.

**Conflicts of Interest:** The authors declare that they have no competing interests.

**Ethics Approval:** The ethical approval was obtained from the Institution' Ethics Committee (CAAE: 53466516.4.0000.5142).

**Acknowledgments:** We would like to thank Mr. Valdemir de Albuquerque and Mrs. Débora Virgínia Rossi (dental technicians) for his assistance in the fabrication of CDs.

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**Received:** 06 March 2024 | **Accepted:** 26 June 2024 | **Published:** 17 July 2024



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