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Oral Healthy Ageing: the impact of oral health and masticatory

performance on nutrition and general health of older adults

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Ai miei nonni Giuseppe e Lidia, Tommaso e Fiorella

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Abstract (ENG)

According to recent studies, the percentage of elderly population will significantly increase over the next few decades, implying the need to pay more attention to the health of elderly to promote healthy ageing. Oral health is an important part of general health, affecting the quality of life of an individual. Oral disorders, associated with a reduced masticatory function, negatively affect the nutritional status of older adults, exposing them to several acute and chronic diseases. The subjects with oral impairment may not have an adequate nutritional status, increasing the risk of general health related adverse effects. During their daily office practice, clinicians should be required to objectively evaluate human mastication, aiming not only at evaluating oral function, but also at providing information about patient impairment.

The Research Project aims at proposing an objective method able to assess human masticatory performance and investigating the impact of masticatory performance on general health status of self-dependent and institutionalized elderly. Overall, even if oral health is deemed to be a crucial factor for general health and well-being, it is often neglected, especially in older adults and in those who are frail and care dependent. Several older adults face difficulties in accessing dental care, so that the development of new strategies aimed at enhancing general and oral health status, such as Teledentistry, should be pursued. In an ageing society, educational interventions about oral health addressed to patients themselves and caregivers, appropriate oral health policies, and citizens empowerment and involvement can contribute to the promotion of oral health in elderly. Geriatric healthcare team members should play an important role in the initial oral health and, thus, taking care of the overall health and well-being of older adults.

Abstract (ITA)

La percentuale della popolazione anziana tenderà ad aumentare nei prossimi anni, implicando la necessità di porre maggiore attenzione allo stato di salute di questa categoria di popolazione, per promuovere un sano invecchiamento. La salute orale è parte integrante della salute generale di un individuo, condizionandone la qualità della vita. Le patologie orali, associate a una riduzione della funzione masticatoria, possono influenzare negativamente lo stato nutrizionale di un individuo, esponendolo a un aumentato rischio di *outcomes* di salute avversi. Durante la pratica clinica quotidiana, i clinici potrebbero avere la necessità di misurare oggettivamente la performance masticatoria del proprio assistito, con lo scopo di valutarne la funzione orale e il grado di disabilità orale.

Lo scopo dello studio è di proporre un metodo oggettivo in grado di misurare la performance masticatoria umana e di verificarne l'impatto sullo stato di salute generale di soggetti anziani autosufficienti e di soggetti istituzionalizzati. In generale, sebbene la salute orale sia ritenuta un fattore determinante per la salute sistemica e per il benessere di un individuo, questa è spesso trascurata, specialmente nei soggetti fragili che hanno bisogno di assistenza quotidiana, a causa delle difficoltà ad accedere alle cure odontoiatriche. Lo sviluppo di nuove strategie (Teledentistry) potrebbe essere utile per promuovere un sano invecchiamento. In una società che sta invecchiando, programmi di educazione alla salute orale, adeguate politiche sanitarie e un aumento della consapevolezza tra la cittadinanza, possono contribuire al raggiungimento di un sano invecchiamento orale. In generale, il team sanitario geriatrico dovrebbe ricoprire un ruolo di primaria importanza nell'iniziale bilancio di salute orale del proprio assistito, co-operando in un ambiente volto a promuovere la salute orale, prendendosi quindi cura della salute generale e del benessere della popolazione anziana.

Preface

Oral health is a factor that highly affects the general health status, the well-being and the quality of life of a subject. To date, it is often neglected, particularly when considering the most vulnerable categories of the population such as frail older adults who face an increased burden of oral pain and discomfort as well as impairments in oral function (e.g. biting, chewing, tasting, swallowing, and speaking) and in psychosocial domains.

Poor oral health outcomes in frail and care-dependent elderly are related to several risk factors that could be enhanced or worsened by personal factors, the lack of professional support, and the lack of appropriate oral health policies. While several attempts trying to explain the reason why care-dependent older subjects have often limited access to dental care were done, the contacts between elderly and other geriatric healthcare team members tend to increase. These care professionals should have some training in oral health-related issues, aimed at performing an initial oral health assessment, referring the patient to a dentist, and knowing and explaining simple oral hygiene and preventive measures.

This Ph.D Thesis is dedicated to all the geriatric healthcare providers and to the frail and unserved population. The Thesis is composed by and divided in six Chapters with different aims: 1. The Ageing Society and Gerodontology, 2. Measuring Oral Function, 3. The Ageing Mouth, 4. Finding links between General and Oral Health, 5. Oral Health and Nutrition, 6. Promoting Oral Healthy Ageing: conclusive remarks. Each chapter links to the others and contains the results of the original research performed during the three-year-long Ph.D Course.

List of Abbreviations

BC: Biceps Circumference

BMI: Body Mass Index

CG: Control Group

C.I.: Confidence Interval

COVID-19: Coronavirus Disease-19

DALYs: Disability Adjusted Life Years

DMFT: Decayed Missing Filled Teeth

dPROS: dental Patient Reported Outcomes

EFP: European Federation of Periodontology

EWGSOP2: European Working Group on Sarcopenia in Older People 2

FDI: Federation Dentaire Internationale

FMPS: Full Mouth Plaque Score

FPD: Fungiform Papillae Density

ICC: Interclass Correlation Coefficient

ICF: International Classification of Functioning, Disability and Health

IDF: International Diabetes Federation

MA: Masticatory Ability

ME: Masticatory Efficiency

MEG: Magnetoencephalography

MF: Masticatory Function

MNA: Mini Nutritional Assessment

MP: Masticatory Performance

NCD: Non-Communicable Disease

OHRQoL: Oral Health Related Quality of Life

PEM: Protein Energy Malnutrition

PhA: Phase Angle

PICOs: Population/Patient, Intervention, Control/Comparison, Outcomes

PRISMA-P: Preferred Reporting Items for Systematic Reviews and Meta-Analyses Protocols

PROSPERO: International Prospective Register of Systematic Reviews

PSR: Periodontal Screening and Recording

QoL: Quality of Life

R: Resistance

ROAG: Revised Oral Assessment Guide

SARS-CoV-2: Severe Acute Respiratory Syndrome Coronavirus 2

SD: Standard Deviation

TG: Test Group

TMJ: Temporomandibular Joint

VAS: Visual Analogue Scale

WC: Waist Circumference

WHO: World Health Organization

Xc: Reactance

Z: Impedance

Chapter 1: The Ageing Society and Gerodontology

1.1 The Ageing Pandemic

The worldwide proportion of older individuals is spreading, thanks to an increase in average life expectancy achievable through a better nutrition, the decrease in fertility rate, vaccine development and medical progress ¹. According to estimates, by 2050 the number of people over 65 will globally surpass the number of people between 15 and 24, growing to twice its proportion in the coming years ². In fact, the old-age dependency ratio (people aged 65 or above relative to those aged 15–64) in the European Union is deemed to spread from 43.1% in 2016 to 68.5% by 2070 ³. The highest old-age dependency ratio in the world in 2019 was registered in Japan (51%), followed by seven European countries (Finland and Italy 39%, Portugal 38%, Greece and France 37%, and Germany and Bulgaria 36%), Martinique (37%), and the US Virgin Islands (37%). Even though the fastest 30-year-long population ageing rate has been projected for Eastern and South-Eastern Asia (Figure 1), also some European countries are expected to be included in the "*top-ten*" list of countries with the highest old-age dependency ratio: Spain (78%), Greece (75%), Italy (74%), Portugal (71%) and Slovenia (65%) ⁴.



Source: United Nations, Department of Economic and Social Affairs, Population Division (2019). World Population Prospects 2019. ** China, Taiwan Province of China.

Figure 1 Top ten countries or areas with the highest old-age dependency ratios, 2019 and 2050

Despite variations, life expectancy is increasing worldwide. In 1990 global average life expectancy at birth was 64.2 years, reaching 72.6 years in 2019. The estimates forecast an increase to 77.1 by 2050 (European life expectancy at birth: 73.5 years in 1990, 78.7 years in 2019 and 83.2 in 2050)⁴. Finally, even though the global 2015–2020 women's life expectancy at birth exceeded men's one by 4.8 years, this difference is expected to shrink in the next few decades ⁵.

1.2 The Ageing Epidemic: Italy and Marche Region

As mentioned above, Italy is one of the European countries with the highest old-age dependency ratio and the estimates clearly indicate that the share is going to increase. Overall, the progressive ageing process of the Italian population is clearly showed when comparing the 2019 and 2011 census age pyramids (Figure 2) 6 .



Figure 2 Italian census age pyramid (2011-2019)

In all age groups under 44 years, the relative weight decreased. Conversely, the opposite happened from the over 45 groups, where an increase of almost 11% was recorded. Moreover, when considering the number of older adults per child and the ageing index, it is showed that the first has a constantly growing trend since 1951, ranging from less than 1 elderly per child in 1951 to 5

in 2019 (3.8 in 2011). When looking at ageing index, given by the ratio of the population aged 65 and over to the one with less than 15 years, the proportion increased significantly, varying from 33.5% in 1951 to almost 180% in 2019 (148.7% in 2001). In 2019, the ageing index assumed the minimum value in Campania (135%) and Trentino Alto Adige (142%, recording 120 percentage points more compared to 1951). On the contrary, the maximum value was observed in Liguria (262%, 195 percentage points more compared to 1951), followed by Molise (226%,) and Friuli Venezia Giulia (224%, +187 points) ⁶.

In the Marche Region, in 2019, there were 1,525,271 people (2.5% of the total resident population in Italy), with more than 1/5 of them residing in the five provincial capitals. Analyzing the age classes, Marche shows a greater presence of population over 75 when compared to the national average (13.4% against 11.7%; when considering the population over 65, the percentage rises to 24.8% against 22.8% national). This incidence is on average higher in some municipalities of inland and rural areas, especially in the central-southern Region.

1.3 Healthy Ageing

Living longer does not imply living better: with life expectancy spread, multimorbidity, frailty, and care-dependency will tend to increase with aging, suggesting that a large percentage of elderly will spend their last years dealing with disease and disability. If on one hand, the increase of life expectancy involves chances for society and families, on the other, all those opportunities are directly associated with health. The World Health Organization (WHO) has defined the term "Healthy Aging" as the capacity of maintaining a functional status that enables well-being in older age ⁷. Functional ability must be supported and maintained during an individual's lifetime. At the same time, barriers should be removed for those people with low-capacity levels. In this context,

the development of supportive environments has the opportunity to maintain well-being and quality of life in older adults, independently from the presence or not of disease and disability. Maintaining physical and mental well-being and a high quality of life as well as building supportive environments for elderly have become important goals to pursue for both physicians and healthcare administrators. Being in good health implies adding life to years. The opportunities that arise from increasing longevity and life expectancy depend strongly on Healthy Ageing. People who experience these extra years of life in good health and continue to participate and be an integral part of families and communities will strengthen societies; however, if the added years are dominated by poor health, social loneliness or dependency on care, the implications for older people and for society are much more negative ⁸. Healthy Ageing spans the life-course and is relevant to everyone, not just those who are currently free of disease. Intrinsic capacity at any time is determined by many factors, including underlying physiological and psychological changes, health-related behavior and the presence or absence of disease.

1.4 Oral Health and Gerodontology

In 2016, the FDI World Dental Federation acknowledged the multifaceted character and nature of oral health. In particular, its importance lies in the fact that it is essential for speaking, smiling, smelling, tasting, touching, chewing, swallowing, and conveying a range of emotions through facial expressions with confidence, and without pain, discomfort, and disease of the craniofacial complex ⁹. Oral Health is a fundamental component of health and physical and mental well-being: it exists along a continuum influenced by the values and attitudes of people and communities, reflecting the physiological, social, and psychological attributes that are essential to the quality of life. In addition, it is influenced by the person's changing experiences, perceptions, expectations,

and ability to adapt to circumstances. In 2015, WHO stated that "oral health is a crucial and often neglected area of healthy ageing" ¹. Even if dental science and oral health prevention recorded a significant progress in recent years, chronic oral diseases are common in older adults, especially in those who are frail and care dependent ¹⁰. Poor oral conditions may be responsible of pain and lead to local and systemic infection, decay, teeth loss, chewing, speaking, and swallowing impairment, food choices constriction and weight loss. Moreover, poor dental appearance and halitosis damage psychosocial well-being and integration in society ¹¹.

Gerodontology is the branch of Dentistry that deals with the oral health of the elderly and the related treatments: in particular, oral health is characterized by the presence of an adequate oral function and the absence of active pathologies. Although oral health is an integral part of general health, contributing to a good physical, psychological and social condition of the subject, WHO states that oral health is the most neglected aspect of overall health during ageing ¹. In fact, oral diseases are one of the most important public health challenges. Good oral health in old age is particularly important not only for maintaining adequate oral function but also for keeping social interaction, self-esteem, personal dignity, and a satisfactory quality of life ¹². Since oral health is an essential part of general health and well-being and chronic and oral diseases have common risk factors, it is essential to identify and prevent the most common oral conditions in the population. In particular, the most common oral diseases (e.g. dental caries, periodontal disease, severe tooth loss, xerostomia, denture-related problems, and oral cancer) frequently affect the most vulnerable, frail, and care-dependent members of society ^{13,14}. Moreover, studies showed a strong relationship between oral diseases and the most common systemic diseases such as diabetes, cardiovascular diseases, aspiration pneumonia, stroke and Alzheimer's disease ¹⁵. Some studies have also described an association between tooth loss, the ability to chew, and nutrition ¹⁶. In frail subjects,

a compromised general health status associated with functional problems (e.g. cognitive disorders) affects the ability to perform good oral hygiene, to follow a healthy diet, and to make regular visits to the dentist, which can lead to severe and rapid oral health deterioration.

Even though they are largely preventable, untreated oral conditions are among the most prevalent non-communicable diseases (NCDs) that affected more than 3.5 billion people globally. The number of individuals with oral diseases increased by 40% from 1990 to 2015, while the oral disability-adjusted life years (DALYs) due to oral conditions increased by 64%, mainly due to demographic changes such as population growth and ageing ¹⁷.

The increasing proportion of elderly living in developed countries with a significant number of natural teeth that require complex daily oral hygiene and frequent professional dental care implies the need to develop and implement innovative and effective oral health policies. Person-related factors as well as limited professional support and lack of appropriate oral health policies contribute to the onset of poor oral health in older people, especially in those who are frail and care dependent. In conclusion, oral diseases should be effectively prevented, detected precociously, and efficiently managed using a collaborative interprofessional approach with the aim to reduce their correlated disabilities and to support "Healthy Ageing".

1.5 Dental Care Access and the Elderly: What Is the Role of Teledentistry? A Systematic Review ¹⁸

1.5.1 Introduction

The crisis imposed by the coronavirus (SARS-CoV-2) pandemic has affected public health systems around the world. The rapid spread of SARS-CoV-2 has constricted many countries to restrict human mobility ¹⁹. During the pandemic, dental care has been limited only to urgent and

not deferrable treatments. Blood and saliva exposure and droplet production put dental practitioners at high risk of contagion during their routine procedures ^{20–22}. The inhalation of droplets and aerosol from SARS-CoV-2-positive subjects and even direct contact with mucous membranes, oral fluids, and contaminated instruments and surfaces could enhance virus transmission during dental procedures ^{23,24}. The implementation of fully digital workflows could be useful in order to limit cross-infection ²⁵. Moreover, this natural pandemic is also responsible for the onset of feelings that may impact the willingness of people to undergo dental appointments²⁶. Oral health is part of general health, with a role in the quality of life ²⁷. In the next few months, health systems will need to treat a large number of patients whose health has been compromised by chronic untreated diseases, including oral diseases. The preservation of oral health is crucial, and the prevention and the treatment of all the diseases that could lead to edentulism should be pursued ^{28,29}. A high level of unmet oral health needs is very common among elderly people, suggesting that enhancing access to dental care is crucial ³⁰. Oral diseases could be also responsible for the triggering or promotion of inflammatory and infectious processes at a systemic level, potentially worsening the clinical picture of subjects with comorbidities ³¹.

Inequalities among different social, ethnic, and economic groups highly affect access to and use of dental care services ³². These inequalities, combined with the current situation imposed by the SARS-CoV-2 pandemic, may become even more critical for elderly people living in long-stay care institutions or with in-home assistance. Elderly people should be isolated to prevent virus infection, but while SARS-CoV-2 infection could be avoided, on the other a worsening of clinical conditions could be observed due to the lack of specialistic check-ups.

The global crisis generated by the pandemic offers a unique opportunity to reshape the traditional approach. Confined and institutionalized elderly people, in particular those with frailty, cannot

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receive conventional dental care. Reduced mobility and SARS-CoV-2 fear prevent older adults from being treated in a conventional way in dental clinics. In order to improve the quality of care for people with a loss of autonomy and institutionalized care, the use of Teledentistry could be a strategy aimed at providing health for elderly adults during and after the coronavirus pandemic. Dentistry and policymakers must reflect on current and subsequent scenarios, considering the needs and emerging opportunities that will be created after these times.

Teledentistry, defined as the use of health information technology and telecommunications for oral care, was addressed to have the potential to identify high-risk populations; facilitate patient access to dental care; and reduce waiting lists, unnecessary travel, loss of productivity, and also inequalities in dental care access and costs for national health systems ^{33,34}. In a society that is getting older and that has been hit so hard by the coronavirus pandemic, the development of new strategies aimed at enhancing general and oral health status should be crucial in order to promote healthy aging. The aim of this systematic review is to assess the feasibility of Teledentistry in communities or in a domiciliary setting where elderly people live. In particular, the present review focused on the evaluation of the accuracy and the effectiveness of Teledentistry compared to traditional face-to-face dental visits, the patient acceptability, and the costs related to the implementation of oral health information technology provision.

1.5.2 Materials and Methods

This systematic review was performed in accordance with the recommendations of the "Preferred reporting items for systematic reviews and meta-analyses protocols (PRISMA-P) statement" ³⁵. In accordance with the guidelines, the present systematic review protocol was registered in the

International Prospective Register of Systematic Reviews (PROSPERO) on 4 September 2020 (registration number CRD42020200827).

Structured and systematic research was performed on the major electronic databases for studies published until 30 June 2020: the PubMed, Cochrane Library, Web of Science, Scopus, and CINAHL databases. The following keywords were used in order to perform database searches: "teledentistry", "elderly", "aged", "older", "elder", "geriatric", "nursing homes", "nursing home", "long term care", "residential care", and "home assistance", in combination with the Boolean operators "AND" and "OR".

A pilot search was undertaken in order to ensure that the search strategy was effective. The study focused on the Population/Patient, Intervention, Control/Comparison, Outcome(s) (P.I.C.O.S.) criteria ³⁶. In particular, studies involving elderly people in nursing homes, in communities, or with in-home assisted were included. Subjects requiring oral health care of any sex, ethnicity, socio-economic status, and comorbidities were considered. Studies assessing the use and the applicability of Teledentistry in such structures were included. Any type of oral health provision through the use of health information technology was examined: screening, diagnosis, support, consultation, education, and any other kind of application in dental medicine. Interventions aimed at assessing the above-mentioned interventions provided both using Teledentistry and the traditional face-to-face dental visits were compared. The primary outcome of the review was the comparison between the accuracy and effectiveness of Teledentistry and face-to-face dental visits. The secondary outcomes were the assessment of Teledentistry advantages and disadvantages with the evaluation of patient acceptability and the cost-effectiveness ratio.

Eligible studies were (a) studies published in the English language; (b) studies published in a peerreviewed journal; (c) studies published until June 30th, 2020; (d) clinical studies. Studies were

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excluded if they were: (a) reviews, editorials, commentaries, letters, book chapters, reports on prospective ideas and futuristic scenarios (protocols included), and dissertations. Two reviewers carried out the evaluations independently. The very first selection was made on the basis of papers title or abstract and eligible ones were selected for full text review. For the assessment of each publication, Excel spreadsheets were compiled. Data were extracted using a standardized form which included (a) authors' names and the year of publication, (b) country in which the study was performed, (c) the type of study, (d) the setting of the study (e.g., home, community, residential aged care facility), (e) the aim of the study, (f) the type of oral health provision using Teledentistry, (g) the sample size, (h) the mean age \pm standard deviation (when applicable), (i) the retrieved article main outcomes, (j) the retrieved article secondary outcomes, (k) the quality assessment score, and (1) the quality of economic evaluation score. Both the authors compared each other and confirmed the data on the basis of the compiled spreadsheets. In case of doubt, concerning the study data, the two reviewers resolved disagreements by discussion. In the case of doubt, a third reviewer solved discrepancies. The quality of the studies included in the review was evaluated by the two independent reviewers using the protocol described by Hailey et al. ^{37,38}. This approach provides a quantitative measure of scientific rigor. The overall quality score, assessing both the performance and study design, provides an indication of the degree of confidence of the studies' findings and their implication for future decision making regarding Teledentistry. Briefly, when reviewing a Telemedicine study, the strength of evidence is defined by the study performance and study design. For the study performance, five different areas of interest are defined: (a) the patient selection, (b) the description/specification of the interventions, (c) the specification and analysis of the study, (d) the patient disposal, and (e) the outcomes reported. For each category, a score of 0, 1, or 2 is given: 0 if relevant information is missing or given in little detail, 1 if reasonable details

are provided but with some important limitations, 2 if the information has no significant limitations. For study design, four different scores are assigned: a score of 5 is allocated to large randomized controlled trials (RCTs) with at least 50 subjects in each arm, a score of 3 to smaller RCTs, a score of 2 to prospective not randomized studies, and a score of 1 to retrospective comparative studies. Basing on the totals of the quality scores, each study is assigned to a category that varies from A to E, where "A" indicates studies with the highest degree of confidence and "E" those with the lowest. In particular, categories A, B, C, D, and E correspond to a total quality score of 11.5–15.0, 9.5–11.0, 7.5–9.0, 5.5–7.0, and 1–5.0, respectively. The quality assessment of the studies that included a cost analysis was performed in accordance with the Drummond et al. 10-point checklist ³⁹. For each eligible paper, a score was assigned for each of the following criteria:

1. Was a well-defined question posed in answerable form?

2. Was a comprehensive description of the competing alternatives given?

3. Was the effectiveness of the programs or services established?

4. Were all the important and relevant costs and consequences for each alternative identified?

5. Were the costs and consequences measured accurately in appropriate physical units?

6. Were the costs and consequences valued credibly?

7. Were the costs and consequences adjusted for different timing?

8. Was an incremental analysis of costs and consequences of alternatives performed?

9. Were allowances made for uncertainty in the estimates of costs and consequences?

10. Did the presentation and discussion of the study results include all issues of concern to users? Each item had three possible answers: "yes", "cannot tell", and "no". The response score was 1.00, 0.50, and 0, respectively. A 0.5 value was set for the "cannot tell" answer because the absence of any information has an equal probability of either a "no" (score = 0) or a "yes" (score = 1). The

lowest and highest possible scores were 0 and 10. A total score of 5.00 was considered the minimum threshold score to consider an economic evaluation of sufficient quality and suitable for the inclusion in an economic evaluation database.

1.5.3 Results

A total of 13 articles were identified through database searching using combinations of keywords. Out of 13 papers, eight abstracts were reviewed to assess if they were coherent with the aim of the study and full texts were retrieved. Five studies were excluded because they were duplicates. After abstract reviews, seven articles were selected for closer inspection. A study was not included because it considered young patients. Of these seven articles, six were assessed for eligibility, while a study was excluded because it did not meet the inclusion criteria.

The studies included in the present review were conducted in three different nations: Australia (n = 4), France (n = 2), and Germany (n = 1). A high heterogeneity was assessed among the retrieved articles, reporting different outcomes. Two studies investigated the feasibility and the accuracy of Teledentistry for diagnosis dental pathology using the traditional face-to-face examination as gold standard ^{40,41}. Four papers were aimed at assessing patients and health practitioners' experiences about Teledentistry using questionnaires ⁴⁰, direct observation of nurses ⁴², surveys and clinical audit charts ^{43,44}. Finally, three studies also reported a cost analysis and a cost description of Teledentistry in residential aged care facilities ^{40,43,45}.

Full-text articles that met the eligibility criteria are included in Table 1. Table 1 shows (a) the authors' names and year of publication, (b) the country in which the study was performed, (c) the type of study, (d) the setting of the study, (e) the aim of the study, (f) the type of oral health provision using Teledentistry, (g) the sample size, (h) the mean age \pm standard deviation (when

applicable), (i) the retrieved article main outcomes, and (j) the retrieved article secondary outcomes.

Regarding the assessment of the feasibility of Teledentistry in screening oral diseases and conditions and in developing treatment plans, Mariño et al. tested both virtual and canonical oral examination ⁴⁰. The intra-examiner agreements, determined by the Kappa index, indicated an excellent agreement, Kappa=0.83. Queyroux et al. compared the accuracy of Teledentistry approach to the conventional face-to-face oral examination ⁴¹. Diagnoses were classified as true positives, as false negatives and false positives and sensitivity, specificity, positive predictive value and negative predictive value were calculated. More receiver operating characteristic analyses were performed to determine the diagnostic performance of Teledentistry. Virtual examination lasted 12 minutes, while the canonical visit took 20 minutes. The results of the diagnostic accuracy for dental pathology showed a sensitivity of Teledentistry of 93.8% (95% CI: 90.7%-96.9%), a specificity of 94.2% (95% CI: 91.2%-97.2%), a positive predictive value of 95.2% (95% CI: 92.4%-98.0%) and a negative predictive value of 92.4% (95% CI: 89%-95.9%). In the receiver operating characteristic analysis, the area under the curve was 0.95 (95% CI: 0.92-0.98). The assessment of accuracy for chewing ability indicated that the sensitivity of Teledentistry was 85.0% (95% CI: 80.0%-90.0%), the specificity 82.8% (95% CI: 77.7%-88.1%), the positive predictive value 92.2% (95% CI: 88.5%-95.9%) and the negative predictive value 69.6% (95% CI: 63.2%-76%). Sensitivity, specificity, positive predictive value and negative predictive value for the assessment of accuracy in the evaluation of dental prostheses rehabilitation status were 87.8%(95% CI: 82.5%-93.1%), 90.3% (95% CI: 85.5%-95.1%), 78.3% (95% CI: 71.6%-85.0%) and 94.9% (95% CI: 91.3%-98.5%), respectively. Overall, Teledentistry had excellent sensitivity and specificity for diagnosing dental diseases among elderlies living in residential aged care facilities.

Queyroux et al.	Mariño et al.	Authors and Year
France and Germany	Australia	Country
2-year, multicenter, cross-sectional study	Pilot feasibility study and cost-analysis	Туре
Nursing homes	Residential aged care facilities	Setting
To assess Teledentistry accuracy using direct examination as gold standard	To assess Teledentistry safety, feasibility, and acceptance	Aim
Dental pathology	Development of treatment plans	Application
237	5	Sample Size (n)
84.4 ± 8.3 years	N/A	Age
Dental pathology • Sensitivity: 93.8% • PPV 1: 95.2% • NPV 2: 92.4%	 High concordance between remote and face-to-face examination Patient satisfaction with Teledentistry 	Main Outcome
 Chewing ability Sensibility: 85.0% Specificity: 82.8% PPV ¹: 92.2% Dental prostheses status Sensibility: 87.8% Specificity: 90.3% PPV ¹: 78.3% NPV ²: 94.9% Use of Teledentistry Not associated with any serious adverse events Excellent acceptability rate (95.3%) Quicker than face-to-face examination (12 vs. 20 min, respectively) 	 Provision of regular and timely oral health checks Travel-associated stress reduction Prioritization of appointments Improvement of efficacy and increment of number of visited residents Improvement of confidence in resident aged care facilities Implementation barriers Implementation barriers 	Secondary Outcome

Table 1. Summary of the studies included in the review.

Authors and Year	Country	Туре	Setting	Aim	Application	Sample Size (n)	Age		Main Outcome	Secondary Outcome
Petcu et al.	France	Report based on the preliminary result of a project	Long term facilities for the elderly and specialized facilities for adults with severe intellectual, motor or somatic disabilities	To evaluate the acceptability of Teledentistry among elderly people	Screening	123	60% of the sample were 65 years old or older	••	Onset of anxiety regarding the procedure Onset of dispositional resistance	N/A
Tynan et al.	Australia	6-months, quality improvement study and cost-analysis	Residential aged care facilities	To describe the development and implementation of Teledentistry model	Screening	116	N/A	• •	Improvement in implementation of oral care plans Minimization of need to attend an oral health care facility	 No adverse events Costs minimization Positive feedback from staff, residents and families Minimization of disruption to high-care residents (particularly those with dementia)
Tynan et al.	Australia	Mixed methods comparative study	Residential aged care facilities and multi-purpose health services	To investigate the impact and the experience of Teledentistry compared to traditional approaches in residential aged care facilities	Screening	252	79.8 years	• •	Improving oral health education among staff and residents Minimization of disruption to residents (especially those with dementia)	 Reduction in inequities in dental care access Improvement of oral care education, promotion, oral diseases prevention and timely intervention Potential reduction in waiting lists and unnecessary travel Familiar setting
Mariño et al.	Australia	Cost-analysis comparison study	Public healthcare	To compare the costs of face-to-face examination with two different Teledentistry approaches in residential aged care facilities	Screening	N/A	N/A	•	Asynchronous Teledentistry model has the lowest cost compared to both real-time Teledentistry and face-to-face examination	 Caregivers training and education

Among the positive aspects that this kind of technology could add to the provision of oral care in residentials, we found: (a) the absence of adverse effects, (b) the reduction in waiting lists and unnecessary travels, and (c) the minimization of disruption to high-care residents, particularly those diagnosed with dementia. Moreover, the use of Teledentistry could be a useful tool also for the improvement of oral care education among residential aged care facilities workers, patients and residentials families. Overall, participants experience about Teledentistry was assessed using questionnaires, direct observation of patient behavior, chart audits and interviews. Only, two papers reported negative experiences among the participants of the studies 40,42 . Fear and anxiety were the two main feelings generated by Teledentistry in patients together with a dispositional resistance, not to the use of technology, but to the whole dental procedure ⁴². According to Mariño et al., controversy than residents, although understanding the opportunities given by technology, the nurses of residential aged care facilities questioned Teledentistry effectiveness, claiming that the proposed method was not capable of recognizing the reality of residential aged care facilities⁴⁰. Nevertheless, when participants were asked to rate their experience with Teledentistry, almost the totality of them were either "very satisfied" or "slightly satisfied" (46% and 38%, respectively), strongly or slightly recommending the remote dental examination to other people of their age (46% and 46%, respectively). Moreover, the participants considered remote communications generally either "very easy" or "easy" to understand, at 46% and 46% respectively. Only 4% of the participants found it "difficult" or "very difficult", complaining about the foreign accent of oral health professional rather than the technology used. The 28% of the residents asserted that the most valuable element of Teledentistry was its convenience in terms of costs savings and disruption and difficulty avoidance ⁴⁰. Finally, Queyroux et al. reported that Teledentistry was not associated with any severe negative effect and an excellent acceptability rate was recorded among

both residents and their families (95.3%)⁴¹. A positive feedback was also evaluated among residential staff, residents and their families by Tynan et al.⁴³.

The quality of the studies included in the review was evaluated using the protocol described by Hailey et al. ^{37,38}. The available literature constricted to include in the present review mostly articles with poor or poor to fair quality, characterized by substantial limitations in the study^{40,42–45}, and only one with fair to good quality ⁴¹. Overall, three studies reported an economic evaluation of Teledentistry and scored five or more according to the criteria suggested by Drummond et al.³⁹. In particular, Mariño et al. ⁴⁵ was given a total score of 7, meeting 5 out of the 10 criteria and receiving a 0.5 value for 4 of them. Mariño et al. ⁴⁰ scored 6, fulfilling 4 out of the 10 conditions and getting a 0.5 score for 4 of them. Finally, Tynan et al. ⁴³ met 3 out of the 10 points and receiving a score of 0.5 for 4 of them, totally scoring 5.

The economic analyses in the reviewed articles showed that the studies were cost-analysis ones. All the pertinent costs from a healthcare perspective were identified and calculated using market values, published official salaries, and expert opinions. Generally, three main categories of costs were defined: (a) training, (b) salaries, and (c) teledental device ⁴⁰. Mariño et al. estimated the unit cost of Teledental consultation, considering both the real-time method and the storage and forward⁴⁰. The cost of training was equal between the two options, while the costs related to intervention provision were different. A higher amount of time was registered in the storage and forward method (20 min vs. 15 min for the real-time oral examination), thus slightly augmenting, but not significantly, the cost of this Teledentistry option, comparing it to the real-time model. Mariño et al. compared costs per treatment plan development among face-to-face oral examination performed by an oral health professional, storage and forward model was fewer than the real-time

one, representing the cheapest option. When considering the conventional face-to-face oral examination, additional costs for dental assistant and travel should be added to the amount. Overall, the storage and forward option is always the cheapest alternative, when comparing it to both the real-time method and the conventional oral examination. The asynchronous model was cheaper than the real-time one because of personnel costs, while for the traditional approach costs increased due to travel for oral professionals and dental assistants. Tynan et al. analyzed the costs generated by the implementation of an oral health therapist in residential aged care facilities, the utilization of a Teledentistry model and the attendance at oral health clinic by resident (via car or ambulance)⁴³. The screening performed by the oral health therapist was shown to be the lowest cost model, while the costs were augmented when considering the Teledentistry model due to the cost of both set-up and dentist time, and residents' attendance at a dental clinic incurred the highest costs due to the cost of transportation. The disruption of high-care patients could be also considered.

1.5.4 Discussion

The present review identified literature regarding the application of Teledentistry in geriatric settings. Overall, there is a strong trend supporting the feasibility of Teledentistry compared to the traditional approaches ⁴⁶. If on one hand, most of papers stated that Teledentistry is comparable to or even better than the conventional alternative, on the other, conclusive statements are not possible to be drawn and publication bias could be met. Only few studies are well constructed and reported a controlled comparison between Teledentistry application and face-to-face alternatives⁴⁷. Regarding the application of Teledentistry in the management of elderly people living in residential aged care facilities, available literature is even fewer. Papers concerning older people

at in-home assistance are null. Only two studies reporting oral home telecare for adults with tetraplegia are available, showing that oral home telecare offers the opportunity to decrease physical barriers and to improve the quality of dental health services for people at in-home assistance ^{48,49}.

Teledentistry belongs to the natural process of the digitalization of modern society and medicine. It is able to improve services, breaking down barriers and allowing also people with limited or no possibility to access to dental care ^{50,51}. The relationship between health and inequalities has been deeply investigated by considering different professional and social conditions, showing that mortality rates increase in proportion to economic and social hardship, lower incomes, education, and social class ⁵². Oral health problems are more prevalent in lower social strata, so the access to dental health services has deteriorated in Europe over the years, especially in countries severely hit by the economic crisis, such as Greece, Spain, Portugal, Italy, and Ireland ⁵³. Beside economic crisis, oral health access could be restricted by the bad clinical conditions of the subjects. In the USA and Australia, the number of elderly people living in rest homes have dramatically risen ^{54,55}. It was estimated that the 65% almost of people who used to live in nursing homes suffered from oral diseases. Moreover, poor oral status is a strong predictor of the onset of adverse health outcomes, including mortality among the community-dwelling elderly ^{56,57}. The individualization and the assessment of factors associated with such conditions will help in the prevention or minimization of their negative consequences on health. The implementation of Teledentistry in residential aged care facilities could assists users to access oral care, providing regular visits using trained caregivers in the first instance.

Overall, the present systematic review identified three main topics, consistent with the main and the secondary outcomes of the present review: (a) accuracy and effectiveness, (b) acceptability,

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and (c) costs. Teledentistry was found to have an excellent accuracy for the diagnosis of dental diseases and good accuracy for the assessment of chewing ability and oral rehabilitation status among elderly people living in nursing homes. Despite the different aims, methods, and outcomes of the studies, virtual dental examination was comparable to the traditional face-to-face dental visit ^{40,41}. If on one hand, Teledentistry could provide general and specialist oral health care support to elderly people, on the other the implementation of Teledental assistance should not substitute the traditional approach in the case of the suspect of more severe diseases that need in-depth diagnostic procedures. As oral health is an indicator of frailty and the improvement of oral conditions among elderly people is needed, Teledentistry could be used for screening and preventing dental pathologies in older people ^{41,58}. Moreover, a previous study stated that Teledentistry has the capacity to reduce appointment waiting lists, triaging, and prioritizing appointments, and to provide a rationalization of time, travel and costs for all parties ⁵⁹. Additionally, teleconsultation could reduce all the stress generated by travelling to a dental office from home or the residential aged care facility of the patients, especially in those with dementia that could lead to a complete lack of collaboration with dental practitioners ⁴⁴.

Overall, a high level of acceptability of Teledentistry was reported in the majority of the articles included in this review among patients, patients' families, and caregivers. Survey-based studies showed satisfaction among the users because of the increased confidence in residential aged care facilities ⁴⁰. Positive feedback was received also because of the augmented awareness of oral health needs and management, savings in residents transportation to oral care facilities, and positive cultural change among caregivers through remote education ⁴³. Moreover, the audits showed an improved compliance and consciousness of the importance of oral health in facilities with access to dental consultation ⁴⁴. Conversely, Petcu et al. reported that psychotic patients perceived

Teledentistry more negatively than nonpsychotic ones ⁴². Among nonpsychotic patients, the total negative experience became more distinct moving from dependent patients to semi-autonomous and to autonomous ones. Fear and anxiety were the main feelings generated by the procedure as a whole and not by the use of technology. The latter, as suggested by a recent review, could have an added benefit in the provision of oral care, particularly in odontophobic subjects ⁶⁰. Other negative feedback could be addressed to the lack of immediate response on the examination and the resistance from some nurses who questioned the effectiveness of Teledentistry in the context of residential aged care facilities ⁴⁰. While, for the first point, the use of a real-time Teledentistry model could solve the problem of immediate feedback, for the second one, the skepticism may be due to the absence of innovative oral care programs in most nursing homes.

When comparing Teledentistry to face-to-face oral examinations, a reduction in costs was likely to be detected ⁶¹. A study with a micro-costing analysis and direct cost measurement was performed from a healthcare perspective, trying to find out the cost of implementing an asynchronous and real-time Teledentistry model in a nursing home setting: no significant differences were detected between the two methods ⁴⁰. Conversely, in a model cost-analysis format, Mariño et al. compared the traditional examinations to the storage and forward Teledentistry method and the real-time one ⁴⁵. The authors stated that the asynchronous model was always the less costly one, followed by the real-time model and face-to-face dental visits. Tynan et al., in turn, estimated the costs of three different scenarios, including the implementation of an oral health therapist in the residential aged care facility, the use of Teledentistry, and patient transportation to a dental facility ⁴³. The first option was considered to be the least-cost scenario, followed by the use of Teledentistry and the third option. Nevertheless, in the latter study, an oral health therapist was supposed to perform the intraoral acquisition and to transmit it to a dentist

placed elsewhere. In addition to the cost of Teledentistry set-up, in this case also the costs of both the oral health therapist and dentist were calculated. Regarding costs, the travel cost also should be considered. The application of Teledentistry in the provision of the oral care of elderly people could allow savings in terms of travel (via car or ambulance), caregiver escort time to accompany the patient, and patient disruption. The additional cost of Teledentistry is associated with training, increasing the total cost amount. However, new skill development among caregivers and the strengthening of health team capacities could be achieved through supportive environments and remote learning sessions ⁵⁹.

1.5.5 Conclusions

The present systematic review is the first that analyzed the feasibility of Teledentistry in the provision of oral care in elderly people living in residential aged care facilities or with in-home assistance. In a society that is getting older, the implementation of Teledentistry in residential aged care facilities and in-home assistance programs could be a viable tool for the management of oral care in people who cannot access dental care. Moreover, in a context in which movement is not recommended for the most vulnerable categories, avoiding unnecessary appointments, and triaging dental visits could be effective. Although the absence of high-quality studies limited the findings, Teledentistry was found to be as accurate as traditional face-to-face dental examinations; cost-effective; and well accepted among patients, patients' families, and caregivers. Well-designed studies aimed at assessing Teledentistry and surroundings are therefore needed in order to increase the body of evidence supporting the feasibility of the digitalization of oral care.

Chapter 2: Measuring Oral Function

2.1 Masticatory function: some definitions

The masticatory process has been deeply investigated since 1901, giving back to the scientific community several papers in the literature ⁶². Many aspects of the masticatory function (MF) have been described, varying from masticatory physiology in dentate individuals ^{63,64} and bolus formation ^{65–67} to masticatory impairments determined by tooth loss ^{68,69} and improved masticatory function after oral prosthodontic rehabilitation ^{70–72} or neurological disorders ^{73,74}. A good MF is not only important for an adequate food fragmentation and bolus preparation, but it is also crucial for both digestion and nutrition ^{75,76}. Moreover, recent studies showed that MF plays a role in exciting brain cortex, stimulating brain function and cognition ^{77–81}. In addition, MF affects dental Patient Reported Outcomes (dPROs) like the dimensions 'Oral Function' or 'Psychosocial Impact' as well as Patient Satisfaction ^{82,83}.

According to the Glossary of Prosthodontic Terms, *masticatory performance* (MP) is defined as "a measure of the comminution of food attainable under standardized testing conditions", while *masticatory efficiency* (ME) is defined as "the effort required to achieve a standard degree of comminution of food" ⁸⁴. In accordance with Bates et al (1976) ⁸⁵, MP refers to a state of chewing outcome following a determined number of chewing cycles, whereas ME denotes the number of chewing cycles needed to attain a particular chewing outcome, suggesting that MP refers to the individual's ability to chew a tester (either artificial or not) after a pre-determined number of masticatory cycles, whereas ME indicates the number of chewing cycles necessary to attain a bolus ready to be swallowed. The term *masticatory ability* (MA), considered as a general term to define the chewing process, has more frequently been associated to self-assessed masticatory function studied by interviewing subjects on their oral function ⁸⁶. Even though the terms referring to MF

were clearly defined, there is still a lack of consensus among researchers on the exact semantics of each term and similar terms are sometimes used to describe different methodologies ⁸⁷.

2.2 The assessment of Masticatory Function

During their daily office practice, clinicians may be required to evaluate patient mastication. Overall, the outcome of masticatory process can be assessed using two different methods: the collection of food bolus after a determined number of chewing cycles and the collection of food bolus at the swallowing threshold. The use of an approach or of the other depends on the research goals. In particular, if the researcher wants to assess the MF of a subject analyzing the food bolus after a fixed number of chewing strokes, the researcher will evaluate how well that subject performed in mixing or fragmenting a tester material (natural or synthetic). In this case, that researcher will assess the MP or the chewing performance of that subject ^{85,88,89}. Conversely, in the second approach, the researcher will evaluate a tester when the subject is ready to swallow it, assessing the ME of that subject ⁸⁹. Food texture and physical properties (e.g. stickiness, cohesiveness, hardness, size, etc ...) as well as oral and the physiological characteristics of an individual (e.g. number of occluding pairs, bite force, tongue mobility, salivary flow rate, neurological status, pain, intraoral sensitivity, age, etc ...) are the main factors affecting the moment of swallowing ^{90–97}. By the way, it is important to bear in mind that MP and ME do not necessarily correspond. In fact, even if the higher fragmentation capacity corresponds to the fewer number of masticatory strokes, subjects with high MP, defined as good chewers, do not necessarily swallow their food after fewer masticatory cycles than those with low MP, defined as bad chewers⁹⁸. At the same time, bad chewers do not necessarily need more chewing cycles before

swallowing than good chewers. They may swallow badly prepared food boluses or even refuse to swallow some types of food ⁹⁹.

Masticatory function can be assessed using different approaches, indicating different parameters which define the masticatory process. Overall, researchers have proposed: direct objective assessment analysis methods, indirect objective assessment analysis methods and subjective assessment analysis methods.

2.2.1 Direct Objective Assessment Analysis

Comminution tests

The comminution tests are performed on a breakable tester material (either natural or synthetic) that is fragmented during chewing cycles. The resulting broken food particles are retrieved from the oral cavity of the subject and then analyzed by sieving or optical scanning. The fragmented tester is characterized by particle sizes distribution, expressed in median particle size ⁸⁸. The distribution of the particle sizes determines the MP of a subject.

Briefly, the subject receives a portion of impression material (e.g. Optosil/optical or hydrocolloid) ¹⁰⁰ or natural food (e.g. peanuts, almonds, carrots) which is chewed for a fixed number of masticatory strokes. The subject is then asked to expectorate the bolus. The particles are dried and sieved for 20 minutes using either a stack of sieves with meshes ranging from 5.6 mm to 0.5 mm or a single sieve and bottom plate ¹⁰¹. Even if the single sieve method is simpler than the multiple sieves one, it is also less reliable, especially if the sieve aperture is not close enough to the median size particle size of the distribution of the crewed tester ¹⁰². Bolus particle size distribution by weight as obtained from the sieves is described by a cumulative distribution function, characterized by the median particle size and the broadness of the distribution. The median particle size reflects

the aperture of a theoretical sieve through which 50% of the weight of the fragmented material could pass ^{88,103,104}. The fragmented particles can be also analyzed by optical scanning, obtaining a discrete particle size distribution ^{101,105}. Finally, when assessing ME, a power function that describes the decrease of the median particle size as a function of the number can be used. The ME test is a more complex version of the MP test using multiple sieves. Moreover, for the use of calculation function, the median particle size should be determined in multiple experiments with different number of chewing strokes.

In conclusion, comminution tests are reliable tests that can be used for the assessment of both MP and ME, also facilitating the comparison within and between subjects or groups of subjects or before/after rehabilitation. In addition, this kind of tests are sensitive to changes in the oro-facial system as they are significantly related to maximum voluntary bite force and dental state ⁶⁸. The limit of this kind of test is referred to the appropriate tester selection. In fact, hard test materials may not be idoneous for children and edentulous subjects as well as for subjects suffering from neurodegenerative diseases. If on one hand, hard tester materials could be not adequately fragmented by these kinds of patients due to a lowered maximum bite force, on the other, very soft test foods for subjects with an adequate MP or ME may not be able to discriminate differences in their MF. Moreover, expensive and special equipment are need, and particles of the specimens have to be completely removed from the mouth after the comminution procedure, which could be very difficult in the case of small fragments. Furthermore, dysphagic patients risk aspirating these particles and comminution test could constitute a health hazard ⁸³.

Mixing Ability Tests

Mixing Ability Tests are performed using a not-nutritive plastic test material that is chewed for a fixed number of chewing strokes. Once retrieved from the oral cavity, the bolus is gently removed
from the excess of saliva using a paper towel, placed in a transparent plastic bag, and flattened to a wafer of, generally, 1 mm thickness ¹⁰⁶. Then, the bolus is analyzed, obtaining digital images of both sides of the wafer under standardized light conditions and subsequentially evaluated in its form and/or color ¹⁰⁷. Moreover, performing masticatory tests using a cohesive test food like chewing gum or wax are useful and indicated for the assessment of MF in dysphagic subjects or in those with assumed severely impaired MF ⁷³.

Generally, mixing ability tests can be performed using a two-colored chewing gum/wax or a color changing chewing gum. In the first case, a two-colored (e.g. blue and red) plastic material is chewed for a fixed number of masticatory strokes, usually 20¹⁰⁸. Twenty is also the number of chewing cycles for which mixing ability tests and comminution tests correlate significantly. The combination between the two portion of the chewing gum reflects the effect of the oro-facial system on bolus preparation and depends on the rheological proprieties of the tester and on the efficiency of the stomatognathic system. Bolus assessment can be performed using a 5-graded scale for a nominal evaluation of color mixture and bolus form, a opto-electronic assessment, or a combination of both ^{109–111}. In the second case, a color-changing chewing gum is chewed between 20 and 200 times and is able to change its color varying from yellow-green to red, depending on the MP of a subject ^{112,113}. As in the first case, chewing gum analysis can be performed using either a visual analysis using a validated and reliable color scale or a colorimeter method using the L*a*b color space model ^{112,114}.

Overall, mixing ability tests are quick, easy, and cheap to perform, requiring very little time and effort. They are able to be performed on demented and dysphagic subjects, avoiding the risk of aspirating tester particle sizes ¹¹⁵ and those subjects deemed to have an impaired MF ¹¹⁶. Moreover, the visual assessment of the bolus allows a fast chair-side evaluation, and the test can be applied

also for the evaluation of children MF. Opto-electronic assessment methods can be applied especially in research settings due to the need of a flat-bed scanner and a computer for the analysis (in some cases, trained evaluators are also needed). However, recently, smartphone application were developed and will further simply the whole procedure ^{111,117}. Of course, as any other tester material, each type of chewing gum or wax must be validated. In particular, chewing gums become softer during the chewing process and their color may be modified after being exposed to saliva. With the increase of masticatory strokes, chewing gums lose their hardness and become easy to chew. In the case of subjects with an adequate MF, mixing chewing gum color can be easily achieved and color saturation may occur, affecting the accuracy of the test ⁸⁶.

In conclusion, mixing ability tests can find their application in the assessment of masticatory impairments in dental offices, hospitals and geriatric facilities because of their performing quickness and simplicity. Moreover, they may be used in large epidemiological studies and in settings in which there is a lack of facilities and know-how for comminution tests. At the same time, they may be not indicated for the indirect assessment of bite force as the tests depend less on the maximum voluntary bite force.

Other Chewing Tests

Other chewing tests use different testers, such as gummy jelly and encapsulate granules. Gummy jelly is chewed for a fixed number of masticatory stokes: the analysis is based on the determination of the concentration of glucose or β -carotene dissolved in saliva or using a photographic analysis of the image of the comminuted tester particles and the posterior calculous of the surface area^{118–121}. The use of encapsulate granules are based on the dye concentration difference of fuchsine, erythrosine or adenosine triphosphate that coat granules prepared and sealed in a rubber or polymerizing vinyl chloride capsule ^{122–125}. The subject freely chews a single capsule containing

the pigment-coated granules for a fixed number of chewing cycles. The capsule is then open and its content is dissolved in water with constant stirring: the obtained solution is filtered and the dye concentration is assessed using a spectrophotometer (each pigment must be analyzed in a specific wave length) ^{122–125}.

Overall, these methods are reasonably fast to perform, with good reproducibility, and can be applied in epidemiological studies. Limits can be characterized by the not representative nature of the rubber capsule in terms of food size, shape and stiffness that can affect the test since chewing an artificial single piece reduces the validity and reliability of these methods in comparison to the other. Moreover, in the gummy jelly method, the comminuted gummy jelly collection and rinsing and the manual ingredients dissolution need skilled personnel even if fully automatic measuring device was proposed ¹¹⁸.

Swallowing threshold

The swallowing threshold is the moment in which a subject is ready to swallow the bolus resulted from the cumulative effect of masticatory cycles on the bolus itself. The number of chewing cycles needed to be prepare a bolus ready to be swallowed and the median particle size of the food bolus just before swallowing can be used as reference parameter to assess ME ^{126,127}.

The number of masticatory strokes needed to comminute a food for swallowing is almost constant within a subject, given a type of food, but vary greatly among different subjects. As stated previously, a good chewer does not necessarily swallow a food after a number of chewing cycles smaller than a bad chewer and vice versa. Food rheological and volumetric characteristics affect the number of chewing cycles and the bolus characteristics at the swallowing threshold. For example, a dry and hard food requires more time before swallowing than soft and moist one ¹²⁸. In conclusion, these tests provide information about how a subject normally swallow a food. They

find their indication especially in subjects suffering from functional dyspepsia with impaired dentition since a decreased comminution can determine a gastric emptying rate reduction, an antral or fundal hypomobility, a lack of antro-pyloric-duodenal coordination and the inhibition of intestinal feedback ¹²⁸.

2.2.2 Indirect Objective Assessment Analysis

Several factors contribute to the masticatory process, such as dentition, jaw muscle activity, bite force, tongue function, lip force and saliva, whose assessment can indirectly provide information about the MF of a subject ^{86,129}. Jaw kinematics and the neuromuscular control of the jaw muscles are important aspects, playing a role in food comminution.

Jaw movement can be recorded using a magnetic, an electromagnetic or an optical motion analysis system, assessing jaw velocity, jaw acceleration, chewing frequency (masticatory cycles per minute) and jaw kinematics ^{130,131}. When evaluating muscle jaw activity and bite force, bipolar surface electrodes are usually used and located on masseter and anterior temporal muscles. Muscle activity can be measured under either dynamic (chewing frequency) or static conditions (maximum voluntary clenching) ¹³¹. Maximum voluntary bite force can be also assessed asking the patient to clench as hard as possible a force transducer placed between the molar teeth ¹³². A force transducer can be also used in order to measure maximum tongue pressure asking the subjects to raise the tongue with maximal voluntary effort ¹³³. When measuring lip function, the so called lip-length and snout indices and maximum lip force measurements can be used ^{134,135}. Finally, salivary flow rate can be measured by the mechanically stimulated salivation using a piece of tasteless parafilm.

between 0.52 and 4.55 mL/min ¹³⁶. In addition to this, also oral moisture can be measure using an electronic tool that is able to assess oral hypofunction. ^{137,138}.

Overall, few modifications in the mean chewing frequency is a suitable criterion for the identification of good masticatory neuromotor control, whereas large changes could indicate an impairment in MF ^{139,140}. Food rheological properties and chewing phase affect the masticatory process and muscular work, especially in the first moments of the chewing process. In fact, hard and plastic foods are chewed slower than soft and elastic ones at the beginning of the process, while no effect on the mean chewing frequency was detected ¹⁴¹. Beside food properties and chewing phase, also saliva plays a crucial role in chewing and swallowing. In fact, its amount and composition are related to bolus formation, the number of chewing cycles and food time in mouth before swallowing. However, no universally recognized value for jaw kinematics assessment is available and studies on jaw kinematics and jaw muscle activity can only be performed in laboratory settings. Moreover, masticatory frequency is generated by a central pattern generator placed in the brainstem and it is specific to each subject. For this reason, chewing frequency can be used for intra- but no for inter-subject comparisons.

2.2.3 Subjective Assessment

Self- or proxy-assessed masticatory function

These masticatory function assessment tests are aimed at evaluating the perceived or self-assessed quality or discomfort of own MF or food intake ability ^{142–144}. They have been defined as the tests aimed at assessing the MA of a subject, through interviews or questionnaires. Several validated tools have been proposed in literature, basing their items on dichotomic answers, 5-point Likert scales, categorical answers or Visual Analogue Scales (VAS). Among the different methods used

to assess MA (e.g. the quality of masticatory function questionnaire¹⁴⁵, the chewing function questionnaire ¹⁴³, the screening for masticatory disorders in older adults questionnaire ¹⁴⁴ and the food intake ability questionnaire ¹⁴⁶), the most comprehensive tool for the self-evaluation of MA is the one developed by the WHO in 2001, the International Classification of Functioning, Disability and Health (ICF) for oral functions ^{147,148}. Overall, ICF provides the human functioning description with regard to body structure and function, activities and participation. ICF is universal as it is applicable to all the categories of population whatever the health condition or the cultural context of the subject. Briefly, with regard to different oral function related items, it is possible to categorize the impairment extension of a subject, following a disability scale. In particular, the following categories can be defined: *category* 0 if there is no impairment; *category* 1 defines a mild impairment (a problem is present less than 25% of the time with a tolerable intensity and happened rarely over the last 30 days); *category 2* describes a moderate impairment (a problem is present less than 50% of the time with an intensity that affects in day to day life which happened occasionally over the last 30 days); category 3 outlines a severe impairment (a problem is present more than 50% of the time with an intensity which partially disrupts day to day life and happened frequently over the last 30 days); category 4 defines a complete impairment (a problem is present over 95% of the time with an intensity that totally disrupts day to day life and happened every day over the last 30 days); category 8 if there is not sufficient information to specify the severity of the impairment (not specified); category 9 (not applicable) if the test is inappropriate (e.g. gastrotomized or tube feeded subjects).

Overall, the self- or proxy-assessed MF tests are able to evaluate also different aspects of human mastication such as adaptation and the psychological aspects. Moreover, they are deemed to be useful screening tools for epidemiological studies and for the assessment of MF with cost and time

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savings ^{149,150}. Even though poor correlation between objective and subjective MF assessment tools was detected, these methods can help practitioners to analyze the effect of different treatments on subjects' well-being and be complementary to the objective tests ¹⁵¹. In any case, choosing an appropriate questionnaire tool is crucial, taking into account the type of answers to questionnaire questions and the type of foods indicated through the questions of the questionnaire that can be specific to a cultural of socio-economic context.

2.3 Bolus sampling device for the objective measurement of Masticatory Performance (Italian Patent)

When considering mixing ability tests, scientific community's attention was focused only on images analysis (software). Only little attention was addressed to bolus flattening technique and procedure (hardware). Bias may accidentally occur during boluses flattening procedure due to samples deformation. Moreover, in a clinical setting, tool miniaturization and dental office space optimization are desirable.

In this sense, during the Ph.D Course, we have developed a novel chewing gum sampling device, the so called "Chew-Meter", able to flatten to a standard size the bolus derived from the application of the two colour mixing ability test. To date, the invention is following the *iter* for intellectual property protection (Italian patent pending).

This invention aims at developing a bolus sampling device for the objective measurement of human masticatory performance. The invention fits within the context of the objective assessment of MP. Among the several methods proposed to assess human mastication, the two-colour mixing test involves the chewing of two-coloured chewing gum and, based on the degree of bolus mixed area, the determination of the MP of an individual.

Although this test is one of the best methods to objectively assess MP, there are several drawbacks in the handling of the obtained boluses. After having obtained the bolus, the procedure described in the scientific literature imposes the insertion of the bolus in a transparent plastic bag, the compression of the bolus with the help of a rectangular template with lateral raises of 1 mm on which applying a force with a foot or with the palm of a hand, and the scan of the compressed bolus. However, the compression of the bolus using a foot or the palm of a hand, without an antirotational system on the template, could cause bolus distortions due, for example, to an unintentional rotation at the time of application of the force on the bolus and a non-uniformity in the pressure applied to the bolus during compression. Finally, the absence of a standardized surface on which flatten the bolus could also be a source of bolus distortion. The assessment depends significantly on the operator and could vary from one to other. In this context, there was the need to set up a device for repeatable and standardized bolus sampling, without causing undesirable distortions.

Briefly, the sampling device includes a first concave body with a bottom surface to support a bolus and a second body configured to be removably inserted within the first body to flatten the bolus placed in the first body. The first body has at least one matching element of the second body placed at a defined distance from the bottom surface, outlining an end-of-travel position of the second body in the first body. Moreover, each body has means of coupling, configured to bind the second body to the first one in order to avoid bolus distortion and the rotation of the second body in the first one and vice versa (Figure 1).



Figure 1 Figures 1 and 2 schematically illustrate a non-sampled bolus and a sampled bolus by means of a the bolus sampling device; Figure 3 shows in a perspective schematic view the device; Figure 4 illustrates a schematic perspective view of the first body of the device of Figure 3; Figure 5 shows a schematic perspective view of a second body of the device in Figure 3; Figure 6 illustrates a frontal schematic view of the second body of Figure 5; Figure 7 illustrates in a perspective schematic view the third body of the wheel sampling device; finally, Figure 8 shows the third body of figure 7 with some parts removed to better highlight the other ones.

With reference to Figure 1 – Fig.1, a bolus to be sampled is indicated with 100. Bolus (100) is obtained by chewing two chewing gum of different colors (e.g. pink and blue chewing gum) for a defined number of chewing cycles. As stated elsewhere, 20 chewing cycles are enough to discriminate human MP performing the two-color mixing ability test ¹⁰⁸. Regardless Figure 1 – Fig.2, 200 indicates a bolus flattened by the bolus sampling device.

Bolus (200) (Figure 1 - Fig.2) is compressed if compared to bolus (100), with a standard thickness (S) determined by the sampling device. With reference to Figure 1 - Fig.3 to Fig.8, the bolus sampling device is indicated with 1. The S of the sample is 1 mm, as required by the scientific literature ¹⁵². Device 1 includes a first concave body (2) with a bottom surface (3) aimed at supporting a bolus (100) and a second body (4) designed to be removably inserted within the first concave body to flatten the raw chewed chewing gum (100) in a flattened bolus (200). The first body (2) has at least one matching element (5) with the second body (4), placed at a defined distance from the bottom surface (3). An end-of-travel position of the second body (4) with respect to the first body (2) is so defined (Figure 1 - Fig.4). The position of the matching element (5) defines the measurement of S of the flattened bolus (200), which preferably is equal to 1mm. The second body (4) is removable from the first body (2). The second body (4) is mobile compared to the first body (2) approaching the surface of the bottom (3) of the first body (2), until the operator finds the matching element (5), when compressing bolus (100). Once the flattened bolus (200) is obtained, the second body (4) is removed from the first body (2), in order to remove also the obtained bolus. The first body (2) includes coupling means 6 designed to constrain the second body (4) to the first body (2) in order to avoid the rotatory movement of the second body (4) with respect to the first body (2). Advantageously, the means of coupling (6) guarantee a correct compression of the bolus 100, given only by a relative translation between the second body (4)

and the first body (2), avoiding unwanted rotations between the second body (4) and the first body (2). With reference to the first body (2), it has an opening of access (7) to the bottom surface (3) and a lateral surface perimeter (8), which develops from the bottom surface (3) to the opening access (7). The means of coupling (6) of the second body (4) with the first body (2) are provided on the lateral surface (8). The means of coupling 6 include a first notch (9) and a second notch (10). The first notch (9) and the second one (10) are designed to contain a respective first portion (11) and a second one (12) of the second body (4). The first body (2) is cylindrical in shape with its own axis of symmetry (2a). According to a preferred realization form, the first body (2) has an external diameter between 7 and 10 cm, extremes included and a height between 3 and 4 cm, extremes included. With reference to the axis of symmetry (2a), the first notch (9) and the second notch (10) develop parallel to it. The first notch (9) and the second one (10) have a straight shape. According to a preferred design, the first notch (9) and the second one (10) are arranged on the opposite side of the axis of symmetry (2a) of the first body (2). With reference to the matching element (5), it has an annular conformation symmetrical to the axis of symmetry (2a) of the first body (2). The matching element (5) has a plane head surface (5a) intended to receive the second body (4).

The bottom surface (3) is black colored in order to photograph the flattened bolus (200), without taking it from the bottom surface (3). With reference to the second body (4), represented in Figure 1 - Fig.5 and Fig.6, it has a bottom flat surface (13) aimed at compressing the raw bolus (100). The bottom surface (13) of the second body (2) is intended to contact the head surface (5a) of the first body (2). The second body (4) has a lateral surface (14) on which a first portion (11) and a second portion (12) are present. Items 11 and 12 can be inserted in the notches (9 and 10) of the first body (2), respectively. The second body (4) has a head surface (15) equipped with a grip (16)

to handle the second body (4). The second body (4) is cylindrical in shape with its own axis of symmetry (4a). According to a preferred design, the second body (4) has an external diameter between 6 and 9 cm, extremes included, and a height between 3 and 4 cm, extremes included. The first portion (11) and the second one (12) develop parallel to the axis of symmetry (4a) of the second body (4). The portions 11 and 12 are in the form of straight ridges.

Device 1 includes a third body (17), which can be removably inserted with the first body (2), represented in Figure 1 - Fig.7 and Fig.8. The coupling of the third body (17) to the first body (2) is of the male/female type. The third body (17) can be inserted within the first body (2) if the second body (4) is removed from the first body (2). The third body (17) includes an image detection medium (18). Preferably, the image detection medium (18) is a photo-camera. The third body (17) is concave and has inside a head surface (19) and a lateral surface (20) defining an inner chamber (21). The inner chamber (21) of the third body (17) holds at least a part of the first body (2) to define the union between the third body (17) and the first body (2). The first body (2) has a border perimeter (26), along the opening access (7), designed to fit into the chamber (21) of the third body (17). The detection devices (18) are placed on the leading surface (19) of the inner chamber (21), preferably in the central position. The head surface (19) shall have at least one through hole (27) for the installation of detection equipment (18). When the third body (17) is associated with the first body (2), the detection medium (18) is configured to detect one or more images of the flattened bolus (200) placed on the bottom surface (3) of the first body (2). In particular, the inner chamber (21) is in communication with the bottom surface (3) of the first body (2). Chamber 21 has one or more light sources (22) placed at a defined distance by the means of detection (18). The light source (22) allows to detect images of the flattened bolus (200) once the third body (17) is coupled to the first body (2) that effectively closes the inner chamber (21).

The defined distance between light source (22) and detection medium (18) allows to guarantee a standardized brightness condition of the internal chamber (21). In this way, the images of the flattened bolus (200) are detected under standard conditions. The third body (17) is cylindrical in shape with its own axis of symmetry (17a). According to a preferred design form, the third body (17) has an external diameter between 7 and 10 cm, extremes included, and a height within an interval between 8 and 11 cm, extremes included.

Preferably, the light source (22) has an annular conformation symmetrical to the axis of symmetry (17a). Advantageously, the ring shape allows the light source (22) to be distributed evenly within the inner chamber (21). The inner chamber (21) is of opaque white color, to favor the illumination and to reduce eventual disturbances in the illumination of inner chamber (21) (e.g. reflection avoidance). Device 1 comprises an activation element (23) for the detection medium (18) and the light source (22).

Recording equipment (18) comprises a temporary storage unit (24) accessible via a data transmission network (29), preferably wireless. The images detected by the detection media (18) are temporarily stored in the storage unit (24) to be exported from it. Once exported they are treated using analysis software to calculate the individual's MP. Device 1 includes the power unit (25) of the detection medium (18) and of the light source (22). Preferably, the power unit (25) is placed in the first body (2).

Device 1 comprises a control unit (28) configured to adjust one or more parameters of both the detection medium (18) and the light source (e.g. focus, contrast, color intensity, intensity of the light beam). These parameters may depend on the staining of the flattened bolus (200) and its reflectance, given the possible presence of saliva. Advantageously, the first body (2) and the second body (4) allow to obtain samples of bolus (200) to be analyzed that have a physical

homogeneity and constant thickness, given by physical distortions avoidance (thanks to coupling media, 6, only a relative translation between the two bodies is allowed). Conveniently, the third body (17) allows to detect digital images of the flattened bolus (200) in repeatable conditions, thanks to the presence of a wireless camera and standardized lighting.

Another not negligible advantage is given by the minimization of the spaces conferred by the conformation and the dimensions of the device 1 according to the present invention. The device 1 is in fact comfortably transportable, allowing it to be placed temporarily in a dental office to the need, replacing in full the location of a scanner, necessary to the state of the art to detect images of the flattened bolus (200).

This invention allows to sample boluses using the sampling device 1, as previously described. The method includes a step of insertion of the raw bolus (100) into a transparent bag, preferably made of a non-reflecting material. When inserting the bolus (100) in the non-reflecting bag, the sample is placed in the center of the bottom surface (3) of the first body (2). Then, the second body (4) is coupled with the first body (2). The second body (4), within the first body (2), arrives at the end of the race position. Subsequentially, the second body (4) is removed from the first body (2) and is replaced by the third body (17). The light source (22) and of the means of detection of image (18) of the third body (17) is activated. Images of both the sides of the flattened bolus (200) are captured. The overturning phase of bolus (200) can be performed manually. Once the image acquisition phase is completed, the image extrapolation phase is performed from the detection (18) for subsequent graphic analysis. Recently, we have developed a smartphone application (it runs both in iOS and Android environments) able to guide the user in the assessment of MP and to analyze automatically the bolus derived from the application of the two-color mixing ability

test. The device has been used in both pre-clinical and clinical studies and the results of its application are reported in the present Ph.D Thesis.

In conclusion, according to the present invention, the device advantageously guarantees the realization of boluses having a physical homogeneity, in particular with constant thicknesses, minimizing as much as possible the known inconveniences that could occur during both the compression and the image acquisition phase. Moreover, the incorporation of a digital camera on the top of the device avoids the use of scanners in the dental office. If on the one hand the present device has the potential to become an instrument that clinicians could use during their daily clinical practice to assess human masticatory performance, on the other the high novelty introduced by the so called "Chew-Meter" may generate skepticism among practitioners, as for all the new technologies. In addition, a high effort should be performed in order to further develop the Chew-Meter.

2.4 A Novel Color-Based Segmentation Method for the Objective Measurement of Human Masticatory Performance ¹⁰⁸

2.4.1 Introduction

Human mastication is a complex biomechanical process that is aimed at properly preparing food for swallowing and digestion ⁸⁴. Quality of life, general health, and social relations could be influenced by impaired mastication. Furthermore, the assessment of mastication could also be able to provide information about the efficiency and status of many oro-facial structures ¹⁵³. During their daily office practice, clinicians may be required to evaluate patient mastication. Thus, it is necessary to have an objective method that is able to assess it. The latter not only should aim

at evaluating oral function but also at providing information about patient disability and a real

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indication for prosthetic rehabilitation. Over the past few years, several methods have been proposed in order to assess MP. MP is defined as the individual ability to grind a test food after a fixed number of chewing strokes ⁸⁵. Most of the studies on MP used comminution tests in order to study the particles of both artificial and natural food by sieving the comminuted food ⁸⁶. Even though these systems are considered to be the gold standard in assessing MP, patient discomfort and high costs hinder their use. Besides the need for expensive and special equipment, particles of the specimens must be completely removed from the mouth after the comminution procedure, which could be very difficult in the case of small fragments. Furthermore, dysphagic patients risk aspirating these particles ⁸³. Previous studies showed that the results of color-mixing ability tests highly correlate with those of comminution ones in assessing MP and proposed that color-mixing tests should be used on patients with chewing deficiencies ^{106,154}.

In 1991 and 1995, Liedberg and Öwall proposed and described for the first time a new method for the study of MP. This test consisted of the chewing of two-colored chewing gum for 10, 20, 40, 60, 80, and 100 strokes. The specimens were then scored visually into a 1 to 5 color mixture scale, in accordance with a reference scale established in pilot tests ^{109,155}. In 1999, Prinz understood the need for simple objective tests for the assessment of oral function. Chewing gum containing two different colors was chewed. Once the bolus was removed from the oral cavity, it was flattened, and a digital image was taken. It was concluded that flattening the bolus before the assessment provided a more reliable evaluation than observing the bolus in its raw state. Nevertheless, the subjective assessment was enough ¹⁵⁶. In 2007, Schimmel et al. tested the reliability and reproducibility of quantitative data obtained from the two-color chewing gum test and found that the visual assessment of the specimens was less reliable than computerized analysis. The authors

concluded that the digital evaluation of the color mixing degree of two-color chewing gum was a precise and reliable method to assess MP ¹¹⁰. As the computerized image analysis was carried out using a commercially available software package, in 2013, Halazonetis et al. proposed novel software for the quantitative assessment of MP ¹⁵⁷. The two-color chewing gum test and the dedicated software were validated in 2015 ¹⁰⁷.

Even though the method proposed by Schimmel et al. is considered to be the gold standard among mixing ability tests, the digital analysis of boluses derived from its application is still controversial. Different software packages were proposed in order to analyze the specimens, highlighting the great variability of those methods and the need to find the best possible one to use both in clinical and research settings ^{157–162}. Unfortunately, the software may introduce errors due to the manual resizing and segmentation of bolus images and the difficulty in distinguishing hue variations between the gum and the background, especially for well-mixed chewing gum. Extracted data highly depend on the accuracy of the segmentation process, which is an essential step in computer vision and in automatic pattern recognition based on image analysis ¹⁶³. Clustering systems could provide high discriminative power that can discern different color regions in each image using color information. This could allow for quantifying the degree of color mixing depending on the number of chewing cycles or different dental patterns.

The aims of the present study were to propose an automatic color-based segmentation method to separate mixed and unmixed colors of images derived from the application of the two-color chewing gum mixing test and to determine the validity of this method for the assessment of MP.

2.4.2 Materials and Methods

The study was performed on a sample of 50 subjects that were enrolled as students at the Dental School of Marche Polytechnic University, Ancona, Italy, from March 2019 to May 2019. They were young adults (mean age: 24.3 ± 2.7 years), homogenous for sex (25 males and 25 females), fully dentate (28 teeth totally, third molars were not considered, 14 occluding pairs), in good general health condition, with a decayed missing filled teeth (DMFT) less than 4, without temporomandibular joint (TMJ) disorders and in angle class I. They were taken as the reference population and were recruited for their similar oral conditions. All participants volunteered for this study. No sample size calculation was made, but a sample size that was 2.5-fold greater than those used in the studies of Schimmel et al. and Yousof et al. was enrolled ^{110,164}. The study was performed in full accordance with the World Medical Association Declaration of Helsinki and it was approved by the Local Review Board of the Dental School (ODO-ID062019). All the tests were performed with the informed and written consent of each subject and in accordance with the abovementioned principles.

The masticatory test was performed using the two-colored chewing gum mixing test as described by Schimmel et al., which involves the use of two-colored chewing gum (Hue-check Gum®, Orophys GmbH, Muri b. Bern, Switzerland)^{107,157}. Each sample was chewed for 5, 10, 20, 30, and 50 chewing cycles. A rest interval of 1 min was set between each masticatory test in order to avoid muscle strain. Briefly, blue and pink gums were roughly stuck together using moderate strength. Specimens were placed on the tongue of the participants who were asked to chew in their usual way. Participants were allowed to change the chewing side during the test. An operator counted the masticatory strokes and at the defined chewing cycle, the operator asked the participants to stop. Boluses were retrieved from the mouth of the participants and gently dabbed with a paper towel in order to eliminate the possible excess of saliva. Specimens were collected by inserting them between two sheets of transparent plastic and flattened to a standard thickness of 1 mm. Figure 2 shows the used chewing gum and the boluses after 5, 10, 20, 30, and 50 masticatory cycles.



Figure 2 Chewing gum specimens: (a) the two-colored chewing gums; (b) the two-colored chewing gums stuck together; (c-g) chewing gum boluses after 5, 10, 20, 30, and 50 masticatory cycles, respectively; (h-l) flattened chewing gum boluses after 5, 10, 20, 30, and 50 masticatory cycles, respectively; (h-l) flattened chewing gum boluses after 5, 10, 20, 30, and 50 masticatory cycles, respectively.

Both sides of each bolus were photographed, and, one side at a time, all images were postprocessed using the segmentation algorithm developed by the Department of Industrial Engineering and Mathematical Sciences of the Polytechnic University of Marche, Ancona, Italy. The algorithm automatically analyzes the images and gives as output as the percentage of the mixed portion of the sample. The proposed algorithm exploits the k-means clustering method and the analysis is carried out by measuring the areas of different colors in pixels. In the laboratory tests, accurate digitization of each sample was performed. The procedure was divided into two main phases:

- An acquisition phase, in which the images of both sides of the boluses were captured using a micro-camera (FREDI HD MINI WIFI 1080P, Shenzhen Jinbaixun Technology Co., Ltd., Shenzhen, China) put at a standard distance of 10 cm. Each wafer was acquired on a dark background in order to make it easier to separate the different colors.
- 2. A processing phase, which was necessary to classify an image into different color groups. A PC (Intel® Core TM i7-6700HQ, 2.6 GHz, 16.0 GB) with Windows 10 Home was used as the electronic equipment to process the acquired data and MATLAB® R2019b (MatLab, MathWorks, Natick, MA, USA) software to enable the color-based segmentation.

K-means clustering was the method adopted for image processing in order to segment the mixed and unmixed areas of the chewing-gum. This method classifies each pixel in a region, according to its properties, such as color, intensity, or texture, to distinguish it from adjacent regions. For each region, called a cluster, it is possible to define k centroids placed as far as possible away from each other ¹⁶⁵.

Each point belonging to a given data set is associated with the nearest centroid until no point is pending. When all objects are assigned to the initial group centroids, this step is executed to recalculate the positions of k new centroids as the barycenter of the clusters. After these k new centroids are found, a new binding needs to be done between the same data set points and the nearest new centroid. Because of this loop, it is possible to notice that the k centroids change their location systematically until the centroids do not move anymore. The goal of the algorithm is to minimize the total intra-cluster variance; this is equivalent to minimizing the pairwise squared deviations of points in the same cluster.

The areas of each chewed gum were divided into a set of four classes according to the pink, blue, mixed-color, and background areas. At the end of the analysis, the proposed software provided the percentage of the chewing gum mixed portion, discriminating the different MPs of the subjects (Figure 3). In particular, a higher percentage indicated a higher MP.



Figure 3 Digital analysis of the samples: (a) first view of the scene; (b) the user has to upload the image for analysis (one side of the bolus per time); (c) once the image is uploaded, it is ready to be analyzed; (d) the software automatically analyzes the image and gives as output the percentage of the mixed portion of the sample.

The specimens corresponding to 20 masticatory cycles were re-analyzed by the same investigator 1 week after the first set of measurements in order to evaluate the intra-rater reliability. All the images were also analyzed by a second investigator to assess the inter-rater reliability. Twenty-five percent of the participants (n = 12) were asked to repeat the test at 20 chewing cycles 1 week after the first test in order to assess the test–retest reliability. In addition, each specimen was classified visually and subjectively into poorly, moderately, or highly mixed categories by an investigator in order to assess the construct validity, as suggested by Montero et al. ¹⁶¹.

The statistical software R (R version 3.5.3 (11 May 2019) - "Great Truth," Copyright© 2020 The R Foundation for Statistical Computing, Vienna, Austria) was used to perform the statistical analysis. The MP values were analyzed using the Pearson correlation coefficient in relation to the number of masticatory strokes. Group comparisons were performed using two-sample t-tests with unequal variances and Mann–Whitney tests. The similarity between the test samples of intra-rater, inter- observer, and test–retest reliabilities were evaluated using the interclass correlation coefficient (ICC) in a two-way mixed-effect model, with absolute agreement. According to Koo and Li, values less than 0.5, between 0.5 and 0.75, between 0.75 and 0.9, and greater than 0.9 are indicative of poor, moderate, good, and excellent reliability, respectively ¹⁶⁶. A value of p < 0.05 was considered statistically significant.

2.4.3 Results

The study was performed on a sample of 50 subjects who were enrolled at the Dental School of Marche Polytechnic University, Ancona, Italy, from March 2019 to May 2019. They were young adults (mean age: 24.3 ± 2.7 years), homogenous for sex (25 males and 25 females), fully dentate (28 teeth totally, third molars were not considered, 14 occluding pairs), in good general health

condition, with a DMFT of less than 4, without TMJ disorders, and in angle class I. Table 1 summarizes the sociodemographic and clinical data of the study sample.

Sociodemographic Data	Value	
Sex		
Male	n = 25	
Female	n = 25	
Age	24.3 ± 2.7	
Clinical Data		
Natural teeth	28 ± 0.0	
Occlusal units	14 ± 0.0	
Filled teeth	1.2 ± 1.0	

Table 1. Sociodemographic and clinical variables of sample size (n = 50). Data values are expressed as the number of participants for sex and mean and standard deviation (SD) for all other values.

A total of 250 specimens were obtained from the subjects enrolled in the present study. Both sides of the images of the samples, 500 images in total, were analyzed. It was possible to estimate the mixed areas of the chewing gums. Each color area was returned as the sum of the partitioned pixels and, for each participant, the mixed fraction of the two colors after 5, 10, 20, 30, and 50 chewing cycles was calculated.

The acquired data and their elaboration through the K-means method were used in order to assess the differences between the MPs of the 50 participants at 5, 10, 20, 30, and 50 chewing cycles. The results are summarized in Table 2.

Table 2. Masticatory performance (MP) values according to the number of chewing cycles. The results are expressed as the mean and standard deviation (SD).

Chewing Cycles	5	10	20	30	50
MP	0.228 ± 0.122	0.319 ± 0.139	0.443 ± 0.138	0.466 ± 0.111	0.830 ± 0.247
Sex					
Male	0.259 ± 0.110	0.352 ± 0.129	0.470 ± 0.157	0.466 ± 0.147	0.880 ± 0.237
Female	0.197 ± 0.128	0.286 ± 0.145	0.416 ± 0.112	0.466 ± 0.059	0.780 ± 0.252

No significant difference was observed between MPs of the male and female populations at 5, 10, 20, 30, and 50 chewing cycles (p > 0.05). A higher number of masticatory cycles corresponded to a higher value of MP. Considering the 50 participants and the different numbers of chewing cycles, the percentage of mixed colors in the samples increased with the increase in the number of strokes (Figure 4). The MP increase was statistically significant when varying from 5 to 10 cycles, from 10 to 20 cycles, and from 30 to 50 (p < 0.05). The MP increase was not statistically significant (p > 0.05) between 20 to 30 masticatory strokes. A ceiling effect was detected at 50 masticatory cycles in 22 participants, thus not allowing for distinguishing between different MPs. The Pearson correlation coefficient explained the relationship between the number of chewing cycles and MP (r = 0.78, p < 0.05).

The ICC results showed good correlations for both the intra- and inter-rater reliabilities (r = 0.85 and r = 0.77, respectively). The ICC showed an excellent test–retest correlation of the proposed

software (r = 0.93). The proposed method was found to have adequate construct validity as statistically significant differences were detected between the MPs of the three different groups classified subjectively into poorly, moderately, and highly mixed (p < 0.05).

2.4.4 Discussion

In the present study, a novel method for the assessment of MP was proposed and described. MP was evaluated using a sample constituted by fully dentate young adults who chewed a two-colored chewing gum for different numbers of chewing cycles. Previous studies stated that a reduction in MP is more pronounced in patients with less than 20 teeth. In this report, 50 fully dentate participants were enrolled to determine the validity of the proposed method as they were considered ideal chewers ^{69,167}. They were fully dentate, in angle class I, with a DMFT < 4, and with no TMJ disorders. The choice of a sample with these characteristics was considered crucial for the validation of the proposed software ^{156,157,160,162,168,169}.

A recent systematic review stated that no established method for the clinical assessment of MP with a high level of evidence is available. Furthermore, all the proposed assessment methods reported in the literature need lab-intensive equipment, such as digital image software or sieves¹⁶⁹. In 2018, Buser et al. tested the accuracy of a custom-built smartphone application, trying to overcome the need for specific and expensive instruments. Unfortunately, further development of that proposed application is not likely to happen ¹¹¹. In the present study, MP was evaluated using the k-means clustering technique. For each chewing gum wafer, mixed areas were evaluated. This clustering system using color information provided a high discriminative power for regions present in each image, reducing the errors due to manual segmentation. In particular, this method

expressed MP as the percentage of mixed areas, where an MP of 1 indicates an optimal MP and an MP of 0 indicates the total absence of it.

Using the k-means clustering method, the MP was evaluated for each bolus by estimating the mixed and unmixed areas. This clustering system is able to provide high discriminative power for the regions present in each image using color information. Moreover, a reduction of the errors due to manual segmentation could be achieved. A significant increase in color mixing was observed in accordance with the different numbers of chewing cycles, as demonstrated in previous studies ^{107,152}. MPs corresponding to 5, 10, 20, 30, and 50 chewing strokes were compared. According to previous studies ^{152,156,170–172}, a significant increase in color mixing depends on the number of chewing cycles, where 20 masticatory cycles were enough to determine the different MPs of subjects with different dental statuses. The variability between the MPs for different numbers of masticatory cycles, as shown in Figure 4, was in accordance with this datum, showing that 20 chewing strokes could be considered the right number of strokes that could show a difference in MPs. Indeed, a statistically significant difference was detected between MPs at 20 and 30 cycles, making it clear that more masticatory cycles are not necessary. Furthermore, increasing the number of strokes after 50 caused a ceiling effect that did not allow for distinguishing between different MPs, and therefore, confirmed the previous theory.



Figure 4 Means and standard deviations of MP in relation to the stepwise increase in the number of masticatory cycles.

According to Koo and Li, ICC scores showed a good correlation concerning both the intra- and inter-rater reliabilities and an excellent correlation between the results of the first test and those of the second one ¹⁶⁶. In addition, a subjective evaluation of the specimens was performed in order to test the construct validity. Each specimen was classified into poorly, moderately, and highly mixed groups to verify whether the obtained MP values were consistent with the objective assessment. According to Prinz, the subjective assessment was coherent with the digital one, but errors can be introduced if a reference scale is not available and, of course, it cannot be as reliable and precise as the computer-assisted objective assessment of MP ¹⁵⁶.

Overall, the results of the present study indicated that the proposed software was able to assess MP objectively and automatically. Nevertheless, this study has some limitations. The proposed method was compared neither to comminution tests nor to other chewing gum analyzer software. Moreover, the algorithm works in MATLAB. This could lead to complications if the user is not familiar with such a programming environment. Finally, low-quality images could have influenced

the accuracy of the results of this study, thus not allowing for drawing conclusive statements regarding the reference MP values. However, the study aimed at describing and evaluating the validity of the novel proposed segmentation method as a proof of concept for the assessment of MP. Furthermore, the algorithm is freely available and, as the algorithm is pasted in MATLAB, the user only needs to upload bolus images. Finally, bolus images were captured using a prototypical tool aimed at standardizing the whole manipulation and acquisition procedure. Even if the used WiFi microcamera was not the most high-quality one, it was able to acquire images, from which, different MPs were assessed. Future research should pursue a double aim: (a) the standardization of the chewing gum manipulation procedure and the miniaturization of the equipment needed, and (b) the assessment of recognized MP values via the daily utilization of chewing mixing ability tests in clinical practice. Moreover, further research is needed to determine the validity and reliability of the proposed method in the assessment of MP in different dental statuses and in comparison to the gold standard tests.

2.4.5 Conclusions

Within the limitations of the present study, the proposed software allowed for analyzing the different MPs corresponding to different numbers of chewing cycles. This method provided an automatic identification of the colored areas, which were perfectly separated from the background of the images. It was demonstrated as being able to quantify the percentage of the mixed color area, providing quantitative data through the computerized analysis by using the best possible segmentation and minimizing human interaction.

Chapter 3: The Ageing Mouth

The stomatognathic system is affected by the aging process as well as any other human apparatus and system. Knowing the anatomical structures and the physiological ageing processes is crucial to understand if the changes that may occur to the oro-facial system belong to a physiological or a pathological process. Overall, ageing is characterized by a progressive, generalized and irreversible degeneration of the biological structures of the organism, affecting the systems and the function of the vital organs ¹⁷³. From a biological point of view, ageing is determined by the loss of the adaptative capacity of the cells that accumulate molecular damages, determining various changes in gene expression and structural changes and becoming more susceptible to diseases development.

According to the literature, the physiological ageing process of a healthy mouth does not necessarily determine a loss in function, having not negative sequalae on Oral Health Related Quality of Life (OHRQoL) or nutritional state. In fact, the physiological adaptative capacity is very large and, as long as a number of at least 10 pairs of occluding teeth are maintained and in absence of otherwise impairment of oro-facial functions, nutritional state and OHRQoL can be preserved. In very old subjects, only in case of masticatory force loss and age-related dysphagia due to sarcopenia, an impairment of MF can be observed ¹⁷⁴.

3.1 Oro-facial physiologic age-related changes

Many structures constitute the mouth and the oro-facial system, ensuring a physiologic function. In particular, teeth, periodontal tissues, mucosa, muscles and temporomandibular joint play a role in the maintenance a healthy oral environment. Moreover, these structures can be monitored, and disease-preventive measures can be achieved. Beside them, also peripheral and central nerve fibers as well as nuclei and central representation areas play a role in maintaining a healthy oral environment, but preventive measures are difficult to be performed.

3.1.1 Teeth

During the lifetime of an individual, the 28 teeth (wisdom teeth are generally excluded) undergo several insults, such as mechanical wear during both normal and parafunctional function (chewing and tooth grinding or habits, respectively) and intrinsic and extrinsic chemical impacts that affect enamel thickness. Due to enamel thickness reduction, teeth tissues become more susceptible for caries development. Moreover, with age, the enamel may become more brittle, exposing teeth to cracks and chippings.

Age-related changes in dentine determine the reduction of the diameter of both dentinal tubules and pulp chamber volume, due to the continuous apposition of tertiary dentine, with a reduction in the organic content and an increase in the mineral one. Dentine and pulp sclerosis can be both age-related (physiologic) and pathologic, due to a defense mechanism against extrinsic insults ¹⁷⁵. In addition, pulp may lose a part of its reactive capacity as a consequence of the general immunescence and signs of connective tissue fibrosis and calcifications can be found ¹⁷⁶.

3.1.2 Periodontal Tissues

Very little evidence about the age-related changes in periodontal tissues is available. Only a thickening of root cementum, a reduced periodontal ligament turnover, a reduction in gingival stippling and a progressive decrease in periodontal ligament width can be assessed during the ageing process without a proven functional impairment ¹⁷⁷. When considering the bony compartment of periodontium, it is important to bear in mind that bone metabolism is heavily

related to individual hormone status, thus determining age-related changes. In particular, a dysregulation in osteoclastic and osteoblastic activity may occur, with an increased activity of osteoclasts and a decreased activity of osteoblasts. Structural changes such as bone trabecula thinning and bone density reduction can be observed with ageing ¹⁷⁸. Even though changes in bone resorption after tooth extraction are well documented, no age-relation was found. Nevertheless, due to the progressive and irreversible nature of resorption, very old subjects might suffer from severe alveolar bone atrophy that depends on the life span edentulism. Finally, generalized osteoporotic processes are deemed to have only a little influence in the alveolar ridges ¹⁷⁹.

3.1.3 Oral Mucosa

Only few are the age-related oral mucosa changes. Elderly's oral mucosa shows little macroscopic and physiological differences compared to the one of younger subjects, if no local or systemic diseases are detected ¹⁸⁰. The reason lays in the fact that, in contrast to the skin of the body, oral mucosa is not affected by environmental factors. The atrophy of oral mucosa, that may occur with age, may result in a decrease in mean epithelium thickness, a decrease in cell density and mitotic activity, and a slowdown in tissue regeneration and healing process, that appears to be slower after the fifth decade of life. Moreover, some neurophysiological functions, such as touch and vibration, are slightly reduced in elderly, while no changes in pain perception are detected ¹⁸¹. The changes in oral mucosa that occur if a removable prosthesis is worn are still debated. Regional differences can be assessed in terms of epithelium thickness, resilience, or load-bearing capacity. Moreover, the age-related changes of the mucosa of hard palate includes a macroscopic flattening of the physiological horizontal elevations. From a histologic point of view, the marginal and median

fibrous areas enlarge at the expense of gland-bearing zones. Hyperkeratosis of the hard palate is likely to be observed in elderly.

3.1.4 Salivary Glands

Physiologically, between 0.5 and 1.5 L of saliva per day are produced, under the influence of the parasympathetic stimulation with acetylcholine neurotransmitter that mediates an increase in saliva production. In addition to general involution, a distinct fibrosis or a fatty degeneration of salivary glands parenchyma can be often observed. The salivary duct may show irregularities with extensions and epithelial growths and inflammatory processes that may result in the duct lumen obstruction. Even though striking histopathological changes may occur, only few age-related physiological changes in salivary glands function can be observed. In fact, the physiologic parameters of saliva secretion are quite comparable between younger and older subjects. Saliva quantity and composition remain stable, with only a slightly increase in the mucous components proportion in elderly. Dry mouth depends almost exclusively on pathological processes ¹⁸².

2.1.5 Oral Function

Chewing and swallowing processes are divided in oral, pharyngeal and esophageal phase. During the oral phase, food undergo a fragmentation process, called masticatory process, aimed at reducing food in particles and transforming it into a coherent bolus ready to be swallowed, once moistened with saliva ¹⁸³. Mastication is driven by a complex interaction between teeth related structures and central and peripheral control centers. Even though age alone cannot determine changes in chewing, bolus formation and transportation or in speaking, in presence of pathological changes of the oral environment or cognitive decline, an impairment of MF can be observed. In

other words, if oral structures are maintained in health, age can only marginally affect the orofacial system thanks to its large physiological spare capacities ¹⁸⁴. Overall, the chewing process can be classified in the following categories: totally healthy, moderately impaired and totally impaired, reflecting the masticatory capacity (mastication is completely achieved), the compensatory adaptation (mastication is slightly disturbed, with a possible increase in the number of chewing cycles for bolus preparation) and the masticatory disability (the function is deficient due to the individual failure in making a proper food bolus; adaptability is overstretched and food is not chewed properly, so that subjects belonging to this category change their diet or swallow unchewed food, with negative nutritional consequences and/or excessive workload to digestive tract) ^{185,186}. Moreover, subjects have a preferred way to process food in their mouths, determining food texture preferences and choices. In particular, some studies suggested that subjects can be divided into four different categories according to the "mouth behavior": chewers, crunchers, smooshers and suckers. In turn, these four groups fell into two different modalities of "mouth action": chewers and crunchers use their teeth to breakdown foods, whereas smooshers and suckers prefer to manipulate food between the tongue and the roof of the mouth ^{187,188}.

Finally, age-related sarcopenia of masticatory muscles, associated with tooth loss, can determine the onset of MF impairment ¹⁸⁹. Sarcopenia is able to reduce the maximum available masticatory force, thus possibly limiting the dietary food intake. Moreover, an age-related sensory function decline can be observed, impairing bolus formation and transportation and reducing taste and smell senses ^{190,191}. In addition, the prevalence of dysphagia increases with age and up to the 69% of institutionalized elderly suffer from different degrees of dysphagia ¹⁹², probably due to age-related sarcopenia but more likely to an underlying neurological disease processes such as stroke and dementia ^{193,194}.

Chapter 4: Finding links between General and Oral Health

Maintaining a good oral health status is crucial for the pursuit of an adequate MF, the prevention of pain and discomfort for what concerns the oro-facial system, the control of local and systemic inflammatory processes, as well as the preservation of a good quality of life. Unfortunately, the assessment of poor oral health status among elderly, especially in frail older adults, is not very usual, and often their conditions remain not diagnosed or not treated. This represents an important health care issue in primary care institutes as poor oral conditions are an indicator of adverse health outcomes including mortality in community-dwelling elderly individuals. The accumulation of a slightly poor status in oral conditions strongly predicted physical frailty, sarcopenia, need for long-term care, and mortality, implying the need to raise awareness about oral frailty and to strengthen the oral health-related literacy to promote healthy ageing ⁵⁶.

4.1 Oral Health and Pneumonia

Aspiration pneumonia is a preventable condition, resulting in clinical deterioration, escalation of preventable hospital admissions, increased mortality, and greater cost of care, particularly for older adults in residential aged care facilities ^{195,196}. Risk is increased by the presence of co-morbidities, including dementia and stroke ¹⁹⁷. Generally, the presence of high amount of bacterial biofilm on tongue and on dental or prosthetic surfaces not only cause local inflammation but may increase the risk of developing respiratory tract infections. Older people's dependence on care, including care for the mouth and assistance with eating, increases the risk of poor oral hygiene with associated colonization of not only the usual oral bacteria, but also exogenous pathogens ¹⁹⁸. A recent systematic review stated that an improvement in oral healthcare significantly reduce the risk of aspiration pneumonia and its risk of death ¹⁹⁹. At the same time, the reduction in Full Mouth

Plaque Score Index determines the decrease of the amount of potential respiratory pathogenic bacteria and of the risk of pneumonia by positively affecting the sensitivity of the swallow and cough reflex ²⁰⁰. Overall, proper oral care provided by older adults themselves or by their caregivers, consisting of brushing teeth after every meal, cleaning dental prostheses, and weekly professional oral care, are the most effective procedures aimed at reducing the risk of aspiration pneumonia, as respiratory infections are one of the most common causes of death, especially in frail older adults. Moreover, wearing dentures during the night increases the risk of aspiration pneumonia occurrence up to twice: removing and cleaning adequately dentures is highly recommended ²⁰¹. Finally, in order to identify subjects at risk for aspiration pneumonia, the revised oral assessment guide (ROAG) can be considered a specific and sensitive tool ²⁰².

4.2 Oral Health and Cardiovascular Diseases

Current literature defines periodontal diseases as a possible responsible of the migration of periodontal pathogens into the bloodstream causing multiple potential bacteremia events and the dissemination of these bacteria in non-oral tissues. Generally, body chronic infections have been mentioned as causal factors of cardiovascular disease: in particular, the migration of periodontal pathogens into the bloodstream could exacerbate or induce atherosclerosis, acting as a pro-inflammatory and procoagulant factor ²⁰³.

Even though a certain causal relation was not found between periodontitis and cardiovascular diseases, an augmented relative risk of developing cardiovascular diseases in periodontopathic subjects was detected, ranging between 1.2 and 1.3 ²⁰⁴. With regards to cerebrovascular episodes, the relative risk for stroke in subjects with periodontitis is raised up to 2.8 ²⁰⁵. When considering people aged over 80 years old, an association between root caries and cardiac arrhythmias was

found, suggesting the need for older adults to be examined for this kind of cardiac disease if root caries are diagnosed ²⁰⁶. Another study investigated the relationship between oral health and mortality as a consequence of cardiac events, reporting that people with sensitive teeth were less likely to manifest cardiovascular events than people without sensitive teeth. At the same time, masticatory impairment, gum inflammation and mouth stickiness were not significantly related to cardiovascular death ²⁰⁷.

The physio-pathologic process that leads to cardiovascular accidents lays in the fact that oral derived bacteremia allows bacteria to colonize and destabilize atherosclerotic plaques. Moreover, bacteremia is also the responsible of the increment of systemic inflammatory burden, witnessed by studies which reported an improvement in cardiovascular diseases biomarkers after oral care procedures ²⁰⁸. In conclusion, the improvement in oral hygiene procedures allows to attenuate the cardiovascular risk related to periodontal diseases, reducing the risk up to 14% ²⁰⁹. The pursuit of information sharing about the correct oral hygiene procedures and regular dental appointments are crucial in order to reduce cardiovascular events in subjects with heart diseases.

4.3 Oral Health and Diabetes Mellitus

Current literature states that periodontal disease can be considered as an early indicator of the development of Diabetes Mellitus, especially Type 2 one ²¹⁰. Generally, a bidirectional relationship between oral health and diabetes was found. In particular, if an insufficient glycemia control is diagnosed, an increased risk of periodontitis, tooth loss, xerostomia and burning syndrome is likely to happen ²¹¹. At the same time, periodontitis in a diabetic subject can complicate glycemic level, increasing the risk of cardiovascular and renal complications.
The physio-pathologic process that lays in both diabetes and periodontal disease is the increased systemic inflammation that both cause. This systemic inflammation is responsible of the onset of the augmentation of insulin resistance, glycemic control difficulties, periodontal damage. High level of glycemia leads to the damage of many organs and processes, such as wound healing and infections defense. Especially due to affected wound healing process and an increase in infection risk, diabetic subjects have a double or threefold risk to suffer from periodontal disease. The latter was consider as the sixth complication of diabetes ²¹². Periodontal therapy is safe and effective in people with diabetes, and it is associated with HbA1C reduction (0.27-0.48% after 3 months). Healthcare professionals play a crucial role in the treatment and prevention of diabetes mellitus and its oral manifestation. Finally, every physician, oral healthcare professional and diabetic subject should follow the European Federation of Periodontology (EFP) and the International Diabetes Federation (IDF) report consensus guidelines for the improvement of early diagnosis, prevention and co-management of diabetes and periodontitis ²¹³.

4.4 Oral Health and Dementia

Alzheimer's disease is considered as the most usual cause of dementia, followed by vascular dementia. Even though "pure" dementia is diagnosed in relatively young patients, subjects older than 75 years old usually suffer from a mixed form. Beside a hereditary disorder that can determine the occurrence of Alzheimer's disease, the most common risk factors are age, ethnic origin, lack of exercise, bad habits, polypharmacy, educational level, BMI, comorbidity and environmental factors ^{214,215}. In accordance with the most universally accepted hypothesis, Alzheimer's disease is caused by an excessive deposition of the so-called beta-amyloid protein that creates plaques outside the brain cells. Moreover, intracellularly, entanglements of Tau protein are usually

observed. Generally, Tau protein is responsible of the transportation of nutrients through the cell, but if it is tangled, it determines cell death.

Recently, many studies about association between poor oral conditions and cognitive decline were published, suggesting different physio-pathological mechanisms ²¹⁶. Dominy et al. suggested that Alzheimer's disease and periodontitis share a common bacterium that is partially responsible of the onset of both conditions: *Porphyromonas gingivalis* ²¹⁷. In addition, gingipains, a class of *P. gingivalis* proteases, were found in association with neurons, Tau protein, and beta-amyloid in specimens from the brains of subjects with Alzheimer's disease ²¹⁸. However, the study was conducted on mice and the results were not replicated on humans. Other authors suggested that periodontitis is associated with an increased amount of amyloid accumulation in brain, indicating that periodontal inflammation/infection may increase the risk for brain amyloid deposition ²¹⁹.

Overall, there is not a consensus regarding the association between oral health and cognitive decline. Periodontal disease could induce systemic inflammation, blood-brain barrier disruption, neuroinflammation, brain amyloid, neurodegeneration, and cognitive impairment. As stated in a recent systematic review, periodontal disease, considering that its sequalae are all pathogenic pathways of Alzheimer's disease, through its inflammatory and bacterial loads, could be considered as a biologically plausible risk factor for Alzheimer's disease ²²⁰. On the contrary, another systemic review stated that the strength of the evidence for the association between oral health and cognitive decline is weak, and the findings were often inconsistent ²²¹.

Overall, older subjects diagnosed with dementia have a poor oral health. When comparing demented patients with their pairs without dementia, a worse oral health status is showed in terms of gingival bleeding, number of periodontal pockets, oral mucosa lesions, reduced salivary flow rate, coronal and root caries and retained teeth root ^{222,223}. On the contrary, for most subjects with

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mild cognitive impairment or dementia, the swallowing ability and chewing ability are generally perceived as good by them-selves or their caregivers ²²⁴.

Many factors can contribute to the worsening of oral health conditions of subjects with dementia, such as cognitive, medical, and functional changes. In fact, agitated behavior, reduced cooperation, and declined motor skills and coordination, can determine difficulties in performing daily oral health care, resulting in plaque accumulation and, in association with reduced salivary flow rate and a modification in dietary habits, a higher risk of caries. Moreover, motor coordination, subjects suffering from dementia face barriers to dental care, especially in residential aged care facilities, due to their reluctance to undergo oral treatments ²²⁵. Also, while recent studies about the prevalence oro-facial pain in cognitive impaired subjects show rates up to 25% ^{226,227}, verbal communication about oro-facial pain can be difficult because of the short-term memory loss and language disturbances, like aphasia ²²⁸.

Finally, the number of retained teeth and the percentage of denture wearers are the same between demented and not demented older subjects ²²². However, in case of severe dementia, a reduction in the percentage of denture use is found, probably due to a lowered tolerance, a decrement in oral musculature control, a worsening in salivary quantity and quality, and an augmented risk of denture loss. In addition to this, when being edentulous for a long time, an extended alveolar process absorption is observed, thus reducing denture retention and stability. Denture diminished retention may increase the risk of aspiration of especially the lower dentures in severe cognitive impaired elderly ²²⁹.

4.5 Oral Health and Frailty

The clinical condition of frailty is one of the most challenging aspects of population ageing that might affect the willingness of elderly to live in health long at home. Frailty is a multidimensional geriatric syndrome and a result of age-related decline in numerous physiological systems, which together produce an increased susceptibility to poor health outcomes such as disability, hospitalization, reduced quality of life and even death ^{230,231}. Moreover, its prevalence ranges between 4 and 59% in institutionalized elderly populations and is higher in women than in men²³². According to Fried et al, who proposed a standardized phenotype of frailty in elderly, frailty is not synonymous with either comorbidity or disability: in particular, comorbidity is an etiologic risk factor for frailty, and disability is an outcome of it ²³³. Overall, four different features can be described concerning frailty: it is associated with an augmented risk for the development of adverse health outcomes; it is associated with reserve capacities or resources loss; it is a dynamic state, and it is potentially reversible by intervening on modifiable factors; frailty risk is proportional to age even if there are also other risk factors.

When dealing with frailty, the evaluation of oral function impairment can contribute to the promotion of a healthy life as the first signs of frailty can be observed in the oral cavity. In fact, considering the high prevalence of frailty and its adverse health outcomes, an early diagnosis is crucial. Hakeem et al. published a systematic review aimed at assessing whether there is a relationship between baseline oral health and change in frailty over time among elderly. They concluded that fewer number of teeth and oral function impairment are longitudinally associated with frailty. On the contrary, periodontal disease is not associated with frailty as studies showed inconsistent results ⁵⁸. Moreover, the new concept of "oral frailty" has been recently introduced in

in Japan. In particular, oral frailty was defined as "a series of phenomena and processes that lead to changes in various oral conditions (number of teeth, oral hygiene, oral functions etc.) associated with aging, and accompanied by decreased interest in oral health, reduced physical and mental reserve capacity, and an increase in oral frailty leading to eating dysfunction; the overall effect is of deteriorating physical and mental function" ⁵⁶. The results of recent studies show that not only maintaining 20 or more teeth, but also oral health indices improvement (e.g. maintenance and improvement of oral function, good diet, and maintenance of nutritional status) are important ⁵⁷. In fact, impaired oral function is an important risk factor for inducing malnutrition and sarcopenia. In addition, elderly who need dental prostheses are significantly more likely to be prefrail or frail as older adults with at least 20 teeth have a lower risk to develop frailty when compared to their edentulous pairs ²³⁴.

4.6 Oral Health and Other Disorders

Even though scientific evidence is scarce, some studies reported a link between oral health and general health conditions such as rheumatoid arthritis, chronic kidney disease, osteoporosis, and cancer ^{235–238}. Oral health affects general health in terms of an increased tissue inflammatory response to mostly microbiological insult. Moreover, recently, periodontitis was found to be associated with a higher risk to develop severe manifestation of COVID-19, in terms of intensive care unit access, need for assisted ventilation, and death, due to an increase in D-dimer, C-Reactive Protein and white blood cells blood levels ²³⁹. Overall, treating oral infections, through bacterial biofilm removal and local inflammation check-up, is recommended in order to lessen the systemic inflammation associated with chronic systemic disorders.

Finally, falls are considered a major problem in elderly, potentially leading to hospital admission, disability, and even death. Tooth loss was shown to be slightly associated with an increased risk of hip-fracture ²⁴⁰. Moreover, dentition and tooth loss have an impact also on gait velocity and stability. In fact, both dentate and fixed restored subjects have a significantly higher gait velocity than the one of denture wearers or edentulous subjects who have also their gait stability affected²⁴¹. Body balance also was showed to be associated with tooth loss: in particular, dentate subjects have a better body stability than edentulous one. Moreover, in edentulous healthy older adults, subjects wearing or not a dental prosthesis does not affect body balance. An explanation of this lies in the fact that with periodontal ligament loss, elders also lose the proprioceptive sensation and feedback that plays a role in balance ^{242,243}.

Chapter 5: Oral Health and Nutrition

"Prima digestio fit in ore": this Latin locution means that the first part of food digestion begins in the oral cavity. In fact, the mouth is the first part of digestive tract, and it is responsible of the preparation of food through biting, chewing, adding saliva for bolus preparation, and bolus transportation into the stomach. It is clear that oral health and nutrition are strictly associated the one to the other as poor nutritional status can affect oral health and poor oral health can influence the dietary intake of a subject, resulting in malnourishment ²⁴⁴.

Poor dietary intake was associated with partial or complete tooth loss: in particular, when teeth are lost, chewing difficulties can be determined due to a decreased MF, restricting food choice and nutritional status ²⁴⁵. Particularly, these subjects experience difficulties in eating hard foods (e.g. fruits, raw vegetables, meat) while preferring soft foods, often rich in calories and fat, or restricting their diet ^{246,247}.

Older adults living in residential aged care facilities have a twofold risk of malnourishment if they have a poor oral condition and difficulties in eating due to both natural teeth and artificial teeth problems, as well as hyposalivation, dysphagia, oral pain, periodontal disease, mucosal lesions or ill-fitting dentures ²⁴⁸. Oral health can be responsible of the maintenance of an adequate nutrition. In fact, dental care and dental treatment in association with tailored nutritional advices may improve the nutritional status of a subject ²⁴⁹.

5.1 Masticatory Function and Food Perception

Mastication is strictly connected to food and nutrient intake, as chewing behavior affects nutritional status ⁷⁹. The perception of sensory and internal texture qualities of food, appreciable during chewing and swallowing, affect food choices and dietary variety. Generally, this perception

generates satisfaction and pleasure of eating. With ageing, food intake quality can be affected due to a reduced hedonic perception, decreased food appeal and appetite, restricting diet. Having a good oral health and maintaining natural teeth are crucial not only for chewing and adequate nutritional status, but also for general health and cognitive function ²⁵⁰.

The retention of natural teeth allows elderly to maintain their own chewing behavior ²⁵¹. Anyway, with ageing process, physio-, para-physiologic and pathologic conditions can determine changes in position, shape and number of teeth, thus affecting chewing performance and efficiency as well as food fragmentation and diet ²⁵². In addition, tooth loss may be linked with nutritional deficits as it influences diet in terms of food selection, modality of cooking, and food preparation. At least 20 well-distributed teeth with 6 occluding teeth by intact premolars and at least one pair of occluding molars guarantee a functional dentition, a satisfactory eating ability in elderly, and play also a role in swallowing ^{10,253,240}. The use of dentures may determine specific difficulties in eating hard consistency foods, foods with seeds, that can be trapped under the denture, causing discomfort and pain, and sticky foods, that can displace the prosthesis if attached to the acrylic denture. These kind of denture-related problems can lead to social isolation, due to embarrassment, pain and discomfort during meals ^{254–256}.

Finally, in older adults wearing complete dentures, MP was found to be 1/6 of the one of younger subjects ²⁵¹. This change is mainly due to lowered force intensity and smaller and slower food processing ²⁵⁷. In particular, when considering the over 85-year-old adults, the prevalence of MP reduction was up to 44% and this decrease can be explained by the physiologic deterioration ^{258–}

5.2 Oral Health and Nutritional Challenges

The oral cavity is able to recover from the constant environmental challenges. In fact, oral cavity is a dynamic ecosystem that mixes together a stable but varied microbiological environment controlled by host immune system, epithelial cells and saliva. Moreover, it is able to lessen the severity of health conditions that may affect the mouth , being of great importance especially in older adults ¹⁴.

In particular, poor oral hygiene status may affect the bacterial biofilm, determining the onset of a dysbiotic microbiome. The latter may lead to the development of caries and tissues inflammation, thus causing attachment loss, tooth mobility, root exposure, and root caries. As a result, the clinical condition of functional dentition loss is obtained, increasing the risk for malnourishment and health challenges ²⁶¹. Tooth loss, derived from poor oral conditions, deteriorates MF and is considered a disability in late life ²⁶². Dietary changes (e.g. lowered intake of fruits, vegetables and fibers) can determine the radicalization or the dysbiotic mutation of periodontal pathogens ^{262,263}. In addition to this inflammation mediators, generated by oral inflammation, can spread systematically, affecting general health ²⁶⁴. Poor oral health affects nutrition, well-being and overall quality of life²⁶⁵.

Tooth loss is an indicator of cumulative stresses in the human body and is a predictor of shortened longevity. Moreover, edentulousness has been considered as a mediator of reduced muscle strength with a subsequent impact in body function, well-being and overall physical activity ^{263,266}. The use of dentures or being edentulous is associated with nutritional status. Studies showed that edentulous subjects who used to wear a pair of complete dentures were able to eat a higher number of foods than those wearing only one denture, with a lessened risk for malnourishment and an increased nutritional status ^{267,268}. The reduction in calorie-protein nutrient intake was associated

with malnourishment and immune deficiencies in elderly, also due to zinc deficiency ²⁶⁹. In addition, immune senescence may determine bacterial proliferation not only from direct bacterial action but also by indirect immune cell pathology ²⁷⁰.

5.3 Impact of Elderly Masticatory Performance on Nutritional Status: An Observational Study ¹⁶⁷

5.3.1 Introduction

According to recent studies ^{1,5}, the percentage of elderly people will significantly increase over the next few decades. The increase in average life expectancy, attributable to the great socio-economic development of the last century, means an aging population and highlights the need to pay more attention to the health of these subjects.

Oral health is an important part of general health, affecting the quality of life ²⁷, and it is therefore important to try to preserve it as much as possible, preventing and possibly treating all the problems most frequently encountered by the elderly that could lead to edentulism ^{11,28,29}. The latter is also the main cause of the reduction in MP and ME. According to previous studies, at least 20 teeth are necessary to maintain an adequate MP. The distribution and the number of teeth, as well as the quality and the type of oral rehabilitation, affect MP ^{69,271,272}.

Nutritional status is an important health factor in elderly patients, and its assessment is crucial to prevent numerous acute and chronic diseases ²⁷³. Oral disorders associated with reduced masticatory function negatively affect the nutritional status of the elderly ²⁷⁴. The inability to chew and shred food properly tends to exclude some basic food from their diets, such as meat, fruit and vegetables, favoring the consumption of refined carbohydrates, fats or soft or overcooked food that risks losing most of its micronutrients ¹⁵.

The limitations on the dietary habits of edentulous subjects do not allow them to have adequate nutrition, exposing them to a greater risk of protein energy malnutrition (PEM) than their peers with an adequate number of natural teeth ²⁷⁵.

As stated by Yoshida et al., tooth loss leads to a change in diet and could, therefore, be linked to eating disorders such as obesity and malnourishment ²⁷⁶. Nevertheless, it is not clear if changes in eating habits and the intake of certain food may determine the onset of conditions that could lead to edentulism and to a further reduction of the masticatory function.

According to Tada and Miura ²⁷⁷, an association between reduced chewing performance and obesity was shown. This could be linked to the fact that reduced masticatory function leads the subject to consume a greater amount of soft food, including food rich in fats or refined carbohydrates, and reduce the intake of fruits, vegetables and meat. However, a high consumption of sweet food in elderly subjects may be due to many other causes, such as the reduction of gustatory and olfactory perception, as well as economic and psychological factors ^{15,278,279}. The aim of this study is to verify the existence of an association between the reduction of MP in the elderly with different degrees of edentulism and the presence of nutritional changes.

5.3.2 Materials and Methods

Participants were enrolled among patients undergoing medical outpatient treatment at the Dental Clinic of the Marche Polytechnic University of Ancona, during the period from April 2017 to December 2017.

Inclusion criteria were: a) \geq 65 years old; b) self-sufficient subjects.

Exclusion criteria were: a) diagnosis of neurodegenerative diseases (e.g., Alzheimer, Parkinson, dementia); b) history of strokes; c) diagnosis of diseases and disorders affecting the muscular

system; d) orofacial pain; e) diagnosis of xerostomia and/or Sjogren syndrome; f) clinical conditions that require a specific diet (e.g., type 2 diabetes mellitus, coeliac disease).

The study was performed in accordance with the principles of the Declaration of Helsinki as revised in 2013, and it was approved by the Local Institutional Review Board (Project identification code: OD2017 01, Date of Approval: 03-16-2017, Dental Clinic Local Institutional Review Board, Ancona, Italy). Written, informed consent was obtained from all participants.

All the patients underwent dental examination. For all participants, individual sociodemographic data, as well as general and oral health data, were recorded.

A masticatory test was carried out, using the two-color mixing test as described by Schimmel et al. ¹¹⁰. Briefly, the test involves the use of two-colored chewing gums (Hue-check Gum®, Orophys GmbH, Muri b. Bern, Switzerland). Each sample was chewed for 20 chewing cycles, as this number of strokes allows the assessment of MP. Boluses were collected, inserted between two sheets of transparent plastic and brought to a standard thickness of 1 mm. Standardized photos were taken from both sides of each bolus, and all the obtained images were processed by computer, analyzing the measure of the area of pixels of different colors using the K-means clustering method ¹⁰⁸. At the end of the analysis, the software revealed the extent to which the chewing gums mixed, and allowed discrimination between the different MPs of the subjects (Figure 1).



Figure 1 Digital analysis of a sample: (a) flattened bolus side A and side B; (b) mixed bolus side A and side B.

Participants underwent both a qualitative and a quantitative food interview to assess their nutritional status. Participants communicated the type of food they generally consume in each meal and the relative quantities in grams. The obtained food data were entered into the appropriate software (WinFood, Medimatica, Martinsicuro, Italy) through which the percentages of carbohydrates, lipids and proteins that the patient consumed were shown, based on the Italian food composition tables ^{280,281}.

Weight (in kg), height (in cm) and waist circumference (WC) (in cm) were measured. Body weight was measured, without shoes and wearing minimal clothes, with a scale to the nearest 0.01 kg. Height was measured to the nearest 0.1 cm with a stadiometer (Seca, Hamburg, Germany) at enrolment. WC was measured with a metric band along a horizontal plane parallel to the floor. It was considered as the minimum circumference between the rig cage and the navel, with the subject standing with relaxed abdominal muscles.

Then, a bioimpedance analysis was performed with a DF50 Body Composition Analyzer (ImpediMed, Brisbane, Australia). The bioimpedance measurement represents the resistance of the body to the passage of a low intensity electric current (800 μ A) at high frequency (50 kHz) and

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is performed by placing a pair of electrodes, connected to the measuring instrument, on the back of the hand and another two on the back of the foot. Impedance (Z), Resistance (R), Phase Angle (PhA) and Reactance (Xc) were evaluated.

Statistical analysis was performed using the statistical software R (R version 3.5.3).

5.3.3 Results

During the period April 2017 to December 2017, 217 subjects attended the Dental Clinic. Only 76 individuals met the inclusion criteria and were enrolled in this study. There were 42 men and 34 women aged over 65 years (mean age: 75.8 ± 5.6). The patients' medical histories revealed the presence of some systemic diseases, such as: hypertension (55 subjects), osteoarthrosis (18 subjects), heart failure (13 subjects), chronic obstructive pulmonary disease (9 subjects), and Hashimoto's disease (1 subject). No association was detected between MP and those pathologies (p > 0.05).

Dental examination revealed that 68.5% of the participants had more than 20 teeth, with a mean of 21.8 \pm 5.7 teeth, with a mean of missing teeth of 6.16 \pm 5.66 and with a mean number of occluding pairs of 10.5 \pm 2.9. Of the participants, 48.7% (n = 37) declared that they had no drinking habits, 38.2% (*n* = 29) declared that they generally consumed less than or equal to 2 daily units of alcoholic drink equivalent and 13.1% (*n* = 10) more than 2. Further, 43.4% (*n* = 33) of the participants were nonsmokers, 10.5% (*n* = 8) were former smokers, 23.7% (*n* = 18) generally smoked less than 10 cigarettes or equivalent per day and 22.4% (*n* = 17) more than 10. A Full Mouth Plaque Score (FMPS) was assessed for each participant, considering six surfaces per tooth and having set 20% as the cut-off. Results showed that 31 subjects had an FMPS lower than 20%.

The MP of the 76 participants in relation to the number of lost teeth was evaluated. Results are shown in Table 1 and Figure 2. The MP was 0.45 ± 0.19 , showing no statistically significant differences between males and females (0.46 ± 0.20 vs. 0.43 ± 0.18 , respectively), as shown in Figure 3.

Table 1. Results of Masticatory Performance by Sex, BMI and Waist Circumference. Data are expressed as mean and standard deviation (SD).

Sex	MP ^a	SD	<i>p</i> -value
-Male	0.46	0.20	
-Female	0.43	0.18	
			>0.05
BMI ^b	MP	SD	
-Normal	0.49	0.18	
-Overweight	0.45	0.17	
-Obese	0.41	0.23	
			>0.05
WC ^c Male	МР	SD	
-<102 cm	0.48	0.11	
-≥102 cm	0.46	0.21	
			>0.05
WC Female	МР	SD	
- < 88 cm	0.50	0.14	
-≥88 cm	0.45	0.17	
			>0.05

^a Masticatory Performance; ^b Body Mass Index; ^c Waist Circumference.

Masticatory Performance and Missing Teeth



Figure 2 Masticatory performance by number of missing teeth.



Figure 3 Participants' masticatory performance in relation to: (*a*) gender, (*b*) BMI, (*c*) waist circumference in males and (*d*) waist circumference in females.

The participants were divided into three groups according to BMI. Of the subjects, 22 were within the normal weight range ($18.5 \le BMI < 25$), 39 were overweight ($25 \le BMI < 30$) and 24 obese ($BMI \ge 30$). For each group, MP was evaluated (Figure 4). Even though obese patients had a lower

MP (0.41 ± 0.23) than both overweight (0.45 ± 0.17) and normal weight (0.49 ± 0.18) patients, a statistically significant difference was not observed among the groups.

As WC has two different cut-offs according to the sex of the patient, the participants were divided into groups on the basis of WC. The relationship between MP and WC in male and female subjects was evaluated, and the results are shown in Figure 3. In the male population, subjects with a WC above the threshold had an MP lower than those under it $(0.46 \pm 0.21 \text{ vs}. 0.48 \pm 0.11)$. Likewise, in the female population those who had a WC above the threshold had a lower MP than those under it $(0.45 \pm 0.17 \text{ vs}. 0.50 \pm 0.14)$. Also in this case, the data did not reach a statistically significant difference.



Figure 4 Participants' masticatory performance in relation to bioimpedance parameters such as: (a) resistance, (b) impedance, (c) reactance and (d) phase angle.

The evaluation was repeated, taking as reference values the parameters of Resistence (R), Impedence (Z), Phase Angle (PhA) and Reactance (Xc). Results are shown in Figure 4. According to statistical analysis, no relationship was noticed between MP and any of the studied parameters (p > 0.05).

5.3.4 Discussion

The study was performed on a sample of 76 patients, who underwent a dental examination during which a masticatory test was performed using the two-color mixing test, and who participated in a nutritional interview.

Comparing the data related to the chewing performance test, it was found that, in accordance with the literature ^{69,271,272}, MP in subjects with less than 20 teeth is lower than in those with more than 20. In particular, Figure 2 shows how dramatically the line representing MP decreased from 0 to 5 missing teeth. Then, the line decreased moderately.

Sheiham et al. investigated the possible relation among oral conditions, the intake of selected nutrients and blood-derived values of key nutrients in adults aged over 65 years old ²⁷⁹. It was demonstrated that a lower nutrient intake was associated with a higher number of missing teeth. In particular, being edentulous was statistically related to the intake of several key nutrients. Food selection could prevent edentulous subjects, and in general subjects with less than 20 teeth, from having adequate nutritional status. In the sample, even if subjects defined as obese had a lower MP than the ones defined as overweight and normal weight, a statistically significant difference among the groups was not found. Moreover, in the sample there were no underweight subjects (BMI <18.5). So, it was not possible to evaluate the influence of MP on undernutrition. Also, comparing MP and WC in male and female populations, no statistically significant differences were detected between subjects within the physiologic range and the ones above it, even if MP was lower in the group of participants with WC above the threshold. A possible explanation for these results lies in the fact that the analyzed sample consisted of independent and self-sufficient subjects.

MP was also evaluated in relation to bioimpedance. Bioimpedance is a non-invasive method that analyzes tissue properties and provides reliable information about body composition by the transmission of a series of alternating electric currents through the body. In a previous study it was proven that bioimpedance parameters are more appropriate for nutritional status assessment than BMI ²⁸². No association among MP and Z, R, PhA or Xc was detected in this study. Nevertheless, it is important to consider that the reduction of lean mass is frequent in elderly patients and is linked to PEM ²⁸³. The latter may also be more pronounced in subjects with high masticatory deficits. The difficulty in chewing meat may lead to a lower consumption of proteins that is not always offset by the intake of other protein bearing foods that are more easily chewed. As reported in previous studies, cases of undernutrition and malnutrition are more frequent in institutionalized or hospitalized subjects. Taste and swallowing difficulties, bad oral conditions, drug consumption, constipation, neurodegenerative diseases and reduced daily life activities are some of the factors that are considered to be strongly related to malnutrition or to the risk of it ^{284–287}.

Another aspect that could affect MP is sarcopenia, defined as a generalized and progressive skeletal muscle disorder that is linked to an increased likelihood of adverse outcomes such as falls, fractures, physical disability and mortality ²⁸⁸. It not only has serious consequences for general health but also affects the oral component, thus reducing the strength of the muscles involved in chewing and swallowing. Musculoskeletal mass loss in subjects with sarcopenia also affects oral musculature, with an associated nutritional deterioration and a worsening of the sarcopenic pathology itself ^{289,290}. Sarcopenia is generally associated with ageing, worsens general health status and increases the risk of hospitalization. It would, therefore, be interesting to extend the sample to institutionalized or hospitalized elderly patients in the future.

5.3.5 Conclusions

The sample consisted exclusively of independent and self-sufficient patients without serious impairment of the general state of health and, apart from a few cases, most of them presented a good oral condition. An association between reduced MP and a worsening of nutritional parameters was not revealed in the results of this study. Furthermore, MP seemed not to negatively affect bioimpedance parameters such as R, Z, PhA and Xc.

Not surprisingly, a statistically significant relation was observed among MP, the number of missing teeth and the number of occluding pairs. Nevertheless, due to the small sample size and the possible adaptive capacity, the results are not conclusive and further studies are needed.

5.4 A Pilot Cross-Sectional Study on Oral Health and Nutritional Status of Institutionalized Older Adults: A Focus on Sarcopenia ²⁹¹.

5.4.1 Introduction

People aged over 60 are expected to grow to twice its proportion in the coming years, implying the need to promote a healthy longevity and ageing, avoiding diseases and functional disability ^{2,292}. If on one hand, the increase of life expectancy involves chances in society and families, on the other, all those opportunities are directly associated with health. The World Health Organization (WHO) has defined the term "healthy ageing" as the capacity of maintaining a functional status that enables well-being in older age ⁷. Thus, constructive efforts are needed in order to foster healthy ageing, especially in institutionalized elderly.

The clinical condition of sarcopenia is one of the most challenging aspects of population ageing. Sarcopenia is described as a progressive and generalized skeletal muscle disorder that is related to an increased probability of unfavorable consequences such as falls, fractures, physical disability

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and mortality ²⁸⁸. According to the European Working Group on Sarcopenia in Older People 2 (EWGSOP2), muscle strength, muscle quantity or quality and physical performance are the three diagnostic criteria on which a diagnosis of sarcopenia is made ²⁸⁸. This condition is deemed to be a major public health issue in the elderly, which also affects their quality of life ²⁹³. Many risks factors could enhance the onset of sarcopenia, such as ageing, sedentary lifestyle, hospitalization, immobilization, chronic diseases, inflammation, metabolic derangements, oxidative stress and nutrition. The latter affects muscle activity by affecting myocyte homeostasis and energy metabolism and takes part in the pathogenesis of sarcopenia ²⁹⁴. In addition to the physiological reduced intake of energy and nutrients that occurs with ageing, other factors play an important role in determining this phenomenon (e.g. loneliness and surroundings, functional ability and autonomy loss, and financial status) ²⁷⁶. In particular, malnourishment and physical function decline are especially noted in institutionalized older adults ²⁹⁵.

Also, tooth loss could worsen nutritional status, being associated with the risk of malnutrition or with malnourishment itself ²⁶⁷. It was stated that poor oral health status is a strong predictor of the inception of adverse health outcomes, including mortality among the community-dwelling elderly⁵⁶. An increased risk of losing food micronutrients is associated with the inability to shred and chew food properly, excluding and/or overcooking some basic foods ¹⁵. A reduced masticatory function could be responsible for inadequate nutrition but this food restriction could be also due to many other causes, including reduced gustatory and olfactory perception, as well as economic and psychological factors ^{15,278,279,296}. However, it is not clear if changes in dietary behaviors and the consumption of certain foods could influence the onset of conditions leading to edentulism and to a further reduction of the masticatory function. When dealing with aging, the interception of oral

health diseases, resulting in a decrease in oral function, should be pursued in order to promote a healthy life and prevent important risk factors for malnourishment and sarcopenia ⁵⁷.

Considering the high risk of diagnosing sarcopenia among community-dwelling and institutionalized elderly, the evaluation of factors associated with such conditions should be pursued in order to lessen their adverse sequelae on health. In fact, the age-related sarcopenia, that affects the masticatory muscles, may be worsened by tooth loss and poor oral conditions ¹⁸⁹. This said, the aim of this pilot cross-sectional study is to evaluate oral health conditions and the prevalence of sarcopenia in subjects living in an Italian residential aged care facility.

5.4.2 Materials and Methods

The present pilot study enrolled adults 65 years and over living in "Casa di Riposo Grimani Buttari", Osimo, Italy, who underwent a comprehensive geriatric health examination from December 2018 to May 2019. The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Institutional Review Board of Dentistry Clinic, Università Politecnica delle Marche, Ancona, Italy (ODO-EXP-107/18, 19-June-2018). Written, informed consent was obtained from all participants.

Individual sociodemographic data and general health data were recorded for all participants. Included subjects had to be ≥ 65 years old and compliant. Subjects were excluded if they suffered from neurodegenerative conditions and were not compliant. The Institute's healthcare team selected participants on the basis of subjects' medical history.

For the definition of sarcopenia the recommendations of the EWGSOP2 were followed ²⁸⁸. For the diagnosis of sarcopenia several measurements were recorded, such as assessment of muscle mass, physical performance or muscle strength.

Muscle mass was measured by using a bioimpedance analysis performed by DF50 Body Composition Analyzer (ImpediMed, Brisbane, Australia; accuracy: $\pm 0.5\%$). Bioimpedance analysis measures the body's resistance to the flow of a low intensity (800 µA) and high frequency (50 kHz) electric current and is carried out by placing a couple of electrodes which are connected to the measuring instrument, on the back of the hand and another pair on the back of the foot. Impedance (Z), Resistance (R), Phase Angle (PhA) and Reactance (Xc) were evaluated. Patients were placed in supine position on a medical examination couch so that the body was parallel to the ground. Patients' arms had to be distanced from the torso at an angle of about 30°, legs had to be distant from each other at an angle of about 45°. Four electrodes were then positioned: 2 electrodes on the back of the hands and feet and another 2 on the bony prominence of the wrists and between the medial malleolus and the lateral ankle ²⁹⁷.

To evaluate muscle performance, reference was made to the usual gait speed test, as it has proven to be rapid, safe and very reliable. To perform the test, patients were asked to follow a straight course of 4m at their usual speed and the journey time was measured in seconds (a single cutoff speed ≤ 0.8 m/s). To evaluate physical strength, the hand grip strength test was performed. The latter can be used as a key indicator both in the evaluation of sarcopenia and of the phenotypes of fragility ²⁹⁸. A digital grip strength dynamometer (Camry Scale, Zhongshan, China; resolution: 0.98 N) was used for the hand grip test to evaluate muscle strength. In order to determine the dominant hand subjects were asked what hand they actively used. Subjects sat on a chair, with their elbows on the table and arms parallel in a 90-degree flexion; measurements were made 3 times with 1-minute rest periods. The maximum value of the three consecutive measurements was recorded. Measurements below 27 kg (265 N) for males and below 16 kg (157 N) for females were counted as low muscle strength ²⁹⁹. With regard to anthropometric measurements, body mass was measured with resolution of \pm 0.1 kg on a balance beam scale with the subject dressed in indoor clothing without shoes. The nursing home's latest recorded weight was used for participants who could not stand, and it was usually up to one month old. Height was measured to the nearest 0.1 cm using a wall-mounted stadiometer (Seca, Hamburg, Germany), or the alternative measurements of knee height and ulna length were used. These values were utilized to calculate the body mass index (BMI). BMI was calculated as weight (in kilogram) divided by square of height (in meter). Subjects were divided into different groups according to BMI and all-cause mortality risk ³⁰⁰. In particular, three different groups were identified: augmented risk of mortality if BMI was < 23, reference range if BMI was 23-30, and increased risk of mortality if BMI was > 30.

Trained staff used a metric band (resolution: ± 1 mm) to record the circumference of the biceps (BC), measured at midpoint between the olecranon process and the acromion with the participant's arm bent 90 ° at the elbow; and waist circumference (WC), measured according to a horizontal plane parallel to the floor, at the natural waist or narrowest part of the torso with a precision of \pm 0.5 cm.

Subjects were asked to participate in both a qualitative and quantitative food interview for the assessment of nutritional status. The nutritional status was assessed using the Mini Nutritional Assessment (MNA). MNA can be completed by nurses and it is used to evaluate the nutritional status of institutionalized older people ³⁰¹. Overall, MNA includes 18 items with an ordinal scale for response and their sum ranges from 0 to 30. Subjects are classified in MNA categories on the basis of the total score: an adequate nutritional status is considered as > 23.5 points, a risk of malnourishment as 17–23.5 points and malnourishment below 17 points ³⁰².

Meals consisted of a continental-style breakfast, lunch which included a choice between two hot dishes, and fruit, and dinner consisting of soup and either a hot or cold food option and dessert, and three snacks daily. The diet was developed by a well-trained dietitian (S.P.) whose task was also to communicate with the kitchen staff.

All patients underwent dental examination. The number of missing teeth and of occluding pairs was recorded, as well as Decayed Missing Filled Teeth (DMFT), Full Mouth Plaque Score (FMPS), Periodontal Screening and Recording (PSR) and self-reported masticatory difficulties using a 0 to 10 visual analogue scale (VAS). Masticatory performance was assessed using a mixing ability test. In short, the test involves the use of two-colored chewing gums (Hue-check Gum®, Orophys GmbH, Muri b. Bern, Switzerland). Each sample was chewed for 20 chewing cycles, as this number of strokes allows to assess the masticatory performance. Boluses were collected, inserted between two sheets of transparent plastic, yielding samples of 1 ± 0.1 mm of thickness. Standardized photos were taken from both sides of each bolus, and all the obtained images were processed by computer, analyzing the measure of the area of pixels of different colors using the k-means clustering method ¹⁰⁸. At the end of the analysis, the software revealed the ratio between mixed and unmixed areas of the boluses, discriminating between the different masticatory performances of the subjects. Figure 5 shows an example of the outcome of the sample analysis.



Figure 5 Example of the digital analysis of a chewing-gum bolus, side A (a) and side B (b). The used software automatically analyzes the images and gives as output the ratio of the mixed portion of the sample.

The rationale for using the overmentioned oral health parameters was their reliability, quickness, and reproducibility. In particular, the assessment of caries index (DMFT) and periodontal health status (PSR) was performed as caries and periodontal diseases are the main responsible of tooth loss in adulthood. FMPS index was carried out in order to evaluate how good the oral hygiene procedure performed by the enrolled subjects were. In addition, masticatory function was evaluated both subjectively (self-reported masticatory difficulties) and objectively (two-color mixing ability test).

Data were analyzed using R statistical software (R Foundation for Statistical Computing, Vienna, Austria). The normal distribution of continuous variables was tested by the Kolmogorov-Smirnov test. For continuous data, Mann-Whitney test and t-student with Welch correction test were used. Chi-square test was used for significance of associations with categorical variables. Pearson correlation coefficient was used to assess correlations between the tested variables. Data were expressed as Mean \pm SD. A value of p <0.05 was considered statistically significant.

5.4.3 Results

During the period December 2018 to May 2019, 190 subjects attended "Casa di Riposo Grimani Buttari", Osimo, Italy. Out of 190 residents, the 16.8% (n = 32) subjects met the inclusion criteria and were enrolled in this study. They were 8 men and 24 women, aged over 65 years (mean \pm SD age: 86.7 \pm 5.7). Patients' medical histories showed the presence of some systemic diseases, such as: hypertension (15 subjects), osteoarthrosis (10 subjects), heart failure (3 subjects), chronic obstructive pulmonary disease (5 subjects), diabetes (7 subjects). Table 2 summarizes both the sociodemographic and clinical data, reporting statistically significant differences between males and females.

Table 2 Sociodemographic and clinical characteristics of the study population. Data values are expressed as the number of participants for sex and mean and standard deviation for all other variables.

Sociodemographic and clinical data				p-value
	Tot	Male	Female	
Sex (n)	32	8	24	0.01
Age (years)	86.7 ± 5.7	83.3 ± 6.0	87.8 ± 5.5	0.07
Drugs (n)	8.2 ± 3.2	9.5 ± 3.2	7.8 ± 3.2	0.32
BMI^{l} (kg/m ²)	27.0 ± 5.2	25.8 ± 5.5	27.4 ± 4.9	0.44
Waist Circumference (WC) (cm)	97.1 ± 11.6	101.8 ± 5.5	95.5 ± 11.6	0.19
Biceps Circumference (BC) (cm)	26.5 ± 3.9	26.3 ± 4.1	26.6 ± 3.9	0.80
Hand Grip Test (kg)	16.0 ± 6.8	25.5 ± 7.2	12.8 ± 5.9	< 0.01
Four meters Test (sec)	8.0 ± 3.0	7.7 ± 3.2	8.1 ± 2.9	0.85
Gait speed Test (m/s)	0.6 ± 0.3	0.7 ± 0.3	0.6 ± 0.2	0.55
Number missing teeth	19.3 ± 9.0	18.9 ± 9.5	19.4 ± 9.2	0.91
Occluding pairs	3.6 ± 4.4	4.1 ± 4.6	3.4 ± 4.5	0.73
Masticatory Performance (%)	28.1 ± 20.1	32.2 ± 21.4	26.7 ± 20.7	0.54
$DMFT^2$	20.1 ± 8.3	19.4 ± 8.8	20.3 ± 8.4	0.81
PSR^3	3.1 ± 1.0	3.4 ± 0.8	2.9 ± 1.0	0.18

¹ Body Mass Index (BMI); ² Decayed Missing Filled Teeth (DMFT); ³ Periodontal Screening and Recording (PSR)

Through the anamnestic interview, 59.4% (n = 19) of the participants declared that they were nonsmokers, 31.3% (n = 10) were former smokers, 6.3% (n = 2) used to smoke less than 10 cigarettes or equivalent per day and 3.0% (n = 1) more than 10. 78.1% declared to have no drinking habits and 21.9% (n = 7) declared that they used to consume less or equal to 2 daily units of alcoholic drink equivalent. No one used to drink more than 2 daily units of alcoholic drink equivalent. 28.1% (n = 9) used to brush teeth more than once a day, while 71.9% (n = 23) did not perform oral hygiene procedures or only once a day (p < 0.01). All the participants were undergoing polypharmacy with a mean \pm SD number of drugs per day per subject of 8.2 \pm 3.2.

Results showed that males recorded a mean strength of 25.5 ± 7.2 Kg (250 ± 70.6 N), significantly higher than that of females, 12.8 ± 5.9 Kg ($126 \pm 57,8$ N), (p < 0.01). In the present study 62.5% of male population scored less than 27 Kg and 87.5% of females scored less than 16 Kg. In regards of gait speed test, out of the 32 participants, 8 did not perform the gait speed test because they were unable to walk. Overall, a mean speed of 0.58 ± 0.25 m/s was recorded. Pearson's correlation coefficient indicated weak positive correlation between gait speed test results and strength (r =0.48, 95% C.I. [0.10; 0.74], p < 0.05). A weak negative correlation was shown between WC and gait speed test results (r = -0.47, p < 0.05, 95% C.I. [-0.73; -0.08]). According to the bioimpedance analysis, no statistically significant differences were assessed between male and female Z, R, Xc and PhA values (p > 0.05). The mean values (mean \pm SD) of bioimpedance parameters were 500.3 ± 98.2 , 499.0 ± 99.3 , 33.8 ± 7.9 and 4.1 ± 2.0 , respectively. In particular, PhA was very low in 19 subjects (PhA = $2-4^{\circ}$), while in 11 patients a PhA range value between 4-6° was recorded. With regard to MNA scores and bioimpedance, a statistically significant difference was assessed between both R and Z scores in participants at risk of malnourishment and those with an adequate nutritional status (p < 0.05). When assessing Pearson's correlation

coefficient among bioimpedance parameters and the other anthropometric data, moderate negative correlations were detected between WC and R and between WC and Z (r= - 0.59, 95% C.I. [- 0.78; - 0.31], p < 0.01).

EWGSOP2 operational definition of sarcopenia ²⁸⁸ defines low muscle strength as the primary criterion of sarcopenia, followed by low muscle quantity/quality and low physical performance. In particular, sarcopenia is probable when low muscle strength is detected. A sarcopenia diagnosis is confirmed by the presence of low muscle quantity or quality. When low muscle strength, low muscle quantity/quality and low physical performance are all detected, sarcopenia is considered severe. Taking this into account, the first criterion was detected in 37.5% of males (n = 3) and in 29.2% of females (n = 7) (probable sarcopenia diagnosis). Low muscle strength associated with low muscle quantity/quality was observed in 25% of males (n = 2) and in 12.5% of females (n = 3) (sarcopenia diagnosis). Finally, the whole criteria were assessed in none of the males and in 45.8% of females (n = 11) (severe sarcopenia diagnosis).

The participants were divided into groups according to BMI. Eight subjects who recorded a BMI score < 23, 17 were within the normal weight range ($23 \le BMI \le 30$), and 7 recorded a BMI higher than 30. Males had a higher WC than females, 101.8 ± 5.5 cm vs 95.5 ± 11.6 cm, respectively. Conversely, females recorded a higher BC than the males one (26.6 ± 3.9 cm vs 26.3 ± 4.1 , respectively). Significant differences were not detected between male and female parameters (p > 0.05). According to Pearson's correlation coefficient, a moderate positive correlation was found between BMI and BC (r= 0.77, 95% C.I. [0.58; 0.88], p < 0.01). Figure 6 graphically outlines the association between BMI and BC: with the increase in the circumference of biceps, also the Body Mass Index increases (R² = 0.63). Similarly, Pearson's correlation coefficient showed a moderate positive correlation between BMI and WC (r= 0.71, 95% C.I. [0.43; 0.87], p < 0.01).



Body Mass Index and Biceps Circumference

Figure 6 Association between Body Mass Index and Biceps Circumference. The figure graphically outlines the association between BMI and BC: with the increase in the circumference of biceps, also the Body Mass Index increases ($R^2 = 0.63$).

The results of MNA revealed that 40.6% (n = 13) had an adequate nutritional status (score \ge 24), 59.4% (n = 19) were at risk of malnourishment (score between 17 and 23.5), while no subjects were malnourished (score < 17). In particular, 15 females and 5 males recorded a MNA score between 17 and 23.5 and 9 females and 5 males had a score \ge 24.

Dental examination revealed that 15.6% (n = 5) of the participants had more than 20 teeth (mean \pm SD: 25.2 \pm 2.2), while 84.4% (n = 27) had a mean \pm SD of 5.7 \pm 5.9 missing teeth. Overall, a mean \pm SD of 8.8 \pm 9.0 teeth and 3.6 \pm 4.4 occluding pairs were detected. A mean masticatory performance of 0.28 \pm 0.20 was calculated among the participants. DMFT scores showed a mean score of 20.1 \pm 8.3. When considering PSR index, a mean value of 3.1 \pm 1.0 was assessed in the whole study sample. Significant differences were not showed between males and females (p >

0.05). FMPS was assessed for each participant, considering 6 surfaces per tooth and having set 20% as cut-off. Results showed that 4 subjects had a FMPS lower than 20% and 28 had a FMPS equal or higher than 20% (p = 0.001). Finally, 7 subjects had neither fixed nor removable dental prostheses, while 8 had fixed ones (bridges and crowns both on natural teeth and dental implants), 22 removable ones (both partial and total) and 5 both. Seventeen (53.1 %) participants reported difficulties in chewing and 65.6% (n = 21) prosthodontic discomfort. According to Pearson's correlation coefficient, a strong negative correlation was detected between masticatory performance and number of missing teeth (r = -0.84, 95% C.I. [-0.92; -0.69], p < 0.01). Figure 7 graphically represents the association between masticatory performance and the number of missing teeth, then the line gently declines ($R^2 = 0.87$).



Masticatory Performance and Missing Teeth

Figure 7 Association between Masticatory Performance and Missing Teeth. The figure graphically represents the association between masticatory performance and the number of missing teeth: the line drops dramatically to 19 missing teeth, then the line gently declines ($R^2 = 0.87$).

When assessing the correlation between the number of occlusal units and masticatory performance a fairly strong positive correlation was recorded (r= 0.85, p < 0.01, 95% C.I. [0.72; 0.93]). A moderate positive correlation was shown between VAS and MP (r= 0.70, 95% C.I. [0.47; 0.85], p < 0.01). When assessing possible associations, among MP and the other studied parameters, no significant associations were found (p > 0.05). Overall, poor oral health was assessed in the enrolled subjects.

5.4.4 Discussion

The present pilot cross-sectional study was performed on a sample of 32 subjects, who were resident in an Italian residential aged care facility. Overall, 81.3% (n = 26) of the sample were diagnosed with probable, confirmed, or severe sarcopenia. Moreover, poor oral health status was assessed among participants in terms of poor oral hygiene, low masticatory performance, and low chewing and prosthetic comfort. MNA showed that almost 60% of the residents were at risk of malnourishment.

The world population is getting older: in Australia and USA, elderly people living in residential aged care facilities has greatly increased ^{54,55}. Moving into residential aged care facilities is more common among women rather than men. This datum is in accordance with a recent systematic review ³⁰³, and with the results of the present study, where the number of males and females differed statistically (p < 0.05). An explanation of this could lie in the fact that women are expected to have a higher life expectancy than men, exposing females to a higher risk of incurring in debilitating diseases and of requiring daily health care.

As stated elsewhere, a significant association was found between nutritional risk and quality of life in the elderly ³⁰⁴. In particular, malnourishment, low physical health, followed by sensory

deficiencies, are the factors that are most strongly associated with a worsening of health in elderly³⁰⁵. According to the results of the present study, the 59.4% of the sample recorded a MNA score that indicates the risk of malnourishment. Females were more commonly affected than males, with a female/male ratio of 1.3, and in agreement with a previous study ³⁰⁶. Nutritional status has a role in the pathogenesis of sarcopenia. EWGSOP2 guidelines defines three criteria on the basis of which sarcopenia is operatively diagnosed. Probable sarcopenia is identified when low muscle strength is assessed. Diagnosis is confirmed if low muscle quantity or quality is evaluated, and sarcopenia is considered severe if low physical performance is added to criterion one and two. A great variety of techniques are available to assess muscle quantity or mass ³⁰⁷. Although Computed Tomography and Magnetic Resonance Imaging are considered to be the gold standards for the assessment of muscle quantity or mass, these methods are not commonly used in primary care due to lack of portability, the requirement for highly trained personnel and high equipment costs ³⁰⁸. Dual-energy X-ray absorptiometry is more commonly used. However, a disadvantage of Dual-energy X-ray absorptiometry is that the instrument is not portable, and measurements can also be influenced by the hydration status of the patient ³⁰⁹. Bioimpedance analysis was used to assess muscle mass. It is a non-invasive method that analyses tissue properties and gives reliable information about body composition by transmitting a series of alternating electric currents through the body. This method is not expensive, requires no specialized staff and is relatively easy to use in clinical practice, either on outpatient subjects or on hospitalized patients ³⁰⁸. Moreover, reference values have been established for the elderly. Low PhA values suggest cell death or reduced cell integrity while high PhA values indicate intact cell membranes: PhA was proposed as a parameter for predicting not only clinical outcomes, but also mortality from various diseases, including sarcopenia ²⁹⁷. Gait speed test was employed to evaluate physical performance. The latter

was defined as the objective measurement of the whole-body function related to locomotion. This test is widely used in practice because it is considered a quick, safe and highly reliable test for sarcopenia.

Overall, sarcopenia was not diagnosed in 18.7% of the sample, while probable sarcopenia, confirmed sarcopenia and severe sarcopenia were diagnosed in 31.3%, 15.6% and 34.4%, respectively. These data are in accordance with those available in literature. The prevalence of sarcopenia is very high among hospitalized older adults and it was found to be directly related to nutritional status and hospital stay time ³¹⁰. In the present study, 65.6% of females were diagnosed with probable, confirmed, or severe sarcopenia, compared to 15.6% of males. This result is in accordance with those of Yalcin et al. and Shen et al., but in contrast with those of Landi et al. and Kim & Won ^{310–313}. An explanation of this could lie in the fact that several discrepancies in sex distribution could be observed among the studies ³⁰³. Several studies considered sarcopenia as a significant predictor of all cause of mortality among community-dwelling residents. In particular, subjects with a diagnosis of severe sarcopenia and those with deteriorated physical performance had a higher risk of death, thus highlighting the need to immediately intervene ³¹⁴.

Poor oral conditions are an important indicator of physical frailty, sarcopenia, need for long-term care, and mortality. Poor oral health conditions and swallowing and masticatory problems, contribute in part to dietary restrictions and to a poor nutritional status in elderly, increasing the risk of frailty and sarcopenia. Similarly, oral conditions may be influenced both by frailty and sarcopenia, probably through the common burden of inflammation and oxidative stress ³¹⁵. Also in the present study, poor oral conditions were assessed among subjects. Masticatory performance was associated both with the number of teeth and the number of occluding pairs (r= 0.84 and 0.85, p < 0.01, respectively). Moreover, masticatory performance was significantly lower in subjects

with less than 20 teeth than in those with more than 20 (p < 0.01), as shown elsewhere ¹⁶⁷. The high prevalence of missing teeth among the participants of the present study is an indicator of unmet dental treatments, in terms of both caries and periodontal disease. As stated by Kassebaum et al., population growth and the increase in life expectancy have determined a dramatic rise in the burden of untreated oral conditions throughout the world. In particular, the loss of natural teeth, as a consequence of periodontal diseases and untreated caries, is the major cause of Disability-Adjusted Life Years (DALYs) due to oral conditions ¹⁷. It becomes crucial to pursued effective preventive and therapeutic programs in order to retain the natural dentition and masticatory function in old age, contributing to the delay of physical and cognitive decline as well as of dependence loss. The relationship between oral health and muscle low physical activity has been considered in several studies, especially in institutionalized older adults ³¹⁶. Also, poor oral health has been associated with a faster decline in handgrip strength, which is an important risk factor for sarcopenia ³¹⁵. The results of the present study showed that, overall, males had a significantly greater hand-grip strength than females' one (p < 0.01). Nevertheless, the 62.5% of the male population and the 87.5% of females scored less than the threshold values, that are 27 kg and 16 kg, respectively. In the sample, no significant associations were detected among masticatory performance and the studied nutritional parameters (p > 0.05). In addition, no statistically significant differences were detected between participants at risk of malnourishment and those with an adequate nutritional status, according to the number of missing teeth. A possible explanation of such a result may be the possible adaptation to the physiological and pathological changes that could occur in ageing and specific diet developed by a well-trained dietitian.

The small number of participants enrolled and the exclusion of those subjects with neurodegenerative diseases could be a limitation of this pilot study. Neurodegenerative conditions

and the non-compliance of most of the residents of the residential aged care facility restricted the sample of this study. In addition, the number of males and females differed statistically (p < 0.05): even if the unequal number of males and females may represent a limit of this study, it could be explained by the fact that women are expected to have a higher life expectancy than men, exposing females to a higher risk of incurring in diseases and disability, thus requiring daily health care and hospitalization. Moreover, some methodological issues may have influenced the results of the present report. The cross-sectional design of the study did not allow to clarify any cause-effect relationships. Furthermore, results may be confounded by unmeasured factors. Being a pilot study, its limited sample size does not allow to draw definitive conclusions. However, we were able to assess the feasibility and the operational acceptability of the study protocol. Further research is needed and the enlargement of the sample size as well as the enrollment of other residential aged care facilities should be pursued in order to deeply investigate the relationship among oral health, nutritional status and sarcopenia in older adults. In fact, even if oral health is considered a crucial element of general health and well-being, it is often neglected, especially in frail older people, determining the occurrence of adverse health outcomes.

5.4.5 Conclusions

Within the limitations of this report, a high prevalence of institutionalized older adults diagnosed as being at risk of developing sarcopenia or of being sarcopenic or severely sarcopenic was showed. Moreover, poor oral conditions were assessed among this kind of subjects. Although a clear association was not showed and the cross-sectional design of this pilot study, the impairment of oral function and the diagnosis of sarcopenia may be considered as factors responsible for the worsening of the general health status. A multidisciplinary approach could help to ensure the maintenance of good oral health status and adequate nutrition, preventing and intervening in the
multiple factors of sarcopenia that could lead to the worsening of the clinical status, especially in elderly. Regular diet, specific physical activity and oral health preventive programs should be crucial goals to be pursued. Both scientific community and policy makers should address great attention to the provision of care to older adults.

5.5 The Influence of Age and Oral Health on Taste Perception in Older Adults: A Case-Control Study ³¹⁷

5.5.1 Introduction

Aging is a progressive, intrinsic, and universal process that occurs in every living being as the result of the interaction between individual's genetics and the environment ³¹⁸. The great socioeconomic development of the last century, medical advances, a better lifestyle, and fertility rate decrease have led to an increase in life expectancy. The World Health Organization (WHO) has calculated demographic projections showing an increase in the population over 65. These predictions will have a significant impact on the delivery of general and oral health care and treatment strategies within the geriatric patient population ³¹⁹.

Oral health is an integral part of general health and affects the quality of life of an individual ²⁷. Poor oral health status is considered to be a strong predictor of the onset of adverse clinical outcomes, including mortality, among the community dwelling elderly ⁵⁶. The maintenance of a healthy mouth is crucial, since the worsening of oral health status leads to functional loss ³²⁰. With regard to poor functional capacity, it has been shown that masticatory performance (MP) decreases with the loss of dental elements. In particular, at least ten pairs of occluding teeth are necessary for adequate chewing capacity ⁶⁸. Effective prosthetic solutions have been proposed to restore dentition and oral function with a consequent greater subjective appreciation. Unfortunately,

prosthetic rehabilitation does not restore chewing performance in its totality and cannot be compared to natural dentition. Therefore, preventive strategies aimed at maintaining a good oral health status are necessary to prevent oral hypofunction ³²¹.

The loss of masticatory function is responsible for diet restriction and impaired bolus formation, leading to two main consequences. First of all, it can cause interference with digestion and nutrient extraction. Secondly, the exclusion of some basic food (e.g., meat, fruit and vegetables), can lead to the exclusion of foods considered difficult to chew in favor of soft, easily chewed foods (e.g., refined carbohydrates and fats), inducing bad dietary practices and poor nutritional intakes ²⁴⁷. As stated in previous studies, malnourishment is prevalent in the elderly population, especially in elderly hospitalized patients ^{322,323}. Overall, the nutritional status is one of the most important modifiable factors capable of affecting the health, the well-being, and the overall QoL of an individual ³²⁴.

The effects of physiological aging on the perception of taste are represented by the alterations of taste cells, the reduction of salivary production, and inability to fully chew food. Taste alterations can be classified as qualitative, which include dysgeusia (e.g., an alteration of taste sensitivity for foods previously enjoyed and which later become unpleasant) and quantitative, which consist of ageusia (total deficit), hypogeusia, and hypergeusia, a decrease or increase in taste sensitivity, respectively ³²⁵. Several geriatric pathological conditions could lead to dysgeusia or ageusia. Among older adults, taste loss is frequently caused by multiple factors, including physiological changes such as impairment in taste receptor cells, poor oral health condition, and a deteriorating olfactory function. In addition, it is worsened by events related to aging such as poor general health, polypharmacy, and systemic diseases. The existence of taste disorders is commonly

observed in elderly hospitalized patients for acute conditions, and it is often associated with poor oral hygiene and infections ^{326,327}.

Researchers are still debating the severity of the taste loss that older adults daily experience. Overall, sour and bitter tastes seem to be the most impaired ones. Several studies report an agerelated decline in the perception of also salty and sweet flavors, with contradictory results, while other papers are in agreement with an increased perception of umami taste ³²⁸. There is still no consensus whether the physiological changes in taste perception could affect food preferences among old people, even though many authors found them less interested in sour tastes and pungent flavors ³²⁹.

Thus, the present study aimed at (i) verifying the impact of oral health on the nutritional status of institutionalized elderly subjects compared to home-living ones; (ii) analyzing the taste discernment by the administration of taste stimuli in all enrolled subjects; (iii) assessing any association between taste perception and presence of removable dentures. Gender differences were also explored.

5.5.2 Materials and Methods

The present cross-sectional study included 90 adults, 65 years old and over, living either at home (control group—CG) or in a residential aged care facility (test group—TG). 58 patients (CG) in outpatient medical treatment were recruited at the Dental Clinic of Università Politecnica delle Marche, Ancona, Italy, during the period April 2017-December 2017, and 32 patients (TG), living at "Casa di Riposo Grimani Buttari", Osimo, Italy, participated in a comprehensive geriatric health examination from December 2018 to May 2019. The study was performed in accordance with the principles of the Declaration of Helsinki as revised in 2013 and was approved by the Institutional

Review Board of Dentistry Clinic, Università Politecnica delle Marche, Ancona, Italy (ODO-EXP-107/18, 19 June 2018). Written informed consent was obtained from all enrolled subjects after the procedures had been fully explained.

Individual sociodemographic data and general health information were recorded for all participants. All included subjects had to be ≥65 years old and compliant. Subjects were excluded if they were <65 years, suffered from neurodegenerative conditions (e.g., Alzheimer, Parkinson, Dementia), had a diagnosis of diseases and disorders affecting the muscular system, had oro-facial pain, or were not compliant. The Institute's healthcare team selected participants on the basis of subjects' medical history. All the enrolled subjects underwent dental examination in which the number of retained teeth, the number of missing teeth, and the number of occlusal tooth unit were recorded. Moreover, the presence or lack of dental prostheses was noted. In addition, a masticatory test was carried out, using the two-color mixing test as described elsewhere ¹⁰⁷. Briefly, the test consists in the chewing of two-colored chewing gums (Hue-check Gum[®], Orophys GmbH, Muri bei Bern, Switzerland). Each sample was chewed for 20 chewing cycles, as this number of strokes allows the assessment of MP. Boluses were collected, inserted between two sheets of transparent plastic, yielding samples of 1 ± 0.1 mm of thickness. Standardized photos were taken from both sides of each bolus, and all the obtained images were processed by computer, analyzing the measure of the area of pixels of different colors using the K-means clustering method ¹⁰⁸. At the end of the analysis, the software revealed the ratio between mixed and unmixed areas of the boluses, discriminating between the different MPs of the subjects.

The taste test was based on filter paper strips as described by Landis et al. ³³⁰ and modified as reported elsewhere ^{331,332}. Briefly, cotton pads, soaked with four substances (sodium chloride, citric acid, sucrose, and quinine hydrochloride) were applied to the protruded tongue, immediately

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posterior to its first third, either to the left or right side, in order to study lateralization too; each basic taste quality (salty, sour, sweet, and bitter) was presented at 4 different concentrations (Table 3).

Stimulus	timulus Substance	
Sweetness	Sucrose	0.05 g/mL
		0.1 g/mL
		0.2 g/mL
		0.5 g/mL
Saltiness	Sodium Chloride	0.016 g/mL
		0.04 g/mL
		0.1 g/mL
		0.25 g/mL
Bitterness	Quinine	0.0004 g/mL
		0.0009 g/mL
		0.0024 g/mL
		0.006 g/mL
Sourness	Citric Acid	0.05 g/mL
		0.09 g/mL
		0.165 g/mL
		0.3 g/ml
Fat	Rapeseed oil	Pure
Neutral	Deionized water	Pure

Table 3. Concentrations of taste stimuli.

In addition, pure rapeseed oil and water were administered to evoke fat and neutral taste, respectively. Rapeseed oil is a neutral oil which has a pale-yellow color and is almost odorless; the rapeseed oil was chosen instead of olive oil since this latter has a specific texture and increased volatility in the oral cavity, making it easily recognizable. Distilled water was used as a solvent, and taste solutions were freshly prepared on the morning of each testing session. Since gustatory stimulation also causes the activation of other sensory system (e.g., touch receptors), the test was performed so as to minimize the activation of other receptors. Subjects were required to wash their mouth with deionized water between samples to avoid carryover effects. Administration was randomized for the four concentrations, and the side of presentation was alternated: 36 cotton pads (18 for the left side and 18 for the right side) were used. The enrolled subjects had to identify the taste by choosing from a list that included eight descriptions: sweet, salty, bitter, sour, water, fat, nothing, I do not know (forced multiple choice). The test took about 20 min.

Data were analyzed using R statistical software (R Foundation for Statistical Computing, Vienna, Austria). The normal distribution of continuous variables was tested by the Kolmogorov-Smirnov test. For continuous data, Mann-Whitney test and t-student with Welch correction test were used. Chi-square test was used for significance of associations with categorical variables. Pearson correlation coefficient was used to assess correlations between the tested variables. The dichotomous dependent variable, taste stimuli perception, was introduced in a multiple logistic regression model to estimate its variation according to the independent variables and to verify the presence of possible confounders. Data were expressed as Mean \pm SD. A value of p < 0.05 was considered statistically significant.

5.5.3 Results

Out of a total of 190 subjects attending "Casa di Riposo Grimani Buttari", Osimo, Italy, only 32 (16.8%) subjects (8 males and 24 females) (TG) met the inclusion criteria and were enrolled in this study. The CG comprises 58 subjects (32 males and 26 females) in outpatient medical treatment recruited at the Dentistry Clinic of Università Politecnica delle Marche, Ancona, Italy. The CG group mean age was 74.6 ± 4.8 , while the TG one was 86.4 ± 7.0 years (p < 0.001). In the CG, a mean loss of 5.3 ± 4.7 teeth was recorded, while in the TG it was 19.3 ± 9.1 , p < 0.001. Table 4 shows oral health related data of the enrolled subjects.

 $\textit{Table 4. Sociodemographic and Oral health related data of the studied groups. Data are expressed as Mean \pm Standard Deviation.$

	Control Group	Test Group	<i>p</i> -Value
Age (years)	74.6 ± 4.8	86.4 ± 7.0	< 0.001
Sex (M/F)	32/26	8/24	
Height (cm)	162.9 ± 8.5	154.1 ± 9.9	< 0.001
Weight (Kg)	74.9 ± 15.1	63.8 ± 11.8	< 0.001
BMI (Kg/m ²)	28.1 ± 4.6	27.0 ± 5.2	NS ²
No. of drugs	3.7 ± 2.3	8.2 ± 3.2	< 0.001
Missing Teeth	5.3 ± 4.7	19.3 ± 9.1	< 0.001
Occlusal Units	10.9 ± 2.5	3.6 ± 4.4	< 0.001
DMFT ¹	13.3 ± 5.4	20.1 ± 8.3	< 0.01
Masticatory Performance	0.43 ± 0.17	0.23 ± 0.18	< 0.001

¹Decayed Missing Filled Teeth; ² Not statistically significant.

The MP test results were analyzed in relation to the number of missing teeth, both in the CG and in the TG. The test showed a negative correlation between MP and missing teeth both in the TG and in the CG, r = -0.87, C.I. [-0.94; -0.76], p < 0.001, and r = -0.51. C.I. [-0.68; -0.28], p < 0.001, respectively. Overall, a negative correlation was showed between MP and missing teeth, r

= -0.77, C.I. [-0.84; -0.66], p < 0.001. Furthermore, when correlating age with the number of missing teeth, a positive relationship was found (r = 0.51, C.I. [0.33; 0.65], p < 0.001). Conversely, when comparing age with MP, a negative correlation was found (r = -0.39, C.I. [-0.56; -0.20], p < 0.001). The multiple logistic regression model showed no influence of the statistically different characteristics of the study groups in the perception of taste stimuli (p > 0.05).

When assessing taste perception, the number of correct answers in the CG and in the TG were analyzed. Table 5 summarizes the results of the taste perception test in the two study groups, also pointing out correct answers for males and females within CG and TG.

	Control	Test		Males			Females		
Stimuli	Group	Group	<i>p</i> -Value	CG	TG	<i>p</i> -Value	, CC	ТG	<i>p</i> -Value
	(CG)	(TG)		cu	10		CU	10	
Sweetness Sucrose (0.05 g/mL)	22.4%	31.3%	NS ¹	19.4%	37.5%	NS ¹	26.1%	29.2%	6 NS ¹
Sweetness Sucrose (0.1 g/mL)	44.8%	50.0%	NS ¹	36.1%	25.0%	NS ¹	56.5%	58.3%	6 NS ¹
Sweetness Sucrose (0.2 g/mL)	56.9%	53.1%	NS ¹	52.8%	37.5%	NS ¹	60.9%	58.3%	6 NS ¹
Sweetness Sucrose (0.5 g/mL)	63.8%	50.0%	NS ¹	52.8%	25.0%	NS ¹	78.3%	58.3%	6 NS ¹
Saltiness Sodium Chloride (0.016 g/mL)	19.1%	12.5%	NS ¹	19.4%	0.0%	<0.01	17.4%	o16.7%	6 NS ¹
Saltiness Sodium Chloride (0.04 g/mL)	46.6%	34.4%	NS ¹	38.9%	37.5%	NS ¹	56.5%	33.3%	6 NS ¹
Saltiness Sodium Chloride (0.1 g/mL)	53.5%	34.4%	NS ¹	50.0%	0.0%	<0.0001	56.5%	645.8%	6 NS ¹
Saltiness Sodium Chloride (0.25 g/mL)	55.2%	34.4%	< 0.05	50.0%	0.0%	<0.0001	60.9%	645.8%	6 NS ¹
Bitterness Quinine (0.0004 g/mL)	24.1%	18.8%	NS ¹	19.4%	0.0%	< 0.001	30.4%	25.0%	6 NS ¹
Bitterness Quinine (0.0009 g/mL)	53.5%	40.6%	NS ¹	41.7%	25.0%	NS ¹	69.6%	645.8%	6 NS ¹
Bitterness Quinine (0.0024 g/mL)	58.6%	62.5%	NS ¹	55.6%	50.0%	NS ¹	60.9%	66.7%	6 NS ¹
Bitterness Quinine (0.006 g/mL)	77.9%	59.4%	NS ¹	66.7%	62.5%	NS ¹	91.3%	58.3%	<i>∞</i> <0.01
Sourness Citric Acid (0.05 g/mL)	37.9%	31.3%	NS ¹	25.0%	12.5%	NS ¹	56.5%	37.5%	\sim NS ¹
Sourness Citric Acid (0.09 g/mL)	53.5%	34.4%	NS ¹	47.2%	12.5%	< 0.05	60.9%	641.7%	6 NS ¹
Sourness Citric Acid (0.165 g/mL)	50.0%	50.0%	NS ¹	50.0%	12.5%	< 0.05	56.5%	62.5%	6 NS ¹
Sourness Citric Acid (0.3 g/mL)	58.6%	46.9%	NS ¹	55.6%	25.0%	NS ¹	60.9%	54.2%	6 NS ¹
Fat Rapeseed Oil	17.2%	9.4%	NS ¹	19.4%	0.0%	<0.01	13.0%	o12.5%	δ NS ^{−1}
Neutral Deionized Water	13.8%	9.4%	NS ¹	13.9%	0.0%	< 0.05	13.0%	o12.5%	ο NS ¹

 Table 5. Taste stimuli test: percentage of correct answer in the study groups.

¹ Not statistically significant.

According to the different types of stimuli, we found that only the salty taste perception was significantly different between the two groups (p < 0.05). In particular, the CG had a better taste performance than the TG. Furthermore, the comparison between the taste perceived by the wearers of removable and non-removable prosthesis was performed. In this case, only the bitter taste was statistically significant between the two groups (p < 0.05). In particular, those who did not formerly wear a dental prosthesis had a better taste perception performance than removable prostheses wearers. Finally, gender differences were analyzed in order to evaluate alterations in the perception of all tastes (Table 6). Bitter, sour, and sweet tastes were better recognized by females than males (p < 0.05).

Stimuli	Contro	l Group	<i>p</i> -Value	Test	Group	<i>p</i> -Value
Sumun	Male	Female		Male	Female	
Sweetness	10 /%	26.1%	NS 1	37 5%	20.2%	NS 1
Sucrose (0.05 g/mL)	17.770	20.170	110	57.570	29.270	IND -
Sweetness	36.1%	56.5%	NS ¹	25.0%	58.3%	NS 1
Sucrose (0.1 g/mL)	50.170					110
Sweetness	52.8%	60.9%	NS ¹	37 5%	58 3%	NS ¹
Sucrose (0.2 g/mL)	52.070	00.970	110	57.570	50.570	110
Sweetness	52.8%	78 3%	< 0.05	25.0%	58.3%	NS 1
Sucrose (0.5 g/mL)	52.070	70.570		23.070		145
Saltiness	19.4%	17 4%	NS ¹	0.0%	16.7%	<0.05
Sodium Chloride (0.016 g/mL)	17.470	17.470				~0.05
Saltiness	38.9%	56 5%	NS ¹	37.5%	33.3%	NS ¹
Sodium Chloride (0.04 g/mL)	50.770	50.570				110
Saltiness	50.0%	56 5%	NS 1	0.0%	45.8%	<0.001
Sodium Chloride (0.1 g/mL)	50.070	50.570	110			~0.001
Saltiness	50.0%	60.9%	NS ¹	0.0%	45.8%	<0.001
Sodium Chloride (0.25 g/mL)	50.070	00.770				\$0.001
Bitterness	19.4%	30.4%	NS ¹	0.0%	25.0%	<0.05
Quinine (0.0004 g/mL)	17.170					-0.05
Bitterness	41 7%	69.6%	< 0.05	25.0%	45.8%	NS ¹
Quinine (0.0009 g/mL)	11.770	07.070				
Bitterness	55.6%	60.9%	NS ¹	50.0%	66.7%	NS ¹
Quinine (0.0024 g/mL)	55.070					
Bitterness	66 7%	91.3%	< 0.05	62.5%	58.3%	NS ¹
Quinine (0.006 g/mL)	00.770	71.570				
Sourness	25.0%	56 5%	<0.05	12.5%	37 5%	NS 1
Citric Acid (0.05 g/mL)	20.070	20.270	-0.05	12.370	57.570	110
Sourness	47 2%	60.9%	NS ¹	12.5%	41.7%	NS ¹
Citric Acid (0.09 g/mL)	17.270	00.770				
Sourness	50.0%	56 5%	NS ¹	12.5%	62.5%	< 0.01
Citric Acid (0.165 g/mL)	50.070	50.570				\$0.01
Sourness	55.6%	60.9%	NS ¹	25.0%	54.2%	NS 1
Citric Acid (0.3 g/mL)	22.070	00.770				110
Fat	19 /1%	13 0%	NS ¹	0.0%	% 12.5%	NS 1
Rapeseed Oil	17.4/0	15.070		0.070		
Neutral	13.9%	13.0%	NS ¹	0.0%	12 5%	NS ¹
Deionized Water	15.770	13.070		0.070	12.3/0	110

 Table 6. Taste stimuli test: percentage of correct answer in the study groups.

¹ Not statistically significant.

5.5.4 Discussion

In the present study, performed on 32 older adults living in an Italian residential aged care facility and 58 autonomous controls, data relating to oral health status, MP, as well as taste perception were analyzed. A relationship between the number of missing teeth and the MP, and impairment in taste perception between CG and TG, between removable and non-removable prostheses wearers, and between genders were found.

In accordance with the literature, and with our previous work, in the present paper we found that as the number of missing teeth increases, the MP decreases ^{333,167,334}. Since in the CG the number of missing teeth was overall smaller than that of the TG, the MP was higher in first group of subjects than in the second one. An explanation of this could lie in the fact that tooth loss is associated with the aging process, and being progressive and cumulative, is more likely to diagnose both partial and total edentulism in the oldest age classes. Tooth loss leads to changes in eating habits, commonly attributed also to changes in the hedonistic quality of food ^{335,336}. With advancing age, it is more frequent that older adults present, even simultaneously, exhibit a low masticatory function and poor appetite ³³⁷. Good oral health is essential for an adequate nutritional status, because the maintenance of a natural dentition can ensure an adequate masticatory function related to a balanced diet, that in turn affect the QoL in the elderly ³³⁸.

Aging is characterized by a reduction in overall sensory perception. The decline in taste and smell can lead to poor appetite and malnutrition, improper food choices, and inadequate nutrient intake ³³⁹. Poor oral health can determine the onset of malnourishment, and the latter can in turn affect oral health. Indeed, insufficient nutrient consumption was associated with a reduction of muscle strength and physical capacity, and, as in a vicious circle, old age can increase the risk of low nutritional intake. In the last decades, several studies investigated the changes in taste perception

that occur with aging with inconsistent results ^{296,340,341}. In the present paper, although in some cases no statistically significant differences were found between groups, CG was able to recognize a greater number of taste stimuli than TG, except for sweet taste that was perceived in the same proportion by both groups. Our findings are in accordance with previous studies, as most of them reported that older subjects required a higher concentration of primary tastes than young people ^{342,343}. However, the decrease in taste perception is not the same for all taste stimuli; in fact, if it becomes difficult to perceive bitterness, the ability to recognize the sweet taste is kept even in advanced age, with a consequent liking of sweet and high-calorie meals.

The reported statistically significant decrease in salty taste between the two analyzed groups may be related to the fact that in Italy the habitual salt intake is well above the recommended amounts ³⁴⁴. The CG was composed by autonomous subjects, and they were able to prepare meals by their own. They used more salt in food than the TG, which on the contrary eats what is prepared by the cooks of the aged care facility. The cooks of the aged care facility reduce dietary salt intake to lower blood pressure and prevent deaths due to stroke or cardiovascular disease. Our findings may indicate that individuals who are living at home may be at risk for injuring their health by involuntary over-intake of a salty diet. On the other hand, institutionalization of older adults can also lead to nutritional deficiency if nutrient intake is not well managed, or taste sensation issues are not properly evaluated.

When comparing taste perception between subjects with and without denture prosthesis, removable prostheses wearers were less sensitive to the recognition of overall taste stimuli than those without prosthesis. These results are in accordance with those by da Silva et al. that showed that the presence of the denture is able to change the recognition of taste when compared to its absence, mainly for the bitter taste ³⁴⁵. Our findings are also in agreement with data present in the

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literature, according to which the taste sensitivity is greatly reduced in people wearing dentures ³⁴⁶. Conversely, some reports have shown that prosthetic carriers have an increased threshold to detect the sweet in solid foods ³⁴⁷. This can be explained by the fact that prosthetic carriers have a reduced ability to grind food and a reduced rate of salivary secretion. In this context, sweet substances dissolve in a non-optimal way and thus reach the taste cells less easily.

The results showed gender differences in the ability to recognize different tastes, showing that on average females are more sensitive in the recognition of all tastes (except fat, p > 0.05).

Overall, in the average of the results between males and females, only bitter and sweet tastes reached statistical significance. These results provide new insights into the identification of the taste sensitivity related to gender. In general, gender differences in taste-related behaviors are associated with circulating estrogen levels, which can modulate the detectability and preference of taste. Hormones, in fact, can modulate the responses to the taste stimuli, performing both organizational and activation roles in the regulation of gustatory responses ³⁴⁸. Regardless of estrogen status, peripheral taste perception differs between males and females, indicating that the same taste stimuli produce differential inputs in the brains of males and females. Mechanisms contributing to differences in gustatory processing, and the extent to which male and female gustatory function vary, are awaiting further clarification, which should include the analysis of electrophysiological responses caused by taste in each of the gustatory nerves. A previous study attempted to analyze the differentiation of the spatial distribution of different taste perception frequencies between the two genders, through the use of magnetoencephalography (MEG) ³⁴⁹. Thanks to this neuroimaging technique it was possible to deduce that females show more channels with high frequencies due to the stimulation with the sweet and bitter taste, guaranteeing a better

gustatory response. On the contrary, as for the salty taste, they show channels with low frequencies and, consequently, a lesser gustatory response.

Beside this, gender differences may be due to the fact that females have higher fungiform papillae density (FPD) than males, in accordance with literature ³⁵⁰. Furthermore, a regular reducing of FPD is observed with age, an effect more evident in males than in females, thus confirming males higher susceptibility to FPD lowering with age ³⁵¹, in agreement with the present results.

In our study, we also analyzed differences between male and female within TG and CG. Remarkably, only males showed alterations inside the two groups, with CG more able to recognize tastes than TG. The data herein presented thus show the interplay of gender and age in defining interindividual variations in gustatory responsiveness.

The present report has some limitations that provide opportunities for future studies. The small number of the enrolled subjects living in the residential aged care facility restricted the sample of this study, due to the non-compliance of most of the residents. The cross-sectional design of the study provided only a picture of the situation, making it difficult to generalize our results to the whole older adult population. Regardless, cross-sectional studies are a useful tool for establishing preliminary evidence in planning future research that should aim at enlarging the sample size and deeply investigating the relationship among taste stimuli perception, age, and oral health status in older adults. Finally, the statistically significant differences between CG and TG and other confounding factors, that might not have been accounted for, may have influenced the results of the study. Although the results of this study should be interpreted with caution, multiple logistic regression models showed no influence of the statistically different characteristics of the study groups in the perception of taste stimuli. The best understanding of the relationship between masticatory performance, taste sensitivity, and nutritional factors is a necessary prerequisite for

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the development of new therapeutic strategies to more effectively address the problems associated with malnutrition of the geriatric patient. In light of these considerations, the importance of continuous training of operators in the field of oral health of elderly patients is warranted in order to implement treatment plans aimed at preserving natural teeth.

5.5.5 Conclusions

Within the limitation of this study, the analyses carried out allow us to confirm that masticatory performance is associated with the number of missing teeth. In particular, masticatory performance is statistically significantly lower in older adults living in a residential aged care facility compared to autonomous subjects, as a result of the fewer number of retained teeth and their poorer oral health status.

Taste results depict a complex interplay of different factors affecting gustatory acuity, among which oral health, age and sex may have a role. Though the association between perception and food intake still requires a more comprehensive analysis, the present data may be important for precision nutrition, as they support the hypothesis that inter-individual differences in taste perception must be taken into account so as to better understand food preferences and food intake and so achieve a tailored adherence to nutritional recommendations.

Chapter 6: Promoting Oral Healthy Ageing: conclusive remarks

The global population is ageing, and important sequelae of such demographic change is the increase in the incidence of chronic illness, multimorbidity, care dependency, and related polypharmacy. The ageing process affects also the oro-facial structures but not necessary lead to functional impairments thanks to the physiologic stomatognathic system spare capacity. When external factors occur, the onset of pathological conditions and severe changes happen, determining a functional loss. Beside the need for elderly of regular dental check-ups, dentists and healthcare professionals should be required to objectively evaluate patient's mastication, aimed at not only evaluating oral function but also at providing information about patient's disability. In this context, the method proposed by our Research Team was able to automatically analyze the different MPs corresponding to different numbers of chewing cycles, quantifying the percentage of the mixed color area, and providing quantitative data through the computerized analysis by using the best possible segmentation and minimizing human interaction. Thanks to the two-color mixing ability test quickness and simplicity, the assessment of masticatory deficiencies can be easily carried out also in healthcare settings as part of the functional assessment of a patient. In addition, MP assessment using this test may be used in large epidemiological studies as it is not invasive to perform on the patient and the bolus derived from this test may be evaluated later after image capture.

Increasing evidence shows clinically significant uni- and bi-directional associations between oral health and general health, arising the importance of oral health preventive and promoting programs. In this sense, the early identification and diagnosis of oral pathologies and oral impairment must be pursued. Overall, elderly people are at risk of food intake quality reduction because of the lessening of hedonic perception, lowered food appeal and appetite. In addition to

this, poor oral health conditions can lead to MF impairment, thus restricting food choices and determining the onset of malnourishment and the worsening of general health conditions. When considering the results of our studies, in independent and self-sufficient patients without serious impairment of the general health status, most of them presented a good oral condition: an association between reduced MP and a worsening of nutritional parameters was not revealed. Not surprisingly, a statistically significant relation was observed among MP, the number of missing teeth and the number of occluding pairs. Conversely, when considering older adults living in a residential aged care facility, most of them were diagnosed as being at risk of developing sarcopenia or of being sarcopenic or severely sarcopenic. Moreover, poor oral conditions were assessed among this kind of subjects. Even if a clear association was not showed, the impairment of oral function and the diagnosis of sarcopenia could be considered as factors responsible for the worsening of the general health status.

Even if oral health is deemed to be a crucial factor for general health and well-being, especially in older adults and in those who are frail and care dependent, it is often neglected. Moreover, a lot of elderly people faces difficulties in accessing dental care due to many barriers, so that the development of new strategies aimed at enhancing general and oral health status, such as Teledentistry, should be pursued in order to promote healthy ageing. The implementation of Teledentistry in residential aged care facilities and in-home assistance programs could be a viable tool for the management of oral care in people who cannot access dental care. Moreover, Teledentistry could be effective in avoiding unnecessary appointments, in triaging dental visits, and monitoring the health of the patients, improving the take-charge process.

In conclusion, in an ageing society, educational interventions about oral health addressed to patients themselves and caregivers, appropriate oral health policies, and citizens empowerment

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and involvement can contribute to the promotion of oral health in elderly. Geriatric healthcare team members should play an important role in the initial oral health assessment, achieving an interprofessional collaborative environment aimed at promoting oral health and, thus, taking care of the overall health and well-being of older adults.

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