MARCIA DIAZ SERRA VICENTIN

AVALIAÇÃO FISIOLÓGICA E MORFOLÓGICA DO SISTEMA MASTIGATÓRIO EM CRIANÇAS

Tese apresentada à Faculdade de Odontologia de Piracicaba da Universidade Estadual de Campinas para obtenção do título de Doutor em Odontologia, Área de Odontopediatria.

Orientadora: Profa. Dra. Maria Beatriz Duarte Gavião

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Salmo 28:7

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Salmo 103

RESUMO

Diversos são os fatores que parecem interferir na mastigação: força de mordida (que pode estar relacionada ao tamanho dos músculos), componentes e quantidade de saliva, características da oclusão, dentre outros. Esta tese está composta de cinco capítulos. O capítulo 1 "*The use of ultrasound in the investigation of the muscles of mastication*" revisou a literatura a respeito das imagens ultrasonográficas (US) dos músculos da mastigação e reforçou que a US é um método confiável de grande utilidade no campo do diagnóstico de alterações dos músculos da mastigação e do estudo de mudanças durante o crescimento e envelhecimento e que permanece como uma técnica promissora para o estudo dos músculos da mastigação. O capítulo 2, "*Correlation between muscle thickness and bite force in children before and after oral rehabilitation – a 2 year logitudinal study*", avaliou a correlação entre força de mordida (FM) e espessura muscular antes e após 6, 12 e 24 meses de reabilitação bucal (T0, T6, T12, T24, respectivamente) em 19 crianças de 6-9 anos que apresentavam perda precoce de dentes decíduos. A FM foi determinada com um transdutor pressurizado flexível. A espessura dos músculos masseter e temporal foi mensurada durante relaxamento e máxima intercuspidação com US digital com transdutor linear de 56mm/10-MHz. Houve aumento significativo da FM e espessura do masseter entre T0 e T12. A FM e a espessura do temporal relaxado e contraído em T6 e temporal contraído em T24 foram significativamente correlacionadas. Foi concluído que houve aumento da FM e espessura do masseter após a reabilitação e também relação entre FM e espessura do temporal, o que sugere que a reabilitação bucal influenciou os aspectos morfológicos e funcionais dos músculos da mastigação. Os capítulos 3 "*Bite force and salivary flow rate in schoolchildren",* 4 "*Bite force, texture perception and chewing parameters in schoolchildren*" e 5 "*Salivary flow rate, protein content, chewing parameters, and taste perception in schoolchildren*" tiveram como objetivos avaliar e correlacionar o fluxo salivar (FS), FM, parâmetros de mastigação, percepção de sabor e textura de alimentos e o papel da proteína na mastigação em crianças de 7-10 anos de idade distribuídas de acordo com fase da dentição, oclusão e sexo. A saliva total não estimulada (NE) e estimulada (E) por *parafilm* foram coletadas por 5 minutos para obtenção do FS. A proteína total foi avaliada pelo método de Lowry *et al.* (1951). A FM foi determinada como um transdutor pressurizado flexível. Os seguintes alimentos foram testados: cenoura,

torrada com e sem margarina, banana, queijo e goma de mascar para determinar os parâmetros mastigatórios (tempo de mastigação, número e freqüência de ciclos mastigatórios), a textura (utilizando uma escala visual analógica de 0 a 100 mm) e o sabor (classificado como amargo, azedo, salgado ou doce). Meninos apresentaram FS e FM (Capítulo 3) significativamente maior que as meninas. Houve correlação significativa entre FS e FM para o grupo com oclusão normal. Não houve diferença significativa na FM, textura ou nos parâmetros da mastigação entre os grupos de oclusão. Os parâmetros da mastigação e a textura foram significativamente menores para os alimentos macios em relação aos duros. Correlação significativa foi encontrada entre FM e freqüência mastigatória no grupo com oclusão normal. Houve correlação significativa entre o FS e os parâmetros de mastigação. Meninas, crianças com maior FS e menor conteúdo de proteína tiveram maior porcentagem de acerto no sabor dos alimentos. Concluindo, houve relação entre FM e FS em crianças com oclusão normal, nas quais observou-se que a maior FM está relacionada a maior freqüência de mastigação de alimentos, o que indica facilidade de mastigação; o FS maior facilitou a mastigação e percepção do sabor de alimentos.

Palavras-chave: força de mordida, fluxo salivar, crianças, espessura muscular, masseter, temporal, ultrassom, mastigação, alimento, textura, sabor, dentição mista, mantenedor de espaço

ABSTRACT

Several are the factors that seem to intefeer on mastication: bite force (which might be related to the size of the muscles), components and quatity of saliva, occlusion caracteristics, among other. This thesis is composed by five chapters. Chapter 1 "*The use of ultrasound in the investigation of the muscles of mastication*" did a literature review about the use of ultrasonography on the investigation of the muscles of mastication and reinforced that US is a reliable method of great utility in the diagnostic field of alterations of the muscles of mastication and in the study of changes during growth and aging and that it remains as a promising technique for the study of the muscles of mastication. Chapter 2, "*Correlation between muscle thickness and bite force in children before and after oral rehabilitation – a 2 year logitudinal study*", evaluated the correlation between BF and muscle thickness before and after 6, 12, and 24 months of oral rehabilitation (T0, T6, T12, T24, respectively) in 19 6-9 years-old children with early loss of primary teeth. BF was determined with a flexible pressurized transducer. Masseter and temporal muscle thickness was measured during relaxation and maximal intercuspation by digital US with a high-resolution real-time 56mm/10-MHz linear-array transducer. There was a significant increase in BF and masseter thickness between T0 and T12. There was a significant correlation between BF and relaxed and contracted temporal thickness at T6, and contracted temporal at T24. It was concluded that there was an increase in BF and masseter thickness after rehabilitation and there was also a relation between BF and temporal thickness, which suggests that the oral rehabilitation influences the morphological and functional aspects of the muscles of mastication. Chapters 3 "*Bite force and salivary flow rate in schoolchildren",* 4 "*Bite force, texture perception and chewing parameters in schoolchildren*" and 5 "*Salivary flow rate, protein content, chewing parameters, and taste perception in schoolchildren*" aimed to evaluate and correlate the salivary flow rate (SFR), BF, chewing parameters, perception of taste and texture of food, and the role of protein on mastication of children 7-10 years-old distributed according to occlusion and sex. BF was determined with a flexible pressurized transducer. Unstimulated (U) and stimulated (S) whole saliva were collected for 5 minutes in order to stablish the salivary flow rate (SFR), and the total protein was evaluated by method of Lowry *et al.* (1951). The following foods were tested: carrot, toast with and without margarine, banana, cheese, and chewing gum to

determine the chewing parameters (chewing time, number of cycles and frequency), texture (using a visual analogue scale from 0 to 100 mm) and the taste (bitter, sour, sweet or salty). Boys presented SFR and BF (Chapter 3) significantly higher than girls. There was a significant correlation between SFR and BF for the group with normal occlusion. There was no significant difference in BF, texture or chewing parameters among groups of occlusion. Chewing parameters and texture were significantly lower for soft than hard foods. Significant correlation was found for BF and chewing frequency in the normal occlusion group. There was significant correlation between SFR and chewing parameters. Girls, children with higher SFR and lower protein content got more correct taste choices. Concluding, there was a relation between SFR and BF in children with normal occlusion, on whom a greater BF was related to a higher chewing frequency, which indicates facility of chewing; a higher SFR facilitated the mastication and taste perception of foods..

Key words: bite force, salivary flow rate, children, muscle thickness, masseter, temporal, ultrasound, chewing parameter, food, texture, taste, mixed dentition, space mantainer

SUMÁRIO

I. INTRODUÇÃO GERAL

A mastigação é o primeiro passo do processo digestivo e é considerada uma das funções mais importantes do sistema estomatognático, sendo um de seus principais objetivos romper os alimentos, preparando-os para a deglutição (Bosman et al., 2004). Consiste de uma atividade rítmica dos músculos elevadores e depressores da mandíbula modulada por informações fornecidas durante toda a sua seqüência, desde a ingestão até a deglutição (Hiiemae, 2004), permitindo ajuste do processo mastigatório às propriedades físicas dos alimentos, de acordo com um *feedback* preciso (Lucas et al., 2004; Hiimae, 2004; Dan & Kohyama, 2007; Dan et al., 2007).

Durante a mastigação o alimento é, em menor ou maior extensão, triturado e quebrado em pequenas partículas. Esta trituração mecânica ocorre com a umidificação e lubrificação do alimento pela saliva (Engelen et al., 2005), pois durante a mastigação mecanorreceptores dos tecidos gengivais, periodonto e mucosa são estimulados, induzindo ao fluxo salivar.

A mastigação também é importante para a manutenção da atividade das glândulas salivares, afetando o tamanho da glândula e, conseqüentemente, a secreção salivar. Sem a função adequada das glândulas salivares, um indivíduo pode experimentar severo prejuízo da saúde bucal, deglutição, fala e prazer na alimentação (Pereira et al, 2006). Componentes da saliva, tais como água, proteínas, mucinas e amilase, participam e facilitam as funções motoras da mastigação, deglutição e fala, assim como as funções sensoriais da percepção do sabor, paladar e textura dos alimentos dentro da cavidade bucal (Engelen et al., 2003; Engelen et al., 2007), que somados à aparência constituem os principais atributos de aceitabilidade na alimentação (Bourne, 2004). As proteínas presentes na saliva podem, possivelmente, ter papel na recepção química do sabor e na percepção de adstringência, viscosidade e outras percepções sensoriais da boca (Guinard & Muzzecchelli, 1996).

A saliva também pode afetar ou facilitar a mastigação. Alimentos secos e duros, por exemplo, requerem mais ciclos antes da deglutição (Engelen et al., 2005). Evidentemente, mais tempo é necessário para a quebra de alimentos e para adicionar saliva e formar um bolo coesivo adequado à deglutição.

A mastigação adequada que estimula as glândulas salivares a secretar saliva para a formação do bolo alimentar ideal à deglutição depende da boa condição dos músculos mastigatórios. A condição destes determina a quantidade de força disponível para cortar e triturar os alimentos durante a mastigação. A avaliação da força de mordida é uma das técnicas utilizadas para avaliar clinicamente as características fisiológicas dos músculos mastigatórios (Kiliaridis et al., 1993). A força de mordida máxima está relacionada principalmente à saúde do sistema estomatognático e acredita-se que quanto maior a força, melhor é o sistema (Helkimo et al., 1975). Além disso, a força de mordida tem sido relacionada ao fluxo salivar em adultos. Yeh et al (2000) e Ikebe et al (2007) relataram que a diminuição da força de mordida foi associada à diminuição do fluxo salivar estimulado e não estimulado.

A força de mordida também tem sido relatada como preditora chave da performance mastigatória (Hatch et al., 2000; Fontijn-Tekamp et al., 2000) e valores altos têm sido considerados como influenciadores da performance mastigatória, especialmente quando da mastigação de alimentos duros (Okiyama et al., 2003).

Por outro lado, a performance mastigatória pode também ser mensurada pelo número de ciclos necessários para deglutir certos alimentos naturais (Owens et al., 2002; Fontijn-Tekamp et al., 2004; Gambareli et al., 2009). A redução das partículas dos alimentos é determinada por um processo multifatorial complexo, que depende de fatores tais como força de mordida, coordenação dos músculos da mastigação e a morfologia e o número de pares de dentes em oclusão (Fontijn-Tekamp et al., 2004). As condições da dentição de cada indivíduo influenciam a performance mastigatória. A literatura tem encontrado resultados divergentes em relação ao número de ciclos necessários para a deglutição em relação à performance mastigatória relatando que uma dentição inadequada determina que sujeitos usem mais ciclos mastigatórios para preparar o alimento para a deglutição do que sujeitos com dentição natural (van der Bilt et al., 1993) ou que quanto mais tempo um sujeito mastiga o alimento, menores são as partículas deglutidas, independentemente da performance mastigatória (Fontijn-Tekamp et al., 2004) e concorda que sujeitos com reduzida performance mastigatória deglutem partículas maiores de alimentos (van der Bilt et al., 1993; Fontijn-Tekamp et al., 2004). Um fator que parece

afetar a performance mastigatória e o limiar de deglutição é o número de pares de dentes em oclusão (Fontijn-Tekamp et al, 2004).

A força dos músculos matigatórios durante a mordida, portanto, está relacionada à qualidade da mastigação e à performance mastigatória. Há múltiplos fatores que contribuem para a força de um músculo, incluindo a espessura das fibras dos músculos, o número total de unidades motoras das fibras musculares e suas propriedades fisiológicas. Fibras maiores e mais numerosas resultam em um músculo maior e com maior área de secção transversal (Zhao & Monahan, 2007). A área e a espessura dos músculos mastigatórios de humanos têm sido relacionadas à máxima força do músculo (Bakke et al., 1992; Pereira et al., 2007; Castelo et al., 2007). Tem sido mostrado que quanto maior o músculo, maior a força de mordida, e que a diminuição no tamanho do músculo masseter pode estar relacionada à redução das forças mastigatórias utilizadas pelos indivíduos no envelhecimento (Newton et al., 1993).

Uma maneira de se avaliar a espessura e a área dos músculos da mastigação é através da ultrassonografia (US), que provou ser um método capaz de fornecer informação pela representação das alterações estruturais dos músculos (Wilson & Crocker, 1985). A US que permite acesso fácil e reprodutível aos parâmetros da função muscular e sua interação com o sistema crânio-mandibular e representa considerável melhoria em relação aos métodos convencionais do acesso à espessura dos músculos da mastigação, particularmente em termos de disponibilidade e custo (Emshoff et al., 2003; Serra et al, 2008) além de permitir estudos longitudinais em larga escala de mudanças da espessura muscular durante o crescimento em relação às mudanças nas propriedades biomecânicas dos músculos mastigatórios (Raadsheer et al., 1994, 1996).

O exame da US geralmente é aplicado somente aos tecidos superficiais da região maxilofacial, porque o esqueleto facial protege os tecidos profundos (Serra et al, 2008). Entretanto, oferece vantagens em potencial porque pode ser realizado não invasivamente, repetidamente, até mesmo em acamados (Serra et al, 2008). Com um transdutor de alta resolução, é possível demonstrar em detalhes estruturas dos tecidos superficiais e quaisquer lesões associadas. Além disso, tem sido demonstrado que a US é uma técnica valorosa para a análise precisa do formato muscular (Kubo et al., 2006).

Outro fator que tem sido relacionado tanto ao menor tamanho dos músculos mastigatórios, verificado por US, quanto a uma menor força de mordida, apesar de ainda não haver um consenso na literatura, é a presença de maloclusões (Tsai, 2004; Kamegai et al., 2005; Kiliaridis et al., 2007; Sonnesen & Bakke, 2007; Castelo et al., 2007). O reconhecimento de condições que predispõem crianças jovens à maloclusão é uma parte importante de qualquer avaliação odontopediátrica completa, já que a detecção destas condições em idades precoces pode permitir intervenções ou monitorações efetivas. Considerando que uma das funções mais importantes dos dentes decíduos é preservar espaço para o sucessor permanente até que este esteja pronto para irromper (Bijoor & Kohli, 2005), no caso de uma perda precoce de dentes decíduos, maloclusões poderiam se desenvolver. Assim, seria de importância a prevenção da maloclusão pela fabricação e uso de de espaço funcionais. Tem sido demonstrado na literatura que a reabilitação protética de molares decíduos pode aumentar a força de mordida (Serra et al., 2007) e melhorar a função mastigatória (Gambareli et al., 2009) anteriormente prejudicadas pela menor quantidade de dentes decíduos em função.

Considerando a interação entre a percepção de alimentos, fluxo salivar, função mastigatória, força de mordida e espessura dos músculos da mastigação, torna-se de interesse o estudo destas relações em escolares, tendo em vista que são escassos os trabalhos na literatura relativos à avaliação muscular e percepção sensorial em crianças durante a mastigação. Isto seria desejável, uma vez que o diagnóstico precoce de alterações pode propiciar intervenções influenciadoras na manutenção da integridade morfofuncional do sistema estomatognático.

II – PROPOSIÇÃO GERAL

Os objetivos desta tese foram:

1. Fazer revisão da literatura sobre o uso da ultrassonografia na investigação dos músculos da mastigação;
2. Avaliar e correlacionar longitudinalmente a força de mordida e a espessura muscular antes e após 6, 12 e 24 meses de reabilitação bucal em 19 crianças de 6-9 anos que tinham perda precoce de dentes decíduos.
3. Verificar a associação entre força de mordida e taxa de fluxo salivar (não estimulado e estimulado) em escolares e sua associação à maloclusão, fase inicial ou final da dentição mista e sexo.
4. Avaliar a força de mordida, percepção da textura dos alimentos e parâmetros de mastigação (tempo de mastigação, número de ciclos mastigatórios, freqüência mastigatória) em escolares e verificar se diferentes forças de mordida influenciam os parâmetros de mastigação, entre diferentes tipos de oclusão.
5. Avaliar as características da taxa de fluxo salivar, parâmetros de mastigação e percepção do sabor em escolares, e avaliar o efeito do fluxo salivar e o papel da proteína nos parâmetro mastigatórios e percepção do sabor dos alimentos.

III – CAPÍTULOS

Esta tese está baseada na Resolução CCPG/002/06/UNICAMP (Anexo 1) que regulamenta o formato alternativo para teses de Mestrado e Doutorado e permite a inserção de artigos científicos de autoria ou co-autoria do candidato . Por se tratarem de pesquisas envolvendo seres humanos os projetos de pesquisas destes trabalhos foram submetidos à apreciação do Comitê de Ética em Pesquisa da Faculdade de Odontologia de Piracicaba, tendo sido aprovados (Anexo 2). Assim sendo, esta tese é composta por cinco capítulos contendo artigos que foram ou serão submetidos à publicação, conforme descrito abaixo:

✓ **Capítulo 1**

"*The use of ultrasound in the investigation of the muscles of mastication*"

Serra MD, Duarte Gavião MB, dos Santos Uchôa MN. Este artigo foi publicado no periódico Ultrasound in Medicine and Biology. 2008 Dez; 34(12): 1875-84. Epud 2008 Sep 5

✓ **Capítulo 2**

"*Correlation between muscle thickness and bite force in children before and after oral rehabilitation – a 2 year longitudinal study*"

Serra-Vicentin MD, Gambareli FR, Gavião MBD. Este artigo será submetido à publicação no periódico Journal of Clinical Investigation

✓ **Capítulo 3**

"*Bite force and salivary flow rate in schoolchildren*"

Serra-Vicentin MD, Gavião MBD. Este artigo será submetido à publicação no periódico Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology

✓ **Capítulo 4**

"*Bite force, texture perception and chewing parameters in schoolchildren*"

Serra-Vicentin MD, Gavião MBD. Este artigo será submetido à publicação no periódico American Journal of Physiology. Regulatory, Integrative and Comparative Physiology

✓ **Capítulo 5**

"*Salivary flow rate, protein content, chewing parameters, and taste perception in schoolchildren*"

Serra-Vicentin MD, Gavião MBD. Este artigo será submetido à publicação no periódico Journal of Physiology (London)

CAPÍTULO 1

The use of ultrasound in the investigation of the muscles of mastication

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Introduction

The field of medical imaging, stimulated by advances in digital and communication technologies, has grown tremendously. New imaging techniques that reveal greater anatomical detail are available in most diagnostic departments (Rasheed et al., 1996).

Among these can be included ultrasonographic imaging (US), which has proven to be a method able to provide information by depicting muscle structural alterations (Wilson and Crocker, 1985). US provides an uncomplicated and reproducible access to parameters of jaw muscle function and its interaction within the cranio-mandibular system, and represents a considerable improvement relative to conventional methods for assessing masseter thickness, particularly in terms of clinical availability and cost (Emshoff et al., 2003; Rasheed et al., 1996; Wilson and Crocker, 1985). US examination is usually applied only to the superficial tissues in the maxillofacial region because the facial skeleton shields the deep tissues. However, it offers potential advantage because it can be performed noninvasively, repeatedly and easily, even at the bedside (Ariji et al., 1994; Martin, 1984; Stewart and Moore, 1984). US with a narrow-surfaced probe is helpful in evaluating whether the hypertrophic portion is located in the lower, middle or upper third of the masseter (Sano et al., 1991). With a high-resolution transducer, it is possible to demonstrate in detail structures of the superficial tissues and any associated lesions. Moreover, it has been demonstrated that US is a valuable technique for the precision analysis of muscle shape (Kubo et al., 2006).

US has been described as an accurate and reliable imaging technique for measuring the thickness and cross-sectional area of the masticatory muscles, and for detecting changes in local cross-sectional dimensions of the head and neck muscles *in vivo*

(Bakke et al., 1992; Bertram et al., 2001, 2003a; Close et al., 1995; Emshoff and Bertram, 1998; Kiliaridis and Kalebo, 1991; Kubota et al., 1998; Raadsheer et al., 1994; 1996; Sano et al., 1991; Wilson and Crocker, 1985) It allows for large-scale longitudinal study of changes in jaw-muscle thickness during growth in relation to changes in biomechanical properties of masticatory muscles (Raadsheer et al., 1994).

US could be used to supplement clinical evaluation in patients with muscle-related temporomandibular disorders (Emshoff and Bertram, 1998), and to visualize muscle movement patterns to analyze motions and to monitor effects of specific splint designs (Bertram et al., 2002). Recently, Ariji et al. *(*2001a) showed that color Doppler sonography may be useful to demonstrate the arteries in and around the masseter muscle and it has the potential of being used to evaluate pathological changes in the muscles and arteries. The same authors (Ariji et al., 2001b) later demonstrated that changes of thickness correlated significantly with minimum blood-flow velocity immediately after exercise.

Aim

The objective of this paper is to critically review the literature concerning ultrasonography of the muscles of mastication, discuss its use, advantages and disadvantages, and give some information that could help the understanding of this technique.

Method

A Publine/Medline search was undertaken using the terms ‘ultrasound’ and ‘muscles’ within the limits of ‘Dental Journals’ and ‘humans’, and the languages ‘Portuguese’, ‘Spanish’, and ‘English’, up to the second week of January, 2008. The articles were selected considering its relation to the use of ultrasound in the muscles of

mastication. The reason for excluding an article was the absence of abstract, absence of full article published or absence of specific relation to the study of muscles of mastication by ultrasound, which was verified by reading the title and the abstract of the paper.

US Advantages and Disadvantages

For soft-tissue imaging, ultrasound is superior to radiographs. It is non-invasive, and has no cumulative biological effects on living tissues (Raadsheer et al., 1994). Therefore, US is often used for muscle examination, especially for large superficial muscle groups (Raadsheer et al., 1994).

Clearer images can be obtained with computerized axial tomography (CT) and magnetic resonance imaging (MRI), however US has no radiation risks, compared with CT, which shows cumulative biological effects; and the exposure times are relatively short, compared with MRI, besides the latter would require sedation in children under 10 years of age (Raadsheer et al., 1996). Ultrasonography can project high-resolution images more easily than MRI and CT (Kubo et al., 2006). Up to now, and despite extensive studies, there is no conclusive evidence of adverse biological effects of use of ultrasound energy at diagnostic power levels, as emphasized by Kiliarids and Kälebo (1991). Additionally, US is a rapid, inexpensive technique, and the equipment can be easily handled and transported, these factors render ultrasound an appropriate technique for assessing muscles for rehabilitation purposes, and make it suitable for large-scale studies, especially in children (Close et al., 1995; Raadsheer et al., 1994, 1996). Moreover, US is a valuable technique for the precision analysis of muscle shape (Kubo et al., 2006).

Ultrasound allows for registration of superficial muscles only, and the cross-sectional areas of the muscles cannot always be covered by the transducer (Raadsheer et al.,

1994, 1996), which could be considered as a disadvantage. Ultrasonographic assessment of cross-sections may also be highly susceptible to technique-related factors (Bertram et al., 2003a). The fact that variables such as transducer pressure exerted on the underlying muscle, transducer orientation, and muscle-site related to the absence of anatomical landmarks may be significantly related to the different ultrasonographic technique of having the patient maintaining slight interocclusal contact, clench, or a physiologic rest position (Kiliaridis and Kalebo, 1991; Raadsheer et al., 1994).

Despite these disadvantages, US is an indicated technique for evaluating muscles *in vivo*, for longitudinal studies, and for evaluations in children, since its advantages suppress the disadvantages. Moreover, it adds valuable information to the conventional methods for the study of muscles of mastication and other muscles and organs of the human body, working also as a diagnose complement, and it is easily accepted by the patients or the patient's parents.

Techniques

The US images are composed of sound wave relations at areas of interface between substances of different acoustic impedance (Kiliaridis et al. 1995). The devices used for US of the stomatognathic system muscles vary greatly among studies. Most of them have been used gray-scale ultrasound systems with real-time scanners and transducers with frequencies varying from 5 to 13 MHz (Table 1). Prabhu and Munshi (1995) considered that the 10 MHz sector scan transducer gives the best possible resolution for the superficial structure. In 1999, Benington et al. studied the muscle volume using a quantitative 3D US system, which is at an experimental stage and requires further development and evaluation.

Ultrasound imaging of masticatory muscles has been used to measure mostly the thickness. The cross-sections, cross-sectional areas, and the transverse dimensions have also been studied (Close et al., 1995; Emshoff and Bertram, 1995, 1998; Emshoff et al., 1999, 2002). The scans have been performed unilaterally or bilaterally, during relaxation and/or contraction (Table 1). The thicknesses of the muscles are usually measured directly on the screen of the scanner with an accuracy of 0.1 mm.

A variety of techniques have been shown in the literature for the registration of the thickness of the muscles. However, it can be drawn a technique which has been used by most of the authors. Usually the registrations are performed two or three times with a time interval between the two measurements of about 2 to 5 minutes and the final thickness is obtained from the mean of the measurements. The trials are conducted in a darkened room with the participants seated upright, heads in a natural position and the Frankfort Horizontal plane parallel to the floor. To avoid tissue compression, a generous amount of gel is used under the probe and the transducer is held against the cheek with light pressure. The angle of the probe is altered until the best bone echo of the mandibular ramus surface is achieved. Contrast between muscle and subcutaneous tissue is enhanced by asking the subject to clench and relax alternately. In the relaxed state, the participants are asked to relax, but to keep slight interocclusal contacts to avoid muscle stretching as a result of mouth opening, and in the contracted state the participants are asked to clench maximally.

It has been reported that the relaxed muscles have lower reproducibility than the contracted ones (Bertram et al., 2003a; Castelo et al., 2007; Emshoff et al., 2002, 2003; Kiliaridis and Kalebo, 1991; Kubo et al., 2006; Raadsheer et al., 1994) due to the higher susceptibility to pressure with which the transducer is held against the cheek in the relaxed state, and to the brightening of the outline resulting from the change of acoustic impedance

with contraction (Kubo et al., 2006). However, Raadsheer et al. (1996) observed no difference in reproducibility between relaxed and contracted muscles and Pereira et al. (2006) found lower reproducibility for the contracted muscles. These differences in results may be due to different techniques or sample age used in these researches. Seral studies have reported that under contraction the thickness increases significantly (Bakke et al., 1992; Kiliaridis and Kalebo, 1991; Kubo et al., 2006; Morse and Brown, 1990; Pereira et al., 2006; Raadsheer et al., 1994). Kubo et al. (2006) demonstrated that not only the masseter thickness increases with contraction but also its volume increases as a whole.

Masseter muscle

The most common muscle studied is the masseter, since the variation in the total cross-sectional area of all masticatory muscles appears to be the result mainly of variation in the masseter cross-sectional area (Raadsheer et al., 1994), followed by the temporal (Table 1). The masseter muscles have been mostly scanned on a level halfway between the zygomatic arch and gonial angle; the scan plane perpendicular to the anterior border of the muscle and perpendicular to the surface of the underlying ramus, in the thickest part of the masseter (the area of the greatest lateral distention), close and approximately parallel to the occlusal plane (Figure 1). The middle (Bertram et al., 2003a; Emshoff et al., 2003; Raadsheer et al., 1994), medio-inferior and lower level of the contracted masseter muscle (Bertram et al., 2003a) were found to be the most reproducible sites.

The normal masseter image has a relatively smooth internal texture of moderate echogenicity. The muscle is clearly demarcated from the more superficial tissues and is seen to abut directly against the mandibular ramus in the scan; the ramus limits the depth of the field. Usually, the wide white shadow on the top depicts the skin echo (Figure 2). The

image of the masseter muscle mass is the darker area under the skin (Jonasson, 2005; Figure 1). Few other significant landmarks are present in the region examined (Wilson and Crocker, 1985). Healthy masseter muscles have a heterogeneous speckled appearance in cross-section on a US scan because of the irregular connective tissue bundles that randomly permeate their structure (Kiliaridis et al., 1995), hyperechoic bands, which probably correspond to the internal fascia, are usually observed and are sometimes referred to as septa (Bakke et al., 1992). These bands diminish or disappear with inflammation; hence, this is an important structural index of masseteric infection (Ariji et al., 1994).

Although the masseter muscle is always easily identified on sonograms as a homogeneous structure lying adjacent to the echogenic band of the mandible, the presence of a nonhomogeneous pattern makes it difficult or impossible to distinguish the superficial from the deep portion in many instances (Emshoff et al., 1999). The diagnostic misevaluation of the deep masseter may be related to the frequently encountered presence of several strong interweaving tendon sheets, which make well-defined depiction of the muscle difficult in many instances, requiring cooperation by having the patient clench for adequate demarcation (Emshoff et al., 1999). This fact has corroborated by Goto et al. (2001) who considered that near the dental intercuspal jaw position this part of the masseter might be at its optimum length, which is clearly seen in the ultrasound image.

In some cases, accumulation of adipose cells results in greater intramuscular echo intensity (Jonasson, 2005) (Figure 3).

Temporal muscle

The temporal muscles have been scanned at the deepest part of the temporal fossa, directly behind the zygomatic ridge of the frontal bone, just in front of the anterior border of the hairline (the area of greatest lateral distention) (Figure 4). On the sonograms

the temporal muscle corresponds to a thin hypoechogenic band lying adjacent to the medial relations of the temporal fossa (Emshoff et al., 1999). The bony landmark is identified as a hyperdense line, whereas the course of the temporal muscle is evidenced by having the patient clench (Emshoff et al., 1999; Figure 4).

Other muscles of the stomatognathic system

The medial and lateral pterigoid muscles have not been studied by US because they are not superficial muscles, and therefore they do not appear clearly on the US scan. Other muscles of the head and neck that have been studied are the digastrics (Emshoff and Bertram, 1995, 1998; Emshoff et al., 1999; Raadsheer et al., 1999, 2004) sternocleidomastoid (Emshoff and Bertram, 1998; Emshoff et al., 1999; Raadsheer et al., 1999), trapezius (Emshoff and Bertram, 1995), and the lip muscle (Kumar and Kuriakose, 2004; Prabhu and Munshi, 1995; Rasheed and Munshi, 1996). US has also been used to determine the value as a diagnostic aid in TMJ dysfunction (Kubo et al., 2006; Nabeih and Speculand, 1991; Pereira et al., 2006), to evaluate the treatment effects of masseteric hypertrophy (Choe et al., 2005), as well as the sonographic appearances of masseter-muscle metastases (Ahuja and Ying, 2000). Emshoff et al. (1999) reported that the anterior temporal and posterior digastric muscles were the only muscles with acceptable repeatability coefficient.

The size of the jaw muscles have been significantly related to that of the limb and trunk muscles (Raadsheer et al., 2004) and this may indicate that the thickness of the muscles are consistent throughout the body.

Considering theses factors, it is essential to compare studies that measure the thickness at the same sites, when considering the data found by different authors.

Moreover, taking into consideration the difficulty of initial visualization of the muscles on the ultrasound equipment, it is imperative, for methodological purposes in researches and as a diagnostic aid, to have a trained person to perform the scans. A more experienced person, who has already worked for a long time with the equipment, should give this training. One person should conduct all the scans, so the inter-observer errors can be eliminated.

Factors of influence sezes measurements

Age

Most of the researches on US have been made in adult subjects (Table 1, 2), showing a great variability of the measured sizes (Table 2). Few authors have studied the thickness of the muscles of mastication by the use of ultrasound in children (Table 1). Only the studies of Raadsheer et al. (1996), Kiliaridis et al. (2003), Castelo et al. (2007), and Kiliaridis et al. (2007) show direct mean values for muscles in children (range of values on Table 2). Moreover, Raadsheer et al. (1996) and Kiliaridis et al. (2003) found that masseter muscle thickness increases with age in growing individuals, whereas Jonasson and Kiliaridis (2004) found a negative correlation between masseter thickness and age in aging individuals. Considering these studies, it seems that the muscles of mastication increase in size during growing, and decrease during aging. It is important to pay special attention to the site used by different authors, when one wants to compare his data with the findings previously reported.

Gender

Concerning gender differences it has been reported that women have thinner muscles than men (Close et al., 1995; Kiliaridis and Kalebo, 1991; Raadsheer et al., 1996, 1999, 2004) and this may be due to the influence of hormones present after puberty, when boys become bigger and stronger then women, with stronger and possibly thicker muscles.

Side of the muscle

Some authors have reported no side differences in subjects with normal occlusion (Castelo et al., 2007; Close et al., 1995; Raadsheer et al., 1996) or signs and symptoms of TMD (Pereira et al., 2006), whereas others found the thickness to be larger in the left side (Raadsheer et al., 1999). Nevertheless, it is necessary to bear in mind the differences in the age of the samples and the occlusal conditions of the subjects.

Bite force

A significant positive correlation between bite force magnitude and the ultrasound thickness of the masseter muscle has been found (Bakke et al., 1992; Raadsheer et al., 1999) as well as with anterior part of temporal muscle in children with normal occlusion (Castelo et al., 2007). Raadsheer et al. *(*1999) showed that the mean masseter muscle thickness was the main contributor to bite force. These correlations may show that the thicker the muscle, the more capable of producing strength it is, and exercising the muscle can increase its thickness and the bite force as well.

Body variables

A positive correlation has also been reported between the jaw muscles and weight (Kiliaridis and Kalebo, 1991; Pereira et al., 2006; Raadsheer et al., 2004), height (Pereira et al., 2006; Raadsheer et al., 1996), and body constitution (Raadsheer et al., 1999; Satiroglu et al., 2005). However, in children the correlation has not been significant (Castelo et al., 2007). This may be due to the fact that girls are as large as boys until puberty, after which boys usually grow more than girls, who stop growing a few years after the menarca.

Type of occlusion

Prabhu and Munshi (1995) found no significant difference in thickness of the masseter and temporal muscles between normal and open-bite subjects. However, Rasheed et al. (1996) found that the open-bite and deep-bite subjects demonstrated greater thickness of both muscles as compared to normal subjects. In the same way, Castelo et al. (2007) found that in subjects in the mixed dentition with posterior cross-bite, the thickness of the temporal muscle was greater in the cross-bite side than in the normal occlusion side, whereas Kiliaridis et al. (2007) found that the masseter thickness on the cross-bite side was thinner than on the normal side, and that this change could be reversible after orthodontic treatment, when the lateral cross-bite and possible asymmetric muscle activity had been eliminated. The differences between studies might be due to the different techniques used, sample size and age of the subjects, and, especially, the severity of the malloclusion, which generates a greater contrast between normal and malocclusion subjects.

Furthermore, masseter muscle thickness has also been correlated positively with alveolar bone mass (Jonasson and Kiliaridis, 2004) and maxillary intermolar width (Kiliaridis et al., 2003). However, other studies must be undertaken in order to confirm these findings.

US and electrical activity of the masticatory muscles

Pereira et al. (2006) found no correlation between muscle activity and thickness in adolescents, on the other hand, Georgiakaki et al. (2007) found positive correlation between eletromyographic maximum activity and thickness only on the right side of female dental students; the authors attribute this difference between sides to the larger method error found for the left side. Rasheed et al. (1996) and Bakke et al. (1992) found positive correlation between anterior temporal muscle activity and thickness, but no correlation was

found in the masseter muscle. This could possibly be due to the dynamic role of the temporal muscle in maintaining the postural rest position of the mandible (Rasheed et al., 1996).

Bakke et al. (1996) found that prolonged static and dynamic activity in healthy masseter muscles results in swelling (increase size) and local pain, corresponding to the responses seen in skeletal muscles in general. This shows that the muscles of mastication function and react the same way to injuries as muscles in general. Therefore, they must be carefully considered when a treatment that involves them is undertaken.

Facial morphology

Significant correlations have been described between the ultrasonographic thickness of the masseter muscle and facial morphology, both in adults (Bakke et al., 1992; Benington et al., 1999; Farella et al., 2003; Kiliaridis and Kalebo, 1991; Kubota et al., 1998; Satiroglu et al., 2005) and in growing individuals (Raadsheer et al., 1996), showing that individuals with longer faces have thinner muscles, while short faces individuals have thicker muscles.

Occlusal contacts

A positive correlation between the number of occlusal contacts and masseter thickness during contraction was reported by Bakke et al. (1992). This result differs from Castelo et al. (2007) who observed that in young children, the number of occlusal contacts had no effect on the masseter thickness during contraction, which may be due to the difference in age of subjects.

TMD

In relation to TMD, Emshoff and Bertram (1995) found a positive correlation between muscle tenderness and muscle hypertrophy, and Ariji et al. (2004) reported that

there would be a muscle thickness increment in patients with TMD. In contrast, Farella et al. (2003) showed that a thicker masseter had a positive influence in delaying the pain onset and prolonging the endurance. In the same way, Pereira et al. (2006) found no difference in thickness between individuals with or without signs and symptoms of TMD, nevertheless they found a negative correlation between masseter thickness and the craniomandibular index, meaning that as the signs and symptoms increase, the masseter thickness decreases. Emshoff and Bertram (1995) considered that US could be used to help locate TMD affected muscles that do not have the symptom of tenderness on palpation and thereby aid treatment planning; they also considered that US may be beneficial to regularly follow the course of TMJ disorders and the response to the treatment regimen. Bertram et al. (2001) confirmed the concept that reduction and equalization of masticatory muscle activity associated with splint insertion may be an important factor in the successful treatment of muscle related TMD, and they verified this by the use of US.

Moreover, US could be a useful first level diagnostic instrument in the study of TMJ disc displacement (Tognini et al. 2005). US has also proven to be accurate in the detection of joints with effusion (Manfredini et al., 2003a; Tognini et al., 2003) and for studying clinically painful joints (Manfredini et al. 2003b), but in some cases MRI is required to gain a better visualization of the joint (Westesson, 1993). In fact, MRI has been accepted as the most advanced imaging modality for the diagnosis of TMJ abnormalities (Liedberg et al. 1996, Okochi et al., 2008) and for evaluating pathological changes of the masticatory muscles in TMD (Bernhardt et al., 2007).

Kiliaridis et al. (1995) verified the internal masseter muscle structure in Myotonic Dystrophy patients, showing a less discernible tendinous structure and fascia, and

a less discernible ramus surface, but greater intramuscular echointensity. Moreover, these patients had atrophy of the muscle. When there are differences in symptoms between sides, the worse the symptoms are on one side, the capability of chewing is impaired, the muscle on that side may be less exercised, and therefore thinner.

Conclusion

US has been show to be a reliable method of great utility in the field of diagnosis of alterations in the muscles of mastication and for the study of changes during growth and aging. It should be preferred in relation to CT and MRI because of its safety and cost advantages, since it is as reliable and precise as those techniques. The US use has grown tremendously and several authors have found new utilities for the technique. In this way, the US is still a promising technique for the study of muscles of mastication.

Summary

The aim of this paper was to review the literature concerning ultrasonography imaging (US) of the muscles of mastication, discuss its use, advantages and disadvantages, and the findings of the authors. A web search was undertaken using the terms ‘ultrasound’ and ‘muscles’. US has been shown to be a reliable method of great utility in the field of diagnosis of alterations in the muscles of mastication and for the study of changes during growth and aging. It should be preferred in relation to CT and MRI because of its safety and cost advantages, since it is as reliable and precise as those techniques. Although various techniques have been used in the ultrasonographic scans, the paper reveals the most adopted by the authors.

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Table 1 – Frequency, side, state of occlusion, muscles, gender, age (years), number of subjects, dental/health state of subjects of several reports in the literature

Author	Frequency	Side	State Of Occlusion	Muscles	Gender	Age	# Subjects	"Dental/Health" State
Ariji et al., 1994	7.5 MHz	Bilateral	Relaxed	Masseter	Both	8-65	32	Swelling In Masseteric Region
Ariji et al., 2001b	12 MHz	Left	Contracted	Masseter	Both	18-30	30	Healthy
Ariji et al., 2004	12 MHz	Bilateral	Relaxed/Contracted	Masseter	Female	18-40 22-36	25 Patient Group 30 Control Group	With / Without TMD
Bakke et al., 1992	7.5 MHz	Bilateral	Relaxed/Contracted	Masseter	Female	21-28 20-31	13 Study Group 29 Control Group	Extraction Of 3rd Molar Indicated/ Healthy
Bakke et al., 1996	10 MHz	Most Convenient	Relaxed/Contracted	Masseter	Female	21-28	14	Helathy
Bertram et al., 2001	7.5 MHz	Bilateral	-	Masseter	Both	19-77	29	TMD
Bertram et al., 2002	7.5 MHz	Bilateral	Contracted	Masseter	Both	19-77	24	TMD
Bertram et al., 2003a	7.5 MHz	Unilateral	Relaxed/Contracted	Masseter	Both	18-59	42	Healthy
Bertram et al., 2003b	7.5 MHz	Bilateral	Relaxed/Contracted	Masseter	Both	19-57	35	TMD
Castelo et al., 2007	10 MHz	Bilateral	Relaxed/Contracted	Temporal	Both	3.5-7	28 Controls 31 Patients	Nornal occlusion/ Posterior cross-bite
Close et al., 1995	5 MHz	Bilateral	Relaxed	Masseter	Both	21-47	39	Healthy
Emshoff & Bertram,1995	7.5 MHz	Bilateral	-	Temporal/Masseter	Both	Mean 26	50	TMD
Emshoff & Bertram,1998	7.5 MHz	Bilateral	Relaxed	Temporal/Masseter	Both	27-48	15	TMD
Emshoff et al., 1999	7.5 MHz	Bilateral	Relaxed	Temporal/Masseter	Both	14-73	46	TMD
Emshoff et al., 2002	7.5 MHz	Bilateral	Relaxed	Masseter	Both	15-57	17	TMD
Emshoff et al., 2003	7.5 MHz	Unilateral	Relaxed	Masseter	Both	19-56	30	Healthy

Farella et al. 2003	10 MHz	Right	-	Masseter	Men	Young	30	Helathy
Georgiakaki et al., 2007	7.5 MHz	Bilateral	Contracted	Masseter	Female	Mean 23.7	52	Full complement of natural teeth
Jonasson & Kiliaridis, 2004	7 MHz	Right	Contracted	Masseter	Female	40-75	62	At Least 9 Mandibular Teeth
Kiliaridis & Kälebo, 1991	7 MHz	Bilateral	Relaxed/Contracted	Masseter	Both	21-35	40	Healthy
Kiliaridis et al., 2007	7.5 MHz	Bilateral	Contracted	Masseter	Both	7.2-22	38 Untreated patients 18 Treated patients 224 Control	Unilateral cross-bite 3 years after correction No malocclusions
Kiliaridis et al., 2003	7.5 MHz	Bilateral	Relaxed/Contracted	Masseter	Both	7-18	60	Angle's Class I Molar Relationship
Kiliaridis et al., 1995	7 MHz	Bilateral	Relaxed/Contracted	Masseter	Both	42-67	16 Patients 16 Controls	Myotonic Dystrophy/ Healthy
Kubo et al., 2006	13 MHz	Right	Relaxed/Contracted	Masseter	Male	25-28	5	Healthy
Kubota et al., 1998	7.5 MHz	Habitual Chewing	Relaxed/Contracted	Masseter	Male	Mean 23	80	Healthy
Morse & Brown, 1990	5 MHz	Bilateral	Relaxed/Contracted	Masseter	-	-	-	Masseteric Hypertrophy
Pereira et al., 2006	10 MHz	Bilateral	Relaxed/Contracted	Temporal/Masseter	Both	12-18	20 Patients/ 20 Controls	TMD/Healthy
Raadsheer et al.,1994	7.5 MHz	Bilateral	Relaxed/Contracted	Masseter	Men	Mean 36	15	Healthy
Raadsheer et al.,1996	7.5 MHz	Bilateral	Relaxed/Contracted	Masseter	Both	7-49	360	Helathy
Raadsheer et al.,1999	7.5 MHz	Bilateral	Relaxed	Temporal/Masseter	Both	18-36	121	Healthy
Raadsheer et al.,2004	7.5 MHz	Bilateral	Relaxed	Temporal/Masseter	Both	18-36	121	Healthy
Rasheed et al., 1996	7.5 MHz	Unilateral	Relaxed/Contracted	Temporal/Masseter	Both	8-12	30	Different Type Of Occlusions
Satiroglu et al., 2005	7.5 MHz	Bilateral	Relaxed/Contracted	Masseter	Both	Mean 24.96	47	Range Of Skeletal Jaw Discrepancies

Table 2 – Measured sizes of masseter and temporal muscles (mm): ranges from the lowest to the highest value found in the literature.

	Relaxed Masseter thickness		**Contracted Masseter thickness**		**Relaxed Temporal thickness**		**Contracted Temporal thickness**	
	Lowest Value	**Highest Value**	**Lowest Value**	**Highest Value**	**Lowest Value**	**Highest Value**	**Lowest Value**	**Highest Value**
Normal Adult subjects	1.9 (Bertram et al., 2003b)	19.0 (Raadsheer et al., 2004)	14.1 (Kiliaridis et al., 1995)	16.7 (Kubota et al., 1998)	1.8 (Emshoff et al., 1999)	19.0 (Raadsheer et al., 2004)	-	-
Normal Children	4.0 (Prabhu and Munshi, 1994)	12.8 (Raadsheer et al. 1996)	2.0 (Rasheed et al., 1996)	15.5 (Raadsheer et al., 1996)	2.0 (Prabhu and Munshi, 1994)	6.0 (Prabhu and Munshi, 1994)	3.0 (Prabhu and Munshi, 1994)	8.0 (Prabhu and Munshi, 1994)
TMD Patients	1.6 (Emshoff et al., 1999)	10.5 (Pereira et al., 2006)	-	13.3 (Pereira et al., 2006)	1.4 (Emshoff et al., 1999)	2.96 (Pereira et al., 2006)	4.2 (Pereira et al., 2006)	4.5 (Pereira et al., 2006)
Open Bite Children	3.0 (Prabhu and Munshi, 1994)	8.0 (Rasheed et al., 1996)	5.0 (Prabhu and Munshi, 1994)	11.0 (Prabhu and Munshi, 1994)	2.0 (Prabhu and Munshi, 1994)	5.0 (Prabhu and Mushi, 1994; Rasheed et al., 1996)	3.0 (Prabhu and Munshi, 1994)	7.0 (Prabhu and Munshi, 1994)
Deep Bite Children	5.0 (Prabhu and Munshi, 1994)	9.0 (Prabhu and Munshi, 1994)	7.0 (Prabhu and Munshi, 1994)	11.0 (Prabhu and Munshi, 1994)	3.0 (Prabhu and Munshi, 1994)	6.0 (Prabhu and Munshi, 1994)	3.0 (Prabhu and Munshi, 1994)	8.0 (Prabhu and Munshi, 1994)
Cross-bite Children	9.8 (Castelo et al., 2007)	10.1 (Castelo et al., 2007)	11.3 (Castelo et al., 2007)	11.9 (Castelo et al., 2007)	2.5 (Castelo et al., 2007)	2.8 (Castelo et al., 2007)	3.2 (Castelo et al., 2007)	3.5 (Castelo et al., 2007)

Figure 1 – Image of tranducer on volunteer. (A) tranducer on masseter; (B) transducer on temporal

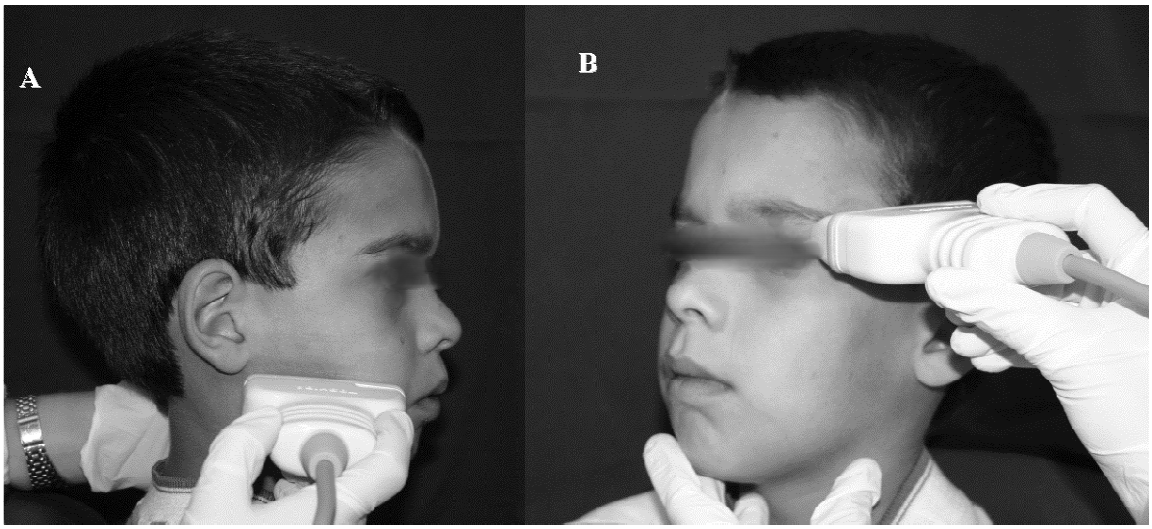


Figure 2 – Image of the masseter muscle during relaxation (a) and contraction (b); (1) surface of transducer, (2) mandibular ramus, (3) thickness of the masseter muscle

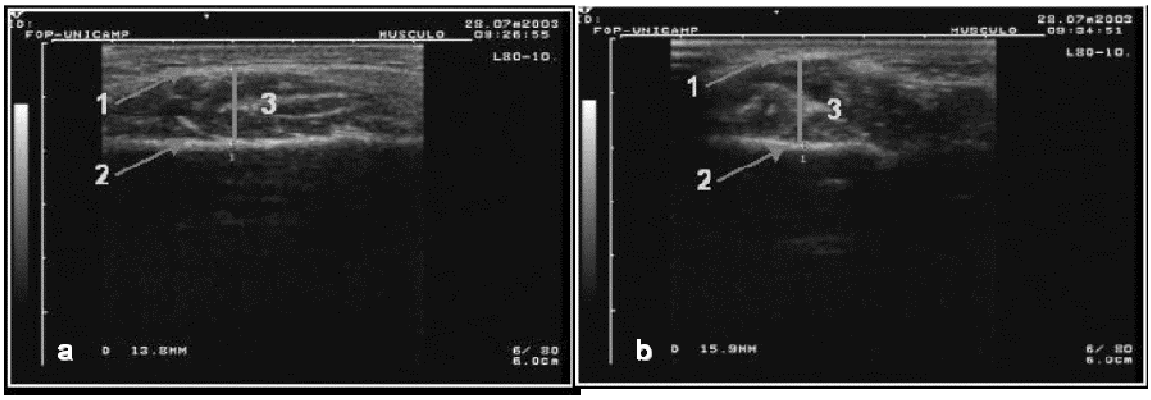


Figure 3 – Image of the masseter with increased echogenicity from adipose tissue

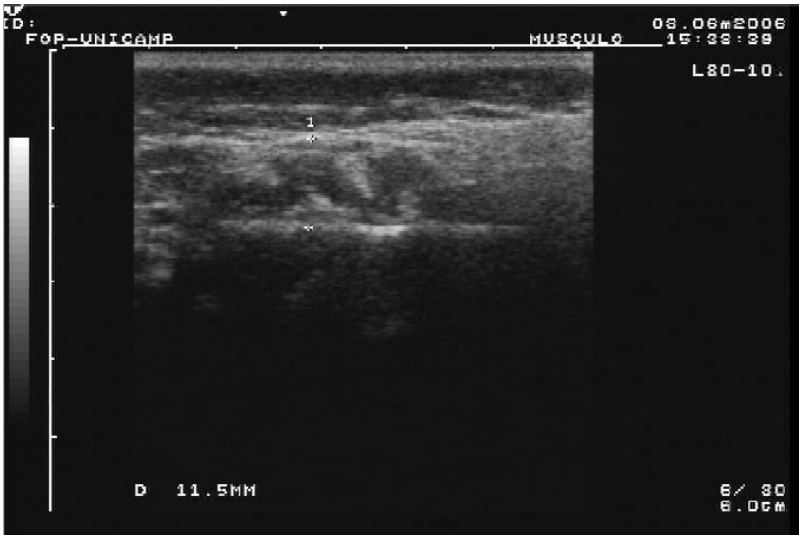
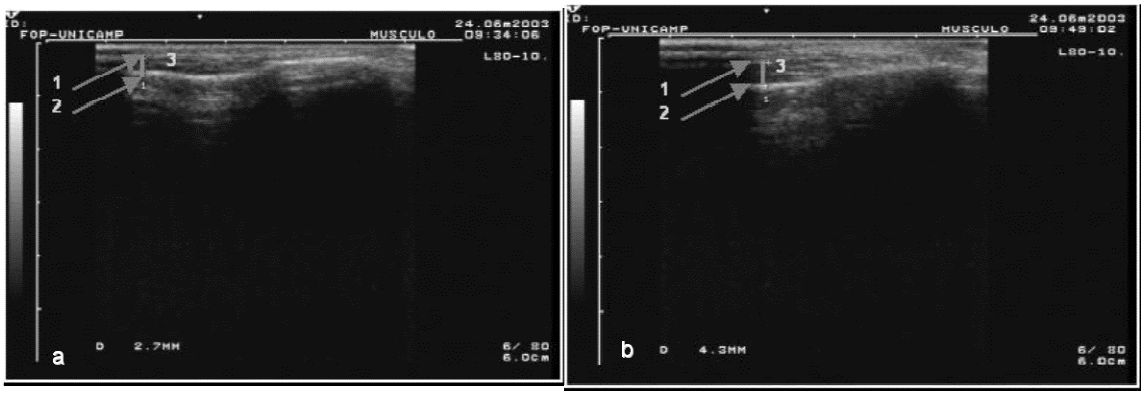


Figure 4 – Image of the temporal muscle during relaxation (a) and contraction (b); (1) surface of the transducer, (2) temporal bone



CAPÍTULO 2

Correlation between muscle thickness and bite force in children before and after oral rehabilitation – a 2 year logitudinal study

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Abstract

Purpose: the aim of this study was to asses the relation between bite force (BF) and muscle thickness before (T0) and after 6 (T6), 12 (T12) and 24 (T24) months of oral rehabilitation in 19 children (6-9 years-old) with early loss of primary teeth. **Methods**: BF was determined with a flexible pressurized transducer. Masseter and temporal muscle thickness was measured during relaxation and contraction bilaterally using Just-Vision 200 digital ultrasonography system. Statistical analysis between genders were done through unpaired *t* test or Mann-Whitney tests, ANOVA for repeated measurements was used for comparisons among evaluations, correlations were assessed by Pearson's coefficients significant at the $p < 0.05$ level. **Results:** No statistical significant difference between genders was found. There was a significant increase in bite force and masseter thickness during the first 12 months of evaluation. There was a significant correlation between BF and relaxed and contracted temporal thickness at T6, and contracted temporal at T24. BF and masseter thickness were not correlated at the four evaluations ($p>0.05$). **Conclusion:** the oral rehabilitation influenced the morphological and functional aspects of the muscles of mastication, increasing bite force and masseter thickness, and BF was related to the thickness of the temporal muscle after rehabilitation.

Key words: bite force, ultrasound, masseter, temporal, children, rehabilitation

Introduction

Premature loss of primary teeth during deciduous and mixed dentition can affect the normal growth and development of the stomatognathic system causing an impairment of its functions, and can affect the intake of nutrients due to a more restricted food choice, because the mastication of harder foods is more difficult (Gambareli et al., 2009). The etiological factors of premature primary teeth loss include dental caries and traumatisms. If the pulpar tissues are affected by an infection, the permanent tooth germ can be injured and its development compromised. Therefore, in order to diminish the probability of injuries in the permanent teeth, infected primary teeth extraction is sometimes necessary, especially if there is a periapical lesion involving the subjacent tooth (Broadbent et al., 2004; Ak et al., 2005). Nevertheless, the extraction of teeth often leads to space loss and the masticatory function can be affected, decreasing the masticatory muscle strength (Shiau & Wang, 1993), which determines the amount of available force to cut or crush the food. In this case, it is indicated the tooth substitution by a device, as a functional space maintainer or a dental prosthesis, which must preserve the space, allowing the permanent tooth to erupt unhindered into proper alignment and occlusion (Bijoor & Kohli, 2005), provide psychological benefit to the child, and preserve oral functions as mastication and phonation.

Bite force is one of the techniques used to clinically evaluate the physiological characteristics of the masticatory muscles. There are multiple factors that contribute to the strength of a muscle, including the size of the individual muscle fibers, the total number of muscle fibers, and their physiologic properties. Larger and more numerous muscle fibers result in a larger muscle with a greater cross-sectional area (Zhao & Monahan, 2007).

One method used to evaluate the area and the thickness of the muscles of mastication is ultrasonography (US), which has been widely used in the study of the muscles of mastication (for a review see Serra et al., 2008). It adds valuable information to the conventional examinations of jaw muscle functions and the interaction within the craniomandibular system (Bakke et al., 1992). This technique allows for large-scale longitudinal study of changes in jaw-muscle thickness during growth, in relation to change in biomechanical properties of masticatory muscles (Raadsheer et al., 1994, 1996).

The midbelly cross-sectional area and thickness of human masticatory muscles have been shown to relate to the maximum muscle strength (Bakke et al., 1992; Raadsheer et al., 1999; Pereira et al., 2007; Castelo et al., 2007). It has been show that the thicker the muscles the stronger the bite forces.

Recognizing conditions which predispose young children to malocclusions is an important part of any comprehensive pediatric dental assessment, since the detection of these conditions in early ages can allow either intervention or monitoring on an effective basis (Ngan & Fields, 1995). One of the main functions of a primary tooth is to hold space for the permanent successor until it is ready to erupt (Bijoor & Kohli, 2005), in the case of early loss of primary teeth malocclusions could develop, which could afect the function and morphology of the stomatognathic system. The prevention of malocclusions may be achieved by the fabrication and use of the functional space mantainers retained by clasps in the first molars.

Considering the influence of the premature primary tooth loss upon the functional and morphological aspects of the masticatory system development, the aim of this study was to asses the relation between bite force and muscle thickness before (T0) and after 6 (T6), 12 (T12) and 24 (T24) months of oral rehabilitation.

Material and methods

Subjects

The subjects, 6-9 years of age at the beginning of the study, were children who were to start dental treatment in the Department of Pediatric Dentistry at the Piraciaba Dental School. At T0 and T6 there were 25 children (13 boys, 12 girls), after one year (T12), there were 3 dropouts (patients who gave up the treatment and did not appear for the appointments), remaining 22 children (12 boys, 10 girls); after two years (T24) there was an additional 4 dropouts (patients who gave up the treatment and did not appear for the appointments), remaining 19 children (11 boys, 8 girls). This study considered only the children who remained throughout the whole study time (n=19). Written and verbal consent was obtained from each child's parents and the Dental School Ethics Committee approved the research.

The selection of the children and the treatment followed the same criteria as described by Serra et al. (2007). The children were in the mixed dentition, and the inclusion criteria were the normality of the oral tissues, presence of maxillary and mandibular first permanent molars in Angle's Class I relationship, absence of signs or symptoms of temporomandibular dysorders (TMD) or parafunctional habits, and premature loss of one or more primary molars, which was substituted by a functional space mantainer (Serra et al., 2007).

The follow up period was two years, and four evaluations of maximum bite force, muscle thickness, body weight and height were performed before the prosthesis placement (T0), at six (T6), twelve (T12) and twenty-four months after (T24).

Bite force measurements

The bite force measurements procedures have been previously described (Serra et al., 2007).

Briefly, bite force was determined with a flexible pressure transducer. For each child, the transducer was placed between the first permanent molars billaterally. As the transducer was placed only in contact with the first permanet molars, the eruption phase of the other permanent teeth in the mixed dentition did not influence the measurement. The force was measured during maximum clenches (2-second duration). Four measurements were made; the first was discarded, and the molar bite force was assessed as the mean of the successive 3 trials. To obtain the highest bite values possible, the subjects were trained before the test (biting the transducer two times before the actual measurement) and they were instructed to bite as forcefully as possible.

This method has already been tested in our laboratory with good results as stated by Rentes et al. (2002). The reliability of the bite force measurement has previously been assessed by double recordings on 10 randomly selected children with an interval of 1 week (Serra et al., 2007) without significant difference between the two sets of measurements and with the method error of the individual double recordings with 1-week interval of 6.55%.

Muscle thickness

The thickness of masseter and anterior portion of the temporal muscles were measured using the Just-Vision 200 digital ultrasonography system (Toshiba Corporation, Japan) and the images were obtained with a high-resolution real-time 56mm/10-MHz linear-array transducer. The recordings were performed bilaterally during relaxation and maximal intercuspation. The location of the muscles was obtained through palpation (the area of greatest lateral distention); for the masseter the site of measurement was close to the level of the occlusal plane, approximately in the middle of the mediolateral distance to the ramus and perpendicular to it (Serra et al., 2008). For the anterior portion of the temporal muscle the transducer was placed in front of the anterior border of the hair line, at the deepest part of the temporal fossa, perpendicular to the underlying bone (Serra et al., 2008). In order to be perpendicular to the bones, the transducer was moved until they were depicted on the screen as a sharp white line. The thickness was measured directly on the screen with an accuracy of 0.1 mm.

All trials were conducted in a properly darkened room, being the subjects seated in an upright, neutral but comfortable position, with the Frankfort Horizontal plane parallel to the floor (Serra et al., 2008). The registrations were repeated twice with an interval of at least 5 minutes. The thickness per side was calculated as the mean of the two measurements. To avoid tissue compression, a generous amount of gel was applied under the probe. Contrast between muscle and subcutaneous tissue was enhanced by asking the subject to clench and relax alternately (Serra et al., 2008).

All scans were carried out by the same observer (MDS), properly trained, to eliminate the inter-observer difference. The reliability of the measurements was determined on 10 ramdomly selected children using the Dahlberg's formula, the method error was 4.1% and 2.8% for the right and left relaxed masseter, and 2.9% and 2.5% for the contracted masseter, respectively; 6.5% and 6.2% for the right and left relaxed temporal muscle; and 4.3% and 7.0% for the contracted temporal muscle.

Body Variables

Body weight and height were determined in order to verify any differences between genders.

Statistical Analysis

The normality of the distributions was assessed by the Shapiro-Wilk's *W*-test. The comparisons between genders were performed through unpaired *t* test or Mann-Whitney test, and the comparisons between evaluations with ANOVA for repeated measures. The correlations among the variables were assessed by Pearson's coefficients significant at the $p < 0.05$ level. All analyses were carried out using BioEstat 5.0 (2007, Belém, PA, Brazil).

Results

There was no significant difference between genders for all variables (muscle thickness, bite force, body weight, and height) in all 4 sessions, therefore, the data were pooled for comparisons and correlations. Moreover, there was no significant difference between sides, thus the mean values between sides was used for statistical analysis.

Bite force increased significantly from T0 to T12 and T24, whereas among other sessions there were no differences (Table 1). There was a significant increase in the masseter thickness from T0 to T6 in both relaxed and contracted states, remaining the same throughout the rest of the period (Table 1). The change in thickness of anterior temporal was seen only in relaxed state from T0 to T12 and T24, and between T6 and T12, as well. (Table 1).

BF was significantly correlated with temporal thickness in both states at T6, and with contracted temporal at T24 (Table 2). There was a significant correlation in thickness between relaxed and contracted masseter; the same was observed for the temporal muscle (Table 2). Moreover, the correlations between the thickness of masseter and the thickness of temporal in both states were significant at T12 (Table 2).

Tables

Table 1 – Mean values and standard deviations (±SD) for the bite force and muscle thickness in all sessions (n=19)

	T0	T6	T12	T24
BF (N)	306.4±59.4^{A}	343.2±44.9^{AB}	354.1±45.9^{B}	381.2±49.1^{B}
Relaxed Masseter (mm)	9.5±0.7^{A}	10.6±0.9^{B}	10.5±0.6^{B}	10.3±0.7^{B}
Contracted Masseter (mm)	10.9±0.8^{A}	11.8±1.0^{B}	11.7±0.6^{B}	11.7±0.7^{B}
Relaxed Temporal (mm)	3.5±0.5^{A}	3.3±0.4^{AC}	3.0±0.1^{B}	3.2±0.2^{BC}
Contracted Temporal (mm)	4.4±0.7^{A}	4.4±0.5^{A}	4.2±0.3^{A}	4.3±0.4^{A}

Different cappital letters in the same line mean statistical significant difference between sessions ($p<0.05$)

Table 2 – Matrix of correlations between variables for the 4 sessions

		Relaxed Masseter (mm)	Contracted Masseter (mm)	Relaxed Temporal (mm)	Contracted Temporal (mm)
BF (N)	T0	-0.125 *0.611*	0.062 *0.801*	0.251 *0.300*	0.323 *0.178*
	T6	0.045 *0.856*	0.109 *0.657*	0.483* *0.036*	0.485* *0.035*
	T12	0.163 *0.506*	0.043 *0.863*	0.074 *0.764*	0.412 *0.079*
	T24	0.435 *0.063*	0.406 *0.085*	0.443 *0.058*	0.700* *0.001*
Relaxed Masseter (mm)	T0	-	0.877** *< 0.0001*	-0.356 *0.135*	-0.227 *0.350*
	T6	-	0.944** *< 0.0001*	-0.110 *0.655*	-0.162 *0.508*
	T12	-	0.917** *< 0.0001*	0.656* *0.002*	0.722* *0.001*
	T24	-	0.959** *< 0.0001*	0.149 *0.542*	0.423 *0.071*
Contracted Masseter (mm)	T0		-	-0.191 *0.433*	-0.105 *0.669*
	T6		-	-0.111 *0.652*	-0.153 *0.533*
	T12		-	0.655* *0.002*	0.587* *0.008*
	T24		-	0.102 *0.679*	0.390 *0.099*
Relaxed Temporal (mm)	T0			-	0.905** *< 0.0001*
	T6			-	0.901** *< 0.0001*
	T12			-	0.748** *0.000*
	T24			-	0.735** *0.000*

* indicates significant correlations at p<0.05; ** indicate correlations significant at p<0.001
p-values in italics

Discussion

There was no significant differences between genders for the variables in this sample, which is in agreement with other studies performed in children (Shiau & Wang, 1993; Kiliaridis et al., 1993; Maki et al., 2001; Sonnesen et al., 2001; Sonnesen & Bakke, 2007; García-Morales et al., 2003), this probably happened because these differences become evident after puberty. Moreover, body variables were not taken into account for correlations with bite force and muscle thickness since in children our previous study (Serra et al., 2007) and studies by other authors show that the correlation has been seen as not significant or weak (Castelo et al., 2007; Kiliaridis et al., 1993; Rentes et al., 2002; Garcia Morales et al., 2003; Gavião et al., 2007;). The expected influences of body size upon masticatory variables must occur later due to the increase in muscle mass (English et al., 2002; Shiau & Wang, 1993).

Bite force increased significantly when comparing T0 with T12 and T24 (Table 1). This gain in bite force could be explained by the normalization of the muscle function with the use of the space maintainer within the 12 months, since this increase was also seen in masseter thickness. All masticatory muscles influence the mandible, but the masseter seems to be a good representative, since the variation in the total cross-sectional area of all masticatory muscles appears to be the result mainly of variation in the masseter cross-sectional area (Raadsheer et al., 1994). Moreover, the lower bite force in T0 could be explained by the restricted chewing function caused by the impaired dentition, considering the results of Gambareli et al. (2009), who observed an improvement in chewing after oral rehabilitation of children with premature tooth losses.

In addition, occlusal contacts promote mandibular stability at maximal intercuspation (Rodrigues et al., 2003), and have an influence on chewing function (Owens et al., 2002). The subjects in this study had absence of teeth prior to the treatment, which were replaced by the artificial teeth adapted on the removable partial dental prosthesis, increasing the amount of contacts. Therefore, they could chew better, improving the muscle function and consequently increasing the bite force and masseter thickness during the first months, adapting to a new oral condition, giving time to the muscle increase its force (Serra et al., 2007) and also the thickness for the masseter muscle. The lack of gain in bite force or muscle thickness on the following period (at the 24 months evaluation) might suggest that after a relatively short time after rehabilitation (6 months for masseter thickness and 12

months for bite force) children achieved stability of function and therefore bite force and muscle thickness remained the same.

In the relaxed state the temporal muscle showed a not linear variability in thickness. This could be due to the lower reproducibility of the relaxed muscles compared with the contracted ones (Raadsheer et al., 1994; Bertram et al., 2003; Kubo et al., 2006; Castelo et al., 2007) because the higher susceptibility to the pressure by which the transducer is held against the cheek in the relaxed state, and to the brightening of the outline resulting from the change of acoustic impedance with contraction (Kubo et al., 2006). The absence of increase in the temporal muscle thickness even in contracted state might be due to the fact that this muscle needs more time to increase its thickness in response to a stimulus, which, in this case, was the prosthesis use, nevertheless this data would have to be further studied.

On the other hand, althought there was not an increase in thickness for the temporal muscle, there was a significant correlation between bite force and thickness of temporal muscle at T6 and T24 that could be attributed to the dynamic role of the temporal muscle in maintaining the postural rest position of the mandible (Rasheed et al., 1996), despite a gain in thickness was not seen by ultrasound. This finding is in accordance with Castelo et al. (2007) who also found a significant correlation between temporal thickness and bite force, but only for the normal occlusion group. Perhaps the normalization of the function was the main contributor of the correlation only after 6 and 24 months of rehabilitation. Nevertheless, this correlation was not found at T12, probably due to some technique related factors such as transducer pressure exerted on the underlying muscle, transducer orientation, and muscle-site factors related to the absence of anatomical landmarks (Serra et al., 2008).

This study failed to find any significant correlation between bite force and the thickness of the masseter muscle. Pereira et al. (2007) found a significant correlation between masseter thickness and bite force only in the TMD group, not in the normal occlusion group. The absence of correlation could be due to the low standard deviation, as stated by Pereira et al. (2007) in the normal occlusion group. Nevertheless, this result is in disagreement with previous studies (Bakke et al., 1992; Pereira et al., 2007; Raadsheer et al., 1999; Castelo et al., 2007) who found a positive correlation between bite force and

masseter thickness. Other reasons for the absence of correlation in this sample could be the size, the conditions or the age of the sample, since significant correlations become stronger after puberty, and only few reports found significant correlations between BF and muscle thickness in children (Castelo et al., 2007).

At T12 there was a significant correlation between masseter and temporal thickness. Probably, after one year of use of the prosthesis, the influence of one muscle on the other reached its maximum. The absence of this correlation in other evaluations might mean that this influence is not linear with time.

In conclusion, the findings of this study suggest that the placement of the removable dental prosthesis influenced the functional and morphological aspects of the muscles of mastication, increasing bite force and masseter thickness. Bite force was related to the thickness of the temporal muscle after rehabilitation in this sample. Nevertheless, bite force did not influence the thickness of the masseter muscle thickness.

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CAPÍTULO 3

Bite force and salivary flow rate in schoolchildren

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Abstract

It is well known that saliva is directly related to oral health. Previous studies in adults have shown that salivary flow rate (SFR) is related to maximum bite force (BF). In the present study, we examined the relationship between SFR and BF in 121 children 7-10-years-old divided by stage of eruption (initial or final phase of mixed dentition) and occlusion. BF was determined with a flexible pressurized tube transducer. Unstimulated (U) and stimulated (S) whole saliva were collected during a 5 min period to stablish the salivary flow rate (SFR). Statistical analysis between genders were done through unpaired *t* test or Mann-Whitney tests. ANOVA was used among groups, correlations were assessed by Spearman's or Pearson's coefficients significant at $p < 0.05$ level. Boys had significantly higher BF and SFR than girls in the total sample. There was no significant differences among groups of stage of eruption and occlusion for BF and SFR. There was a significant correlation ($p<0.05$) between S-SFR and BF for the initial mixed dentition group, and between U-SFR and BF for the group with normal occlusion. In conclusion, the results of this study suggest that BF is related SFR, in children with normal occlusion but not in children with malocclusion.

Key-words: bite force, saliva, salivary flow rate, occlusion, children

Introduction

The strength of the jaw muscles determines the amount of available force to cut or crush the food and various techniques have been used to clinically evaluate the physiological characteristics of the muscles and the mechanics of mastication. One method is to measure the bite force (Kiliaridis et al., 1993), which is one of the components of the chewing function. Maximum bite force is mainly related to the health of the masticatory system, and it is believed that the stronger the bite force, the better the system (Helkimo et al., 1975). The dental morphological health status of children can be evaluated by dental and craniofacial characteristics and the functional health status by conditions of the masticatory system (Tsai & Sun, 2004).

Saliva has manifold functions in protecting the integrity of the oral mucosa: it participates in the clearing of the oral cavity of food residues, debris ('Schmutz') and bacteria; it buffers, as far as possible, the deleterious effects of strong acids and bases; it provides the ions needed to remineralise the teeth; it has antibacterial, antifungal and antiviral capacity. Additionally, salivia facilitates the motor functions of chewing, swallowing and speaking, as well as sensory and chemosensory functions in the oral cavity (Serebny et al., 1992).

Mastication is important for the maintenance of salivary gland function. While food mastication affects salivary gland size, it also affects the secretion of saliva. Thus, mastication and bite force are both involved in the secretion of saliva (Yeh et al., 2000). Without adequate salivary gland function, an individual may experience severe impairment in dental health, swallowing, speech, and enjoyment of food (Ship et al., 1991).

In humans, there are studies that point to mastication as a factor in the regulation of salivary secretion. Feeding humans a liquid diet for a week or more results in a decreased salivary flow of saliva in response to a stimulus (Johansson & Ericson, 1989; Johansson et al., 1984). Increasing mastication by daily gum-chewing (Dodds et al., 1991) or by the institution of a diet that is more firm in texture (de Muñiz et al., 1983) enhances salivary secretion. Moreover, the moisture content of the diet and the dryness of the mouth alters the volume of parotid saliva secreted in rats (Ito et al., 2001).

While salivary gland function responds to increases and decreases in mastication, bite force, too, appears to be responsive to mastication. Maximal bite force can

be increased by 'exercise' of the masseter muscle, i.e. by the chewing of hard gum for an hour each day (Kiliaridis et al., 1995). Yeh et al. (2000) and Ikebe et al. (2007), reported, in adults, that a decrease in bite force strength is associated with a decrease in salivary unstimulated and stimulated flow rate. To the best of our knowledge, this relation has not yet been studied in children.

Therefore, the aim of this study was to verify the association between bite force and salivary flow rates (unstimulated and stimulated) in schoolchildren, and their relation to malocclusion, age, and gender.

Material and methods

Subjects

The subjects, 7-10 years of age, were conviniently selected from a public school in the city of Santa Barbara D'Oeste, São Paulo, Brazil. All the children had the same social-economic profile. Written consent *pro formas* were sent to 250 subjects and their parents, and consent was obtained from 131 subjects (67 girls and 64 boys). Ten children were excluded from the study because either they were absent in the school on the day of the examinations (because they changed schools or period of classes) or because they had teeth with large caries that affected their form and structure. Therefore, 121 children participated in the study (62 girls and 59 boys). The Ethics Committee of the Dental School of Piracicaba approved the research (protocol number 033/2006).

The inclusion criteria were: the children should be in the mixed dentition stage, have a healthy state of the masticatory system, the presence of the teeth (primary and/or permanent) without anomalies and alterations of form, structure or number, and the normality of oral tissues. In addition, the teeth should have no pain because of dental caries or pulpal involvement that could interfere with molar bite force measurements. The exclusion criteria considered any type of orthodontic treatment prior to or during the research examination period, systemic disturbances in general (e.i. neurological problems, Sjögren's syndrome, cistic fibrosis) and ingestion of medicines that could directly or indirectly interfere with muscular activity or salivary flow rate (e.i. antidepressants, anti-hypertensives, aniolytics), and uncooperative behaviour.

A clinical examination was performed for the verification of the normality of the oral soft tissues, the condition of the teeth, and functional evaluation of the masticatory system for further classification into normal occlusion or mallocclusion groups considering: normal occlusion – no alteration in the anterior relation; overjet – anterior overjet 4≤7 mm; crossbite – unilateral posterior crossbite; overbite – anterior overbite 4≤7 mm; openbite – anterior openbite 4≤7 mm. The children were also classified according to stage of eruption – initial phase of mixed dentition (7 and 8 years old); and final phase of mixed dentition (9 and 10 years old).

Bite force measurements

The bite force measurements procedures have been previously described (Serra et al., 2007).

Briefly, bite force was determined with a flexible pressure transducer. Measurements of maximum bite force were made with the children standing in a posture such that their head was relaxed. For each child, the tranducer was placed between the first permanent molars billaterally, and was maintained approximately parallel to the floor in frontal view. As the transducer was placed only in contact with the first permanet molars, the eruption phase of the other permanent teeth in the mixed dentition did not influence the measurement. The force was measured during maximum clenches (2-second duration). Four measurements were made; the first was discarded, and the molar bite force was assessed as the mean of the successive 3 trials. To obtain the highest bite values possible, the subjects were trained before the test (biting the transducer two times before the actual measurement) and they were instructed to bite as forcefully as possible.

This method has already been tested in our laboratory with good results as stated by Rentes et al. (2002). The reliability of the bite force measurement has previously been assessed by double recordings on 10 randomly selected children with an interval of 1 week (Serra et al., 2007) without significant difference between the two sets of measurements and with the method error of the individual double recordings with 1-week interval of 6.55%.

Salivary flow rate

For determination of the salivary flow rate (SFR), unstimulated (U) and stimulated (S) whole saliva samples were collected in pre-weighted sterile disposable containers. The collections were all taken at least 2 hours after meals and 1 hour after brushing, to minimize effects of the circadian rithym, between 8:30 and 9:30 am. All the saliva samples were also taken in the same season (spring) to avoid climate influences and in a different day from the bite force measurements. The collections were taken first for the unstimulated followed by the stimulated saliva.

The children were instructed to swallow immediately before the period of saliva collection, and not to swallow during the next 5 minutes of collection. They were instructed to spit out each 30 seconds into the container. For the stimulated saliva they had to chew on paraffin film of 0.3 g (*Parafilm 'M"®, American National Can™, Greenwhich, CT, USA*) for the total period of 5 minutes.

After collection, the cointainers were re-weighted, the weight of each saliva sample (g) was equated to volume (mL), since the specific gravity of saliva is 1.0 (Shannon, 1973). Salivary flow rate was calculated by measuring the total volume of saliva and dividing this by the collecting time. Salivary flow rates are expressed as mL/min.

Body Variables

Body weight and height were determined in order to verify any differences between genders or groups. Body weight was measured in kilograms, to the nearest tenth. The children were measured without shoes to determine height; the measurements were then rounded to the nearest tenth of a meter.

Statistical Analysis

The normality of the distributions was assessed by the D'Agostino-Pearson's test, Lilliefor's test or Shapiro Wilk's W test, depending on the size of the sample for the specific test. The comparisons between genders and dental stages were performed through unpaired *t* test or Mann-Whitney test. ANOVA was used among groups of occlusion. The correlations among the variables were assessed by Spearman's or Pearson's coefficients at

the $p < 0.05$ level. All analyses were carried out using BioEstat 5.0 (2007, Belém, PA, Brazil).

Results

Table 1 presents the mean values and standard deviations for bite force (BF), unstimulated salivary flow rate (U-SFR), stimulated salivary flow rate (S-SFR), weight and height for the dental stage groups and the whole group.

The groups according to dental stage of eruption were composed as following: Initial mixed dentition (IMD) – 51 children (29 girls, 22 boys); Final mixed dentition (FMD) – 70 children (33 girls, 37 boys).

The children were classified into 5 occlusion groups: normal occlusion (NO; n=46); overjet (OJ; n=27); crossbite (CB; n=15); overbite (OB; n=13); openbite (OP; n=20). The values for the occlusion groups are presented on Table 3.

Considering the whole sample (n=121) there was a significant statistical difference between boys and girls for bite force (p=0.042), U-SFR (p=0.014), and S-SFR (p=0.008), the boys showing higher values (Table 1). On the other hand, there was no significant statistical difference between boys and girls for age (mean 8.8 ±1.0), weight, and height (Table 1; p>0.05).

There was a statistical significant difference between girls and boys SFR (both unstimulated and stimulated) in the IMD (p=0.012 and p=0.014, respectively) (Table 1). No significant differences between boys and girls were found among the other variables and dental stages (p>0.05) (Table 1).

There were no significant differences among occlusion groups for the studied variables (Table 2). When the malocclusions were pooled into one malocclusion group (Table 3), there was no statistical difference between normal occlusion and malocclusion groups (p>0.05). Nevertheless, it was observed a significant difference between genders for BF in the normal occlusion group; U-SFR in the overjet group; S-SFR in overjet and overbite groups; weight in the normal occlusion group (p<0.05; Table 2); and both U-SFR and S-SFR in the malocclusion group (Table 3).

The correlation analysis for the whole sample (n=121), girls (n=62) and boys (n=59) showed no significant correlations between the variables BF, U-SFR and S-SFR

(Table 4). In relation to the dental stages, there was only a significant correlation for S-SFR and BF in the IMD ($p=0.0123$, $r=0.459$). Considering the occlusion groups, there was only a statistical significant correlation for BF and U-SFR in the group with normal occlusion ($p=0.045$, $r=0.293$). There was no other significant correlations among the variables (BF, U-SFR, S-SFR) in the other groups, not even when considered the malocclusions summed up ($p>0.05$).

Tables

Table 1 – Mean values and standard deviation (±SD) of BF, U-SFR, S-SFR, weight and height for girls and boys in the dental stage groups – initial mixed dentition (IMD) and final mixed dentition (FMD) and the whole sample

Age	IMD			FMD			Whole Sample		
	Girls (n=29)	Boys (n=22)	Total (n=51)	Girls (n=33)	Boys (n=37)	Total (n=70)	Girls (n=62)	Boys (n=59)	Total (n=121)
BF (N)	487.25 ±51.81	511.97 ±66.37	497.92 ±59.21	491.65 ±58.02	505.61 ±59.36	499.03 ±58.73	489.59* ±54.80	507.98* ±61.58	498.56 ±58.69
U-SFR (mL/min)	0.58* ±0.24	0.79* ±0.35	0.67 ±0.30	0.64 ±0.36	0.75 ±0.37	0.70 ±0.36	0.61* ±0.31	0.76* ±0.36	0.69 ±0.34
S-SFR (mL/min)	1.06* ±0.43	1.53* ±0.76	1.26 ±0.63	1.12 ±0.60	1.32 ±0.64	1.23 ±0.63	1.09* ±0.52	1.40* ±0.69	1.24 ±0.63
Weight (Kg)	29.16a ±6.86	32.55a ±8.05	30.62A ±7.51	35.72b ±8.63	35.90a ±8.60	35.81B ±8.55	32.65 ±8.46	34.65 ±8.48	33.62 ±8.50
Height (m)	1.31a ±0.08	1.31a ±0.06	1.31A ±0.07	1.39b ±0.07	1.40b ±0.07	1.40B ±0.07	1.35 ±0.09	1.37 ±0.08	1.36 ±0.09

* significant difference between genders ($p<0.05$)

Different small letters in the same line mean significant statistical difference between dental stages in the same gender

Different capital letters in the same line mean significant statistical difference between dental stages considering girls and boys together

Table 2 – Mean values and standard deviation (±) for girls and boys within the different occlusion groups

	NO Group (n=46)			OJ Group (n=27)			CB Group (n=15)			OB Group (n=13)			OP Group (n=20)		
	Girls (n=22)	Boys (n=24)	Mean	Girls (n=13)	Boys (n=14)	Mean	Girls (n=10)	Boys (n=5)	Mean	Girls (n=6)	Boys (n=7)	Mean	Girls (n=11)	Boys (n=9)	Mean
BF (N)	469.47* ±53.20	515.26* ±56.06	493.36 ±58.84	488.76 ±41.42	501.63 ±69.27	495.43 ±58.47	486.11 ±83.89	514.27 ±88.00	495.52 ±83.22	530.21 ±40.26	508.28 ±62.48	518.40 ±53.78	511.81 ±23.93	494.71 ±52.95	504.12 ±39.47
U-SFR (mL/min)	0.70 ±0.37	0.73 ±0.28	0.72 ±0.32	0.44* ±0.17	0.88* ±0.50	0.67 ±0.44	0.60 ±0.34	0.72 ±0.32	0.64 ±0.32	0.62 ±0.17	0.77 ±0.26	0.70 ±0.24	0.64 ±0.26	0.68 ±0.37	0.66 ±0.31
S-SFR (mL/min)	1.36 ±0.66	1.23 ±0.52	1.29 ±0.58	0.86* ±0.35	1.67* ±0.76	1.28 ±0.74	0.91 ±0.40	1.11 ±0.41	0.98 ±0.40	1.14* ±0.50	1.67* ±0.44	1.42 ±0.54	0.98 ±0.25	1.38 ±1.05	1.16 ±0.73
Age (years)	8.6 ±0.8	8.8 ±1.2	8.7 ±1.1	9.1 ±1.0	9.2 ±1.1	9.1 ±1.1	9.2 ±1.0	9.2 ±0.8	9.2 ±0.9	8.8 ±0.9	8.9 ±1.1	8.8 ±1.0	8.3 ±1.0	8.6 ±0.9	8.4 ±0.9
Weight (Kg)	31.02* ±7.26	36.33* ±9.20	33.79 ±8.66	33.61 ±8.07	34.64 ±8.64	34.14 ±8.40	33.04 ±9.80	34.00 ±8.22	33.36 ±9.02	36.23 ±8.46	31.77 ±8.50	33.83 ±8.79	32.45 ±10.17	32.77 ±6.32	32.59 ±8.44
Height (m)	1.34 ±0.07	1.36 ±0.09	1.35 ±0.08	1.37 ±0.10	1.38 ±0.08	1.38 ±0.09	1.36 ±0.10	1.39 ±0.05	1.37 ±0.09	1.39 ±0.06	1.38 ±0.12	1.39 ±0.09	1.33 ±0.09	1.35 ±0.06	1.34 ±0.08

* Significant difference between genders within the group ($p<0.05$). There were no significant differences among the other variables.

NO= normal occlusion; OJ=overjet; CB=crossbite; OB=overbite; OP=openbite

Table 3 – Mean values and standard deviation (±) for girls and boys considering the normal occlusion group and the malocclusion pooled

	NO			Malocclusion		
	Girls (n=22)	Boys (n=24)	Total (n=46)	Girls (n=)	Boys (n=)	Total (n=75)
BF (N)	469.47* ±53.20	515.26* ±56.06	493.36 ±58.84	500.66 ±53.09	502.99 ±65.41	51.75 ±58.76
U-SFR (mL/min)	0.70 ±0.37	0.73 ±0.28	0.72 ±0.32	0.56* ±0.25	0.78* ±0.41	0.66 ±0.35
S-SFR (mL/min)	1.36 ±0.66	1.23 ±0.52	1.29 ±0.58	0.95* ±0.37	1.51* ±0.77	1.21 ±0.65
Age (years)	8.6 ±0.8	8.8 ±1.2	8.7 ±1.1	8.85 ±1.05	8.97 ±1.04	8.91 ±1.04
Weight (Kg)	31.02* ±7.26	36.33* ±9.20	33.79 ±8.66	33.54 ±9.01	33.49 ±7.88	33.52 ±8.45
Height (m)	1.34 ±0.07	1.36 ±0.09	1.35 ±0.08	1.36 ±0.09	1.37 ±0.08	1.37 ±0.09

*Significant difference between genders

There was no statistically significant difference between the group with normal occlusion and the group with malocclusion

Discussion

The mean values of bite force in this study (498.56N) were similar to those found by Kiliaridis et al. (1993) but higher than in other studies in children (Castelo et al., 2007; Rentes et al., 2002; Serra et al., 2007; Kamegai et al., 2005). When comparing values from one study with another, extreme caution should be exercised unless the same apparatus is used (Helkimo et al., 1975). In addition, differences in the caracteristics of the sample such as age and dental/occlusion status could also influence the different results. If all the caracteristics are not similar, the attention should be given to the results, correlations and comparisons found within a study, instead of comparing the values with other studies.

In the present study boys presented higher BF values than the girls considering the whole sample (Table 1). Boys also presented higher BF values in the NO group (Table 2), which indicates that this trait is more regular in children with normal occlusion. The difference between genders is in agreement with previous studies in children (Ingervall & Minder, 1997; Tsai, 2004) and in contrast with others (Kiliaridis et al., 1993; Sonnesen et al., 2001).

This study found no difference in bite force between the dental stage eruption groups (Table 1). Probably, bite force can be in line with later stages of dental eruption (Sonnesen et al., 2001; Sonnesen & Bakke, 2005).

No differences in bite force values for children with normal occlusion and malocclusion were found; this result could be attributed to the fact that malocclusions were not severe in this sample. In spite of that, this result agrees with Rentes et al. (2002), Sonnesen & Bakke (2005), Miyawaki et al. (2005), who showed that BF did not vary significantly among Angle malocclusion types. On the other hand, these results are in contrast with Sonnesen & Bakke (2007), Castelo et al. (2007), Tsai (2004) and Kamegai et al. (2005) who found a positive correlation between malocclusion and reduced bite force. In this context it is important to consider that, along the time the malocclusion might worsen, since in adults the correlation between malocclusion and reduced bite force has been found to be stronger (Miyawaki et al., 2005). One explanation might be that in adults the mallocclusions might have been installed for a longer period, giving the muscles of

mastication time to adapt to the condition, which causes the bite forces to change over time, showing differences when comparing individuals with normal occlusion and mallocclusions. Therefore, the effects of the malocclusion might be seen further in the future.

In relation to the U-SFR, the mean values found in this study (0.69mL/min) are comparable to those found by Bretz et al. (2001) in a specific site in Brazil and also in accordance with Dezan et al. (2002) and Negoro et al. (2000). Nevertheless, they were higher than those found by other studies (Watanabe & Dawes, 1990; Watanabe et al., 1995). This difference in flow may be due to differences in ages of samples, origin of children, site of study and technique used for saliva collection. The mean S-SFR was 1.24 mL/min considering the whole sample, similar to that of Torres et al. (2006) and Alamoudi et al. (2004).

Boys had significantly higher SFR than girls (Table 1), as seen previously (Bretz et al., 2001; Söderling et al.1993; Tukia-Kulmala & Tenovuo, 1993). Considering the dental stage groups, this difference was only significant for the IMD, which indicates that even in young children a difference between genders could exist. Although this difference might not be consistent throughout childhood, since it was not observed for the FMD. Moreover, in contrast with BF, this difference in gender for SFR was seen only in the malocclusion group (Table 3), which could indicate that this trait might be influenced by the dental occlusion status. Nevertheless, this result could not be compared to others in the literature because of the absence of similar studies considering flow rates and occlusion in children.

There was no significant difference in relation to SFR between the two dental stage groups that indicates no gain in flow with growth. Perhaps the age range of this study was not so wide to show significant increase in flow during growth, contrasting with previous studies that show that SFR increases with age (Dezan et al., 2002; Bretz et al., 2001; Söderling et al., 1993; Tukia-Kulmala & Tenovuo, 1993), nevertheless there have been controversies in other studies (Kavanagh et al., 1998; Rosivack, 2004; Rotteveel et al., 2004). Torres et al. (2006) found significant correlation between age and SFR in children

from 6-12 years of age, however, when the data from the extreme ages were eliminated, significant statistical correlation ceased to exist between age and mean SFR.

When we considered the whole sample, girls, and boys we failed to find a correlation between SFR and BF, which is in contrast with the results found by Yeh et al. (2000) and Ikebe et al. (2007), who found a significant correlation between SFR and bite force in adults. Ikebe et al. (2007) even reported that bite force, which is a functional variable, is more important for S-SFR than the number of remaining teeth, which is an anatomical variable, although bite force is also related to the number of residual teeth. We suggest that the lower age of our sample could be an explanation for the different results. Nevertheless, when we considered seperately girls and boys in the different dental stage groups, we found a positive correlation between S-SFR and BF in the IMD girls. Possibly this isolated correlation may be due to the fact that at this point of childhood both SFR and BF may be undergoing a transition phase and might not be yet stablished or stable.

Interestingly, there was a positive correlation between bite force and U-SFR in the normal occlusion group, althought it was weak. The absence of correlation between BF and SFR on the other groups could be due to the sample sizes. On the other hand, this result could indicate that the malocclusions might interfere with the proper saliva gland function, SFR and its relation to BF since there was also no correlation when the malocclusions were pooled; nevertheless this interference might be obscure in children, since there was no significant differerences among groups in SFR and BF (Table 2). We put forward that correlations between bite force and SFR could be greater in older groups of individuals. Yeh et al. (2000) reported that denture wearers had lower SFR than individuals with natural dentition which reinforces the importance of preserving the teeth in good health. In this way, hypothetically, normal occlusion could generate higher bite force values. To test this hypothesis more studies are needed in larger samples and with a wider age ranges, including younger children and adolescents.

Previous studies in adults and in children show that chewing training increases bite force (Ono et al. 1992; Kiliaridis et al., 1995) and salivary secretion (Dodds et al., 1991; de Muñiz et al., 1983; Dawes & Kubieniec, 2004). Since SFR is an important factor in oral host defense, and is regulated in large measure by mastication, attempting to

enhance mastication may be an important factor for maintenance of both masseter muscle strength and salivary gland function (Yeh et al., 2000). Hence, it would be important to correct the malocclusion as early as possible in children, and exercise the muscles of mastication, especially in children with low SFR, in order to increase the SFR, and, therefore, the health of the oral cavity and also enhance the masticatory performance. This would stress the importance of maintaining a healthy dentition throughout childhood, permitting a proper mastication, which, in turn, would play a role in oral host defense against disease.

Conclusion

There is a relationship between bite force and salivary flow rate, in children with normal occlusion but not in children with malocclusion.

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CAPÍTULO 4

Bite force, texture perception and chewing parameters in schoolchildren

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Abstract

The aim of this study was to asses bite force (BF), perception of food texture and chewing parameters in 115 children (58 girls, 57 boys) 7-10-years-old, who were stratified according to occlusion, and to verify if different BF influences the chewing parameters. BF was determined with a flexible pressurized tube transducer. The following foods were tested: carrot, toast with and without margarine, banana, cheese, and chewing gum. Chewing time, number of chewing cycles and chewing frequency were evaluated. The texture of those foods was analysed using a visual analogue scale from 0 to 100 mm. Statistical analysis between genders were done through unpaired *t* test or Mann-Whitney test. ANOVA was used among groups and foods, correlations were assessed by Spearman's or Pearson's coefficients significant at the $p < 0.05$ level. There was no statistically significant difference in bite force, perception of texture or chewing parameters among groups ($p>0.05$). The frequency, number of chewing cycles, chewing time, and texture were significantly lower for soft foods (cheese, banana) than for hard foods (carrot, toasts) ($p<0.05$). Significant correlations were found for BF and chewing frequency in the normal occlusion group. We conclude that BF, chewing parameters and perception of food texture do not vary among children with normal or malocclusions. Nevertheless, in children without malocclusion a greater bite force is related to a higher chewing frequency of foods, which could indicate a facility to chew foods.

Key-words: food, texture, bite force, children, chewing frequency, chewing time, chewing cycles

Introduction

Mastication, the first step of digestion, is a sensory-motor activity to prepare food for swallowing (Bosman et al, 2004). Humans can modify their habitual masticatory patterns to adjust to the physical properties of food (Lucas et al, 2004; Hiiemae, 2004; Dan & Kohyama, 2007; Dan et al, 2007).

The strength of the jaw muscles determines the amount of available force to cut or crush the food and one method used to clinically evaluate the physiological characteristics of the muscles and the mechanics of mastication is to measure the bite force (Kiliaridis et al, 1993), which is one of the components of the chewing function. Bite force is reported to be a key predictor for masticatory performance (Hatch et al, 2000) and a large bite force has been found to cause a high masticatory performance, especially when chewing hard food (Okiyama et al, 2003).

Masticatory performance can be measured as chewing ability, or the number of chews necessary to swallow certain natural foods (Owens et al, 2002; Fontijn-Tekamp et al, 2004; Gambareli et al, 2009). The reduction of food particles is determined by a complicated multifactorial process, which depends on such factors as bite force, the coordination of chewing muscles, the morphology and the number of occluding pairs of teeth (Fontijn-Tekamp et al, 2004). The dental conditions of each individual may influence the masticatory performance. The literature has found diverging results in relation to the number of cycles before the first swallow in relation to the masticatory performance, reporting that an inadequate dentition determines that subjects may use more cycles to prepare food for swallowing than subjects with natural dentition (van der Bilt et al, 1993) or that the longer a subject chews the food, the smaller are the particles swallowed, independently from the masticatory performance (Fontijn-Tekamp et al, 2004) and agrees that subjects with reduced masticatory performance swallow bigger food particles (van der Bilt et al., 1993; Fontijn-Tekamp et al., 2004).

Food texture is sensed during mastication and affects human chewing patterns (Nakazawa & Togashi, 2000; Bourne, 2002), modifying masticatory forces (Bishop et al, 1990), mandibular jaw movements (Thexon et al, 1980), duration of the mastication cycle, and number of cycles preceding the first swallow (Hiiemae et al, 1996; Gaviao et al, 2004;

Engelen et al, 2005). Dry and hard products require more chewing cycles before swallowing (Engelen et al, 2005). Evidently, more time is needed to break the food down and to add enough saliva to form a cohesive bolus suitable for swallowing. Buttering dry foods significantly reduces the number of chewing cycles until swallowing (Engelen et al, 2005).

Therefore, the aim of this study was to asses bite force, perception of food texture and chewing parameters in schoolchildren and to verify if different bite forces influence chewing parameters among children with different types of occlusion.

Material and methods

Subjects

The subjects, 7-10 years of age, were conviniently selected from a public school in the city of Santa Barbara D'Oeste, São Paulo, Brazil. All the children had the same social-economic profile. Written consent *pro formas* were sent to 250 subjects and their parents. One hundred and fifteen children participated in this study (58 girls and 57 boys). The Ethics Committee of the Dental School of Piracicaba approved the research (protocol number 033/2006).

The inclusion criteria were: the children should be in the mixed dentition stage, have a healthy state of the masticatory system, the presence of the teeth (primary and/or permanent) without anomalies and alterations of form, structure or number, and the normality of oral tissues. In addition, the teeth should have no pain because of dental caries or pulpal involvement that could interfere with molar bite force measurements. The exclusion criteria considered any type of orthodontic treatment prior to or during the research examination period, systemic disturbances in general (e.i. neurological problems, Sjögren's syndrome, cistic fibrosis) and ingestion of medicines that could directly or indirectly interfere with muscular activity or salivary flow rate (e.i. antidepressants, anti-hypertensives, ansiolytics), and uncooperative behaviour.

A clinical examination was performed for the verification of the normality of the oral soft tissues, the condition of the teeth, and functional evaluation of the masticatory system for further classification into normal occlusion or mallocclusion groups considering:

normal occlusion – no alteration in the anterior relation; overjet – anterior overjet 4≤7 mm; crossbite – unilateral posterior crossbite; overbite – anterior overbite 4≤7 mm; openbite – anterior openbite 4≤7 mm.

Bite force measurements

The bite force measurements procedures have been previously described (Serra et al., 2007).

Briefly, bite force was determined with a flexible pressure transducer. Measurements of maximum bite force were made with the children standing in a posture such that their head was relaxed. For each child, the transducer was placed between the first permanent molars billaterally, and was maintained approximately parallel to the floor in frontal view. As the transducer was placed only in contact with the first permanet molars, the eruption phase of the other permanent teeth in the mixed dentition did not influence the measurements. The force was measured during maximum clenches (2-second duration). Four measurements were made; the first was discarded, and the molar bite force was assessed as the mean of the successive 3 trials. To obtain the highest bite values possible, the subjects were trained before the test (biting the transducer two times before the actual measurement) and they were instructed to bite as forcefully as possible.

This method has already been tested in our laboratory with good results as stated by Rentes et al. (2002). The reliability of the bite force measurement has previously been assessed by double recordings on 10 randomly selected children with an interval of 1 week (Serra et al., 2007) without significant difference between the two sets of measurements and with the method error of the individual double recordings with 1-week interval of 6.55%.

Chewing parameters

The following foods were tested: carrot, toast with and without margarine (Bauducco®, Guarulhos, São Paulo, BR), banana, cheese (Polenghi®, Angatuba, São Paulo, BR), and fruit flavor chewing gum (Trident - Adams®, Bauru, São Paulo, BR). Each food was offered to the child in a 2g portion in duplicate (two equal portions of each food

were presented in two different plates). The natural test foods were given to the subjects in a predetermined sequence. The subjects were asked to chew the food in their usual manner and were instructed to signal when they were ready to swallow. In between the foods, subjects were allowed to sip water. The chewing time and the number of cycles were controlled by the examiner. Thus, the chewing time (time - sec), number of chewing cycles (# cycles) and chewing frequency (frequency - cycles/sec) were evaluated (Gambareli et al, 2009). The chewing gum was chewed during 60 s, assessing the number of chewing cycles and chewing frequency. Since the weight and chewing time of the chewing gum differed from the other foods, it was only compared to them in relation to texture and chewing frequency. Banana was classified as soft and wet, cheese as soft and fat, carrot as hard and wet, toast with margarine as hard and fat, toast without margarine as hard and dry, and the chewing gum as springy.

A visual analogue scale (VAS) from 0 to 100 mm was used for analyzing the perceived texture. The foods were classified as 0-extremely soft and 100-extremely hard. The quantification was determined by means of a milimetrical ruler. The values obtained for the two portions were added and the mean value was used as the percentage of perceived hardness (texture).

Body Variables

Body weight and height were determined in order to verify any differences between genders or groups. Body weight was measured in kilograms, to the nearest tenth. The children were measured without shoes to determine height; the measurements were then rounded to the nearest tenth of a meter.

Statistical Analysis

The normality of the distributions was assessed by the D'Agostino-Pearson's test, Lilliefors test or Shapiro Wilk's W test. The comparisons between genders were performed through unpaired *t* test or Mann-Whitney tests. ANOVA was used among groups and foods. The correlations among the variables were assessed by Spearman's or

Pearson's coefficients with significant values when $p < 0.05$. All analyses were carried out using BioEstat 5.0 (2007, Belém, PA, Brazil).

Results

There were no differences between genders in relation to age (8.9±1.1 years), weight (33.9±8.5 kg) and height (1.36±0.08 m).

Table 1 presents the mean values and standard deviations for all variables. There was a statistically significant difference between genders for texture of cheese; chewing frequency of banana, toast with margarine, toast without margarine, and chewing gum; chewing time of both toasts, and number of cycles of toast with margarine and chewing gum (Table 1).

The soft foods (banana and cheese) were considered as having similar hardness, and some children considered their texture similar to the gum (Table 1). For the two toasts the hardness was considered equal, and for girls toast with margarine was similar to carrot. Hard and soft foods had similar chewing frequencies with little variation between genders. Boys and girls chewed the gum with the same frequency as toasts, but this was not observed when the results were pooled. Chewing time was similar for carrot and toast with margarine. Boys also chewed banana and cheese with the same times. Carrot and toast with margarine had similar number of chews for the total sample. For girls and boys banana and cheese also had similar number of chews ($p<0.05$).

The children were classified according to occlusion into 5 groups: normal occlusion (NO; n=44); overjet (OJ; n=28); crossbite (CB; n=12); overbite (OB; n=11); openbite (OP; n=20). The values for the occlusion groups are presented on Table 2. No significant differences in all variables were found among occlusion groups ($p>0.05$, Table 2).

Texture, frequency, chewing time and number of cycles among foods in most groups followed the same parameters: hard foods (carrot and both toasts) were similar and soft foods (banana and cheese) were similar. Some groups, however (Table 2) had different chewing time and number of cycles between toast with and without margarine. Chewing gum in some cases was similar to hard foods and in other cases similar to soft foods.

When the difference between genders was significant ($p<0.05$) the correlations were also done separately for girls and boys. Despite the numerical values obtained for texture, the examiner observed that children had great difficulty in rating the food in relation to its texture, as confirmed by the high standard deviations (Tables 1 and 2). Therefore, this attribute was considered only for comparisons among foods, but was not used for correlations.

Table 3 shows the significant correlations for BF and chewing parameters for total sample (♂♀), girls (♀) and boys (♂). There was only a significant correlation ($p<0.5$) between BF and banana and cheese frequencies considering the total sample. For the occlusion groups, there was only significant correlations for chewing frequency and bite force in NO (Table 3). There were no significant correlations for the other groups. BF significantly correlated with chewing frequency of banana, cheese and both toasts in NO.

Tables

Table 1 – Mean values and standard deviation (±SD) of bite force and chewing parameters for girls and boys and the total sample

		Girls (n=58)	Boys (n=57)	Total (n=115)
	Bite force (N)	492.95±53.46^{A}	508.62±62.65^{A}	500.72±58.47
Texture (% of perceived texture)	Carrot	59.61±24.91^{Aa}	55.94±24.56^{Aa}	57.79±24.69^{a}
	Banana	8.46±8.46^{Ab}	7.81±8.53^{Ab}	8.14±8.46^{b}
	Cheese	13.13±15.65^{Ab}	7.31±8.92^{Bb}	10.25±13.4^{bd}
	Toast+marg.	69.49±23.25^{Aac}	71.73±22.29^{Ac}	70.60±22.71^{c}
	Toast	71.96±20.89^{Ac}	74.88±20.17^{Ac}	73.41±20.50^{c}
	Chewing gum	15.77±18.06^{Ab}	15.91±17.16^{Ab}	15.84±17.54^{d}
Frequency (cycle/s)	Carrot	1.50±0.19^{Aa}	1.56±0.23^{Aa}	1.53±0.21^{a}
	Banana	1.09±0.19^{Ab}	1.17±0.19^{Bb}	1.13±0.19^{b}
	Cheese	1.18±0.23^{Ab}	1.20±0.18^{Ab}	1.19±0.20^{b}
	Toast+marg.	1.40±0.20^{Ac}	1.50±0.20^{Bac}	1.45±0.21^{c}
	Toast	1.40±0.18^{Aac}	1.51±0.17^{Bac}	1.46±0.18^{ac}
	Chewing gum	1.33±0.16^{Ac}	1.41±0.18^{Bc}	1.37±0.17^{d}
Time (s)	Carrot	19±7^{Aa}	17±6^{Aa}	18±7^{a}
	Banana	5±1^{Ab}	6±2^{Ab}	5±2^{b}
	Cheese	8±3^{Ac}	8±3^{Ab}	8±3^{c}
	Toast+marg.	20±5^{Aa}	17±5^{Ba}	19±5^{a}
	Toast	25±7^{Ad}	21±6^{Bc}	23±7^{d}
	Chewing gum	60	60	60
# Cycles (n)	Carrot	28±10^{Aa}	26±9^{Aa}	27±10^{a}
	Banana	6±2^{Ab}	7±3^{Ab}	6±3^{b}
	Cheese	9±3^{Ab}	9±4^{Ab}	9±4^{c}
	Toast+marg.	28±7^{Aa}	26±7^{Ba}	27±7^{a}
	Toast	35±9^{Ac}	31±8^{Ac}	33±9^{d}
	Chewing gum	80±10^{A}	84±11^{B}	82±10

Different capital letters in the same line mean significant statistical difference between genders ($p<0.05$); differente small letters in the same column mean significant statistical difference among foods in texture, chewing frequency, chewing time or number of chewing cycles. The time of chewing gum was constant for all children (60 sec). This food was compared to the other only in relation to chewing frequency.

Table 2 – Mean values and standard deviation (±SD) of bite force, texture, and cehwing parameters for the different occlusion groups

		Normal Occlusion (n=44)	Overjet (n=28)	Crossbite (n=12)	Overbite (n=11)	Openbite (n=20)
Bite force (N)		493.06±60.52	495.09±57.40	516.14±79.01	522.65±57.59	504.12±39.47
Texture	Carrot	55.63±24.68[a]	61.03±26.51[a]	59.76±17.80[a]	63.11±17.58[a]	53.90±29.57[a]
	Banana	10.36±10.59[b]	5.52±4.65[b]	6.24±4.55[b]	5.54±5.03[b]	9.50±9.50[b]
	Cheese	9.78±11.95[b]	9.88±15.53[b]	11.40±13.39[b]	9.91±10.59[b]	11.27±13.75[b]
	Toast +marg.	73.20±18.68[c]	63.13±28.90[a]	72.85±15.25[a]	70.88±27.52[a]	73.82±21.64[c]
	Toast	73.68±19.77[c]	71.36±24.00[a]	77.88±17.86[a]	80.91±17.71[a]	68.87±19.88[ac]
	Chewing gum	17.48±18.52[b]	13.04±14.51[b]	21.42±22.83[b]	14.8±18.201[b]	13.37±15.84[b]
Frequency	Carrot	1.50±0.20[a]	1.56±0.22[a]	1.48±0.24[a]	1.52±0.17[a]	1.59±0.21[a]
	Banana	1.15±0.21[b]	1.07±0.22[b]	1.10±0.18[b]	1.16±0.12[b]	1.16±0.21[b]
	Cheese	1.17±0.20[b]	1.20±0.20[bd]	1.17±0.18[bc]	1.23±0.15[bc]	1.23±0.25[bc]
	Toast+marg.	1.48±0.20[a]	1.40±0.23[c]	1.34±0.16[ac]	1.51±0.18[a]	1.47±0.19[a]
	Toast	1.48±0.16[a]	1.44±0.20[ac]	1.38±0.23[ac]	1.50±0.12[a]	1.46±0.19[a]
	Chewing gum	1.40±0.18[a]	1.33±0.16[cd]	1.27±0.18[ab]	1.35±0.12[ac]	1.41±0.18[ac]
Time	Carrot	19±6[a]	18±7[a]	21±8[a]	15±6[a]	17±6[a]
	Banana	5±2[b]	6±2[b]	6±1[b]	5±2[b]	5±2[b]
	Cheese	8±3[b]	8±3[b]	7±2[b]	7±2[b]	8±2[b]
	Toast+marg.	19±5[a]	19±5[a]	21±7[a]	17±4[ac]	19±5[a]
	Toast	23±7[c]	23±6[c]	25±10[a]	20±5[c]	23±6[c]
	Chewing gum	60	60	60	60	60
# Cycles	Carrot	27±9[a]	27±10[ac]	30±12[a]	23±13[a]	27±8[a]
	Banana	6±3[b]	6±3[b]	6±2[b]	6±2[b]	6±2[b]
	Cheese	9±4[b]	10±4[b]	8±3[b]	9±3[b]	9±3[b]
	Toast+marg.	27±6[a]	26±8[a]	27±9[a]	24±5[a]	28±7[a]
	Toast	34±8[c]	33±9[c]	33±12[a]	30±8[a]	34±9[a]
	Chewing gum	84±11	80±10	76±11	81±7	84±11

There was no difference among groups for BF and for the same food within the same parameter ($p>0.05$)
Different small letters in the same column mean significant statistical difference among foods in texture or chewing parameters (ANOVA). The time of chewing gum was constant for all children (60 sec). This food was compared to the other only in relation to chewing frequency.

Table 3– Significant correlation coefficients ($p<0.05$) for bite force (BF) and chewing frequency of for the total sample (♂♀; n=115) and normal occlusion group

	Frequency (cycles/sec)			
	Banana	Cheese	Toast with margarine	Toast without margarine
BF	0.213 (♂♀)	0.214 (♂♀)	-	-
	0.335 (NO)	0.350 (NO)	0.405 (NO)	0.394 (NO)

Discussion

The main purpose of the masticatory system is the mechanical reduction of food (masticatory performance) into small particles that can be more easily digested. Malocclusions impair breakdown of at least some foods, and selection of foods is limited in patients who have poor masticatory performance (Zhao & Monahan, 2007).

Food can vary widely in, for example, thickness, hardness, fat, and moisture content. Theses differences are reflected in the force needed to shear food, the breakdown pattern of the food, the ease by which it is manipulated, formed into a bolus, and swallowed. In this study we tested if the children who had higher bite forces would have less dificulty in chewing natural foods.

The mean values of bite force in this study (Table 1) were higher than some studies in children (Castelo et al, 2007; Rentes et al, 2002; Serra et al, 2007; Kamegai et al, 2005; Hatch et al, 2000), however, they were similar to another study (Kiliaridis et al, 1993). Although there could be found different values among studies, it must be payed attention to the results, correlations and comparisons found within a study, instead of comparing the numerical values with other studies; these differences in values might be due to a number of factors such as placement of transducer, age of sample, dental eruption stage of sample, dental status of subjects, among others.

Considering the total sample there was no significant difference between genders for bite force (Table 1) wich is in accordance with studies in the literature (Kiliaridis et al, 1993; Braun et al, 1996; Sonnesen et al, 2001). The difference between genders becomes more evident with puberty, since it is believed that continued muscle development may account for gender-related bite force differences in the postpubertal population (Braun et al, 1996).

There was no differences in bite force values for children with normal occlusion and malocclusion, agreeing with Rentes et al (2002), Sonnesen & Bakke (2005), Miyawaki et al (2005), who showed that BF did not vary significantly among Angle malocclusion types. One reason for this absence of difference might be the lower severity of the malocclusions in children, considering that, with time, the malocclusion might worsen, since in adults the correlation between malocclusion and reduced bite force is stronger (Miyawaki et al, 2005).

A higher bite force was related to a higher chewing frequency of banana and cheese (soft foods), but not the hard foods (carrot, toasts) considering the total sample. This result could indicate that a higher bite force facilitated the chewing of the soft foods, considering that the frequency is the rate between number of chewing cycles and time. This result was unexpected, since it is thought that bite force should account more for the chewing of harder foods, nevertheless even the significant correlations were weak (Table 3).

Interestingly, the correlations between bite force and chewing frequency were more evident in the NO, including the mastication of harder foods (toasts) (Table 3). This suggests that in children with better occlusion status the bite force might account more to the easiness to crush a food until it is ready to be swallowed. This could indicate that bite force might be important to provide a better chewing pattern. In this respect, Zhao and Monahan (2007) stated that restoring the normal anatomic occlusal relation translates functionally to increased ability of the patients to generate a maximal bite force. The lack of correlation between bite force and chewing frequencies of gum and carrot might be related to the fact that the higher bite forces are probably only present in the beginning of the chewing process as food will be softened by saliva after a few chewing strokes (Gavião et

al, 2004), moreover carrot has a high percentage of water (90% of water, Ottenhoff et al, 1992), and its wetness facilitates the food bolus formation suitable for swallowing.

This research did not test the understanding of the children of hard and soft foods, which would be an interesting data, since it was noted a very wide range of perceived hardness (high standard deviations). For this reason this attribute was not analyzed in relation to the other variables, but only on comparison of foods. Possibly texture is a food attribute that is not correctly interpreted by children, probably because food consistency changes throughout mastication, and they cannot distinguish the texture of the first bite from the following bites. It would be interesting to create a training session to the children on this attribute before following researches and to compare the results with the ones found in this study.

Despite the difficulty on rating foods in relation to texture, we can see on Tables 1 and 2 that mostly, children rated as harder the carrot and both toasts, and softer the banana and cheese. Chewing gum was considered as soft as banana and cheese in all cases (Tables 1 and 2), except for the total sample, who rated gum similar only to cheese (Table 1).

The chewing frequency (which measures mastication rate, or how fast a subject chews a determined food), number of cycles and chewing time, in general, was similar for soft foods (banana and cheese) and this value was lower than that of the hard foods (carrot and toasts). This trait was almost the same for all children (Tables 1 and 2), with exception of chewing time for girls and the total sample, as well as of the number of chewing cycles for the total sample. The general finding is in agreement with previous findings on the relationship between chewing rate and food hardness (van der Bilt et al, 2006; Gavião et al, 2004; Hiiemae & Palmer, 1999; Gambareli et al, 2009).

The chewing frequency, time and number of cycles of carrot was similar to either toast with or without margarine (Tables 1 and 2). This finding is in disagreement with the results found by Pereira et al (2007) who found higher number of chewing cycles for carrot compared with melba toast. They stated that foods that are relatively difficult for chewing are chewed at a higher chewing rate than foods easily chewed, in this study this

was true when comparing the soft and hard foods (Tables 1 and 2), but not when comparing carrot with toasts.

Although the chewing frequency of both toasts did not differ significantly in all cases, the chewing time and number of chewing cycles differed significantly in almost all of them (with exception of time for CB and OB, and frequency for CB, OB, and OP), (Tables 1 and 2). This result is in accordance to the findings of Gavião et al (2004), who found that the number of chewing cycles needed before swallowing toast significantly decreased when the toast had 2 g of margarine on it. The margarine facilitates bolus formation and lubricates the food, which makes it easier to swallow (Gavião et al, 2004).

Chewing gum was chosen as a test food because it forms a coherent bolus and the mastication could be considered more uniform (Gambareli et al, 2009). Nevertheless, no significant difference was observed among occlusion groups in number of cycles and frequency, perhaps due to the facility that children have of chewing gum, since it is a commom food in the childhood context (Table 2). In this respect, Gambareli et al (2009) found no significant difference in frequency and number of chewing cycles of chewing gum before and after the oral rehabilitation of children.

From Table 1 we can see that the chewing frequency of gum for boys and girls was similar to both toasts, but when considering the total sample, the chewing frequency was statistically different from all other foods, having an intermediate frequency between soft and hard foods, this finding is in line with its texture, that is a little hard in the beginning, but becomes really soft after a few bites. This is the reason why some groups (OJ, CB, OB, and OP) had frequencies similar to both soft and hard foods, or similar only to hard foods (NO).

Conclusion

Within the limits of this study, the results suggest that in children 7-10 years-old, bite force, chewing parameters and perception of food texture do not vary among children with normal or malocclusion. Nevertheless, in children with absence of malocclusions a greater bite force is related to a higher chewing frequency of foods, which could indicate a facility to chew foods.

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CAPÍTULO 5

Salivary flow rate, protein content, chewing parameters and taste perception in schoolchildren

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Abstract

Saliva plays an important role in mastication and in the formation of a food bolus suitable for swallowing. The aims of this study were to assess the characteristics of salivary flow rates (SFR), chewing parameters (chewing time, cycles and frequency) and taste perception in 115 7-10-year-old schoolchildren, and to assess the effect of SFR and the role of protein on chewing parameters and taste perception. Unstimulated (U) and stimulated (S) whole saliva were collected during a 5 min period to stablish the salivary flow rate (SFR). The following foods were tested: carrot, toast with and without margarine, banana, cheese, and fruit flavor chewing gum. Chewing time, number of chewing cycles and chewing frequency were evaluated. Children rated the foods as bitter, sweet, salty or sour and the percentage of correct answers were evaluated: banana, carrot and gum were considered sweet; both toasts and cheese as salty. Protein content was asseyed by the method of Lowry. Statistical analysis between genders were done using unpaired *t* test or Mann-Whitney test. ANOVA was applied among foods and the correlations were assessed by Spearman's or Pearson's coefficients, with significant level at $p < 0.05$. Boys had SFR significantly higher than girls ($p<0.05$), as well as lower chewing frequency of both toasts and gum. There was a significant correlation between SFR and chewing parameters of carrot, both toasts and cheese. Girls and children with higher SFR and lower protein content had higher percentage of correct taste choice. Concluding, a higher salivary flow facilitated the chewing of foods and taste perception, which means that it is indeed important for the efficient mastication and proper food bolus formation and perception.

Key-words: Salivary flow, Food, Texture, Taste, Flavor, Children, Protein

Introduction

Fragmentation and moistening of food is the main function of mastication, but it also imparts enjoyable sensations related to taste and the pleasure of eating (Pereira et al, 2006). During mastication, food particles are reduced in size, while saliva is produced to moisten and lubricate them. The water in saliva moistens food particles, whereas the salivary mucins bind masticated food into a coherent and slippery bolus that can be easily swallowed (Pedersen et al, 2002). Dry and hard products require more chewing cycles before swallowing (Engelen et al, 2005). Evidently, more time is needed to break the food down and to add enough saliva to form a cohesive bolus suitable for swallowing.

Without adequate salivary gland function, an individual may experience severe impairment in dental health, swallowing, speech, and enjoyment of food (Ship et al, 1991). Mastication is important for the maintenance of salivary gland function. Masticatory performance can be measured as chewing ability, or as the number of chews necessary to swallow certain natural foods (Gambareli et al, 2009).

Saliva is also thought to be involved in our perception of taste and flavor of foods. Researchers have investigated the effect of saliva on selected attributes and have found that saliva may exert diverse effects on food, resulting in changes in the way it is perceived (Guinard et al, 1998). The proteins present in saliva may possibly play a role in taste chemoreception and in the perception of astringency, viscosity, and other mouth feel attributes (Guinard & Muzzecchelli, 1996).

In this context, the study of the effect of saliva on chewing parameters and taste perception in children becomes important for the better understanding of the relations among the variables that are implicated in the masticatory process during the developmental phase. Therefore, the aims of this study were to assess the characteristics of salivary flow rates, chewing parameters (chewing time, number of chewing cycles, chewing frequency) and taste perception in schoolchildren, and to assess the effect of the salivary flow rate and the role of protein content on chewing parameters and taste perception.

Material and methods

Subjects

The subjects, 7-10 years of age, were conveniently selected from a public school in the city of Santa Barbara D'Oeste, São Paulo, Brazil. All the children had the same social-economic profile. Written consent *pro formas* were sent to 250 subjects and their parents, and 115 participated in this study (58 girls and 57 boys). The Ethics Committee of the Dental School of Piracicaba approved the research (protocol number 033/2006).

The inclusion criteria were: the children should be in the mixed dentition stage, have a healthy state of the masticatory system, the presence of the teeth (primary and/or permanent) without anomalies and alterations of form, structure or number, and the normality of oral tissues. In addition, the teeth should have no pain because of dental caries or pulpal involvement that could interfere with molar bite force measurements. The exclusion criteria considered systemic disturbances in general (e.i. neurological problems, Sjögren's syndrome, cistic fibrosis) and ingestion of medicines that could directly or indirectly interfere with muscular activity or salivary flow rate (e.i. antidepressants, anti-hypertensives, ansiolytics), and uncooperative behaviour.

Salivary flow rate

For determination of the salivary flow rate (SFR), unstimulated (U) and stimulated (S) whole saliva samples were collected in pre-weighted sterile disposable containers. The collections were all taken at least 2 hours after meals and 1 hour after brushing, to minimize effects of the circadian rithym, between 8:30 and 9:30 am. All the saliva samples were also taken in the same season (spring) to avoid climate. The collections were taken first for the unstimulated followed by the stimulated saliva.

The children were instructed to swallow immediately before the period of saliva collection, and not to swallow during the next 5 minutes of collection. They were instructed to spit out each 30 seconds into the container. For the stimulated saliva they had to chew on

paraffin film of 0.3 g (*Parafilm 'M"®, American National Can^{TM}, Greenwhich, CT, USA*) for the total period of 5 minutes.

After collection, the cointainers were re-weighted, the weight of each saliva sample (g) was equated to volume (mL), since the specific gravity of saliva is 1.0 (Shannon, 1973). Salivary flow rate was calculated by measuring the total volume of saliva and dividing this by the collecting time. Salivary flow rates are expressed as mL/min.

Total protein concentrations

For protein content measurements, the unstimulated and the stimulated saliva samples were frozen at -40^{o}C and thawed and mixed shortly before analyses. The total protein was assayed by the method of Lowry et al (1951) using bovine albumin as standard. Protein content is expressed as mg/mL.

Chewing parameters

The following foods were tested: carrot, toast with and without margarine (Bauducco®, Guarulhos, São Paulo, BR), banana, cheese (Polenghi®, Angatuba, São Paulo, BR), and fruit flavor chewing gum (Trident - Adams®, Bauru, São Paulo, BR). Each food was offered to the child in a 2g portion in duplicate (two equal portions of each food were presented in two different plates). The natural test foods were given to the subjects in a predetermined sequence. The subjects were asked to chew the food in their usual manner and were instructed to signal when they were ready to swallow. In between the foods, subjects were allowed to sip water. The chewing time and the number of cycles were controlled by the examiner. Thus, the chewing time (sec), number of chewing cycles (# cycles) and chewing frequency (cycles/sec) were determined. The chewing gum was chewed during 60 s, assessing the number of chewing cycles and, further, the chewing frequency. Since the weight and chewing time of the chewing gum differed from the other foods, it was only compared to them in relation to chewing frequency. Banana was classified as soft and wet, cheese as soft and fat, carrot as hard and wet, toast with margarine as hard and fat, toast without margarine as hard and dry, and the chewing gum as springy.

The subjects indicated the taste of the foods as bitter, sour, sweet or salty. The correct answers considered were: banana, carrot and chewing gum as sweet; both toasts and cheese as salty. The reliability of the taste evaluation was assessed by double recordings on 50 randomly selected children with an interval of 1 week without significant difference between the two sets of measurements and with the method error of the individual double recordings with 1-week interval of 8.0%

Body Variables

Body weight and height were determined in order to verify any differences between genders. Body weight was measured in kilograms, to the nearest tenth. The children were measured without shoes to determine height; the measurements were then rounded to the nearest tenth of a meter.

Statistical Analysis

The normality of the distributions was assessed by the D'Agostino-Pearson's test. The comparisons between genders were performed through unpaired *t* test or Mann-Whitney test. ANOVA was used among the foods tested. The correlations among the variables were assessed by Spearman's or Pearson's coefficients with significant values when $p < 0.05$. For taste, descriptive analysis was undertaken considering the number of correct answers. All analyses were carried out using BioEstat 5.0 (2007, Belém, PA, Brazil).

Results

There were no differences between genders in relation to age (8.9±1.1 years), weight (34.0±8.5 kg) and height (1.36±0.08 m).

Table 1 presents the mean values and standard deviations for unstimulated salivary flow rate (U-SFR), stimulated salivary flow rate (S-SFR), and protein content (mg/mL) for the total sample (n=115), girls (n=58) and boys (n=57) (Table 1). There was a significant difference between unstimulated and stimulated SFR for girls, boys and the total

sample and a significant difference in protein content of unstimulated and stimulated saliva for the total sample ($p<0.05$). Salivary flow rates were significantly different between genders, boys presenting higher values ($p<0.5$).

Table 2 presents the mean values and standard deviations for chewing time, number of chewing cycles, and chewing frequency for the total sample. The data are pooled for gender, but it was previously observed significant differences between genders in chewing time, number of chewing cycles, chewing frequency for both toasts and texture of cheese ($p<0.05$) (data previously described in details in Chapter 2).

The chewing time and the number of chewing cycles was similar ($p>0.05$) for toast with margarine and carrot (Table 2). There was a significant difference ($p<0.05$) between toasts in chewing time, as well as in number of chewing cycles. The chewing frequency was similar ($p>0.05$) for carrot and toast without margarine. The chewing frequency of both toasts did not differ significantly ($p>0.05$) as well as for the soft foods (banana and cheese; $p>0.05$; Table 2). The frequency of gum was different from all other foods ($p<0.05$).

The significant correlations between U-SFR, S-SFR, chewing time, number of chewing cycles, and chewing frequency for total sample, girls and boys are shown on Table 3. Salivary flow rates correlated negatively to chewing time and number of chewing cycles (carrot, cheese, toasts), and positively with chewing frequency (carrot and toasts) ($p<0.05$), meaning that children with higher salivary flow rates chewed the food faster.

The significant correlations between S-SFR, cycles, frequency, and protein content for the total sample, girls and boys can be seen in Table 4. The protein content of stimulated saliva correlated negatively to its flow ($p<0.05$), and positively to number of chewing cycles (carrot and toast with margarine). The total protein content of unstimulated saliva correlated negatively with chewing frequency (carrot) (Table 4).

Table 5 presents the percentage of children who chose the right flavor of the foods in the first and second portions, and in both portions in relation to flow rate (U-SFR and S-SFR), gender and protein content on unstimulated (U) and stimulated (S) saliva. It can be seen that children with U-SFR above average chose the right flavor more often than the children with U-SFR under average for carrot, banana, and both toasts. For the cheese

and gum, the opposite occurred. The S-SFR above average determined more right answers for carrot, banana, and toast without margarine. Girls rated the right taste more times than boys. Children with under average protein content in the unstimulated saliva had more right answers on the taste of food (Table 5). The same happened to the stimulated saliva, except for cheese (Table 5). In the case of chewing gum, for both unstimulated and stimulated saliva, there were more right answers when the protein content was above average (Table 5).

Table 1 – Mean values and standard (±SD) deviation of salivary flows and total protein content

		Girls (n=58)	Boys (n=57)	Total (n=115)
Salivary flow (mL/min)	Unstimulated	0.62^{Aa} (±0.31)	0.78^{Ba} (±0.36)	0.70^{a} (±0.34)
	Stimulated	1.11^{Ab} (±0.54)	1.41^{Bb} (±0.69)	1.26^{b} (±0.63)
Protein (mg/mL)	Unstimulated	0.83^{Aa} (±0.55)	0.87^{Aa} (±0.62)	0.85^{a} (±0.58)
	Stimulated	0.91^{Aa} (±0.48)	1.02^{Aa} (±0.67)	0.97^{b} (±0.58)

Different capital letters in the same line mean significant statistical difference between genders ($p<0.05$); differente small letters in the same column mean significant statistical difference ($p<0.05$) between unstimulated and stimulated flow rates, and between unstimulated and stimulated protein

Table 2 – Mean values ±standard deviation for chewing parameters of all foods in the total sample

	Chewing time (s)	Chewing cycles (n)	Chewing frequency (cycles/s)
Carrot	$18±7^{a}$	$27±10^{a}$	$1.53±0.21^{a}$
Banana	$5±2^{b}$	$6±3^{b}$	$1.13±0.20^{b}$
Cheese	$8±3^{c}$	$9±4^{c}$	$1.20±0.21^{b}$
Toast with margarine	$19±5^{a}$	$27±7^{a}$	$1.45±0.20^{c}$
Toast without margarine	$23±7^{d}$	$33±9^{d}$	$1.46±0.18^{ac}$
Chewing gum	60	82±10	$1.37±0.17^{d}$

Different small letters in the same column mean significant statistical difference among foods ($p<0.05$)
Chewing time for chewing gum was the same for all children, the comparison with other foods was done only for chewing frequency

Table 3– Significant correlation coefficients (r) ($p<0.05$) for unstimulated salivary flow rate (U-SFR), stimulated salivary flow rate (S-SFR), chewing time (s), number of chewing cycles, and chewing frequency (cycles/sec) of carrot, cheese, toast with margarine (t+m), toast without margarine (t-m) for the total sample (♂♀; n=115), girls (♀; n=58) and boys (♂; n=57)

	Chewing time (s)				Chewing cycles (n)				Chewing frequency (cycles/s)		
	Carrot	Cheese	T+m	T-m	Carrot	Cheese	T+m	T-m	Carrot	T+m	T-m
U-SFR	-0.426 ♂	-0.250 ♂♀	-0.245 ♂♀	-0.253 ♂♀	-0.302 ♂	-0.189 ♂♀	-	-	0.285 ♂	0.255 ♂♀	0.303 ♂♀
	-	-0.342 ♂	-0.281 ♂	-0.279 ♂	-	-0.321 ♂	-	-	-	0.284 ♂	0.328 ♂
S-SFR	-0.273 ♂	-0.187 ♂♀	-0.291 ♂♀	-0.350 ♂♀	-	-	-0.220 ♂♀	-0.235 ♂♀	-	0.188 ♂♀	0.283 ♂♀
	-	-	-0.335 ♂	-0.336 ♂	-	-	-	-	-	0.274 ♂	0.376 ♂

Table 4 – Significant correlation coefficients (r) ($p<0.05$) for S-SFR, cycles, and frequency of chewing carrot and toast with margarine (T+m) in relation to protein content for unstimulated and stimulated saliva for the total sample (♂♀; n=115), girls (♀; n=58) and boys (♂; n=57)

		S-SFR		Cycles			Frequency	
				Carrot		T+m	Carrot	
Protein mg/mL	Unstimulated	-	-	-	-	-	-0.219 ♂♀	-0.333 ♂
	Stimulated	-0.210 ♂♀	-0.290 ♂	0.207 ♂♀	0.283 ♂	0.274 ♂	-	-

Table 5 – Percentage (%) of children who chose the right flavor of the foods in the first (1st) and second (2nd) portions, and in both portions in accordance to flow rate above average (⇧) and below average (⇩) in U-SFR and S-SFR, and to gender

		Total sample	U-SFR		S-SFR		Gender		Protein-U		Protein-S	
			⇧average (n=44)	⇩average (n=71)	⇧average (n=49)	⇩average (n=66)	Girls (n=58)	Boys (n=57)	⇧average (n=44)	⇩average (n=71)	⇧average (n=47)	⇩average (n=68)
Carrot	1st	31.3	34.09	29.58	30.61	31.82	32.76	29.82	34.09	29.58	31.91	30.88
	2nd	26.09	31.82	22.54	28.57	24.24	25.86	26.32	25.00	26.76	23.40	27.94
	Both	25.22	29.55	22.54	26.53	24.24	25.86	24.56	25.00	25.35	23.40	26.47
Banana	1st	94.78	95.45	94.37	95.92	93.94	96.55	92.98	90.91	97.18	95.74	94.12
	2nd	92.17	97.73	88.73	93.88	90.91	96.55	87.72	86.36	95.77	91.49	92.65
	Both	90.43	95.45	87.32	91.84	89.39	94.83	85.96	84.09	94.37	89.36	91.18
Cheese	1st	61.74	59.09	63.38	61.22	62.12	63.79	59.65	61.36	61.97	63.83	60.29
	2nd	55.65	52.27	57.75	55.1	56.06	58.62	52.63	54.55	56.34	59.57	52.94
	Both	57.39	54.55	59.15	55.1	59.09	62.07	52.63	56.82	57.75	59.57	55.88
T+M	1st	83.48	86.36	81.69	83.67	83.33	86.21	80.7	79.55	85.92	76.60	88.24
	2nd	85.22	86.36	84.51	81.63	87.88	86.21	84.21	81.82	87.32	82.98	86.76
	Both	79.13	81.82	77.46	77.55	80.3	81.03	77.19	75.00	81.69	72.34	83.82
T-M	1st	87.83	93.18	84.51	91.84	84.85	87.93	87.72	81.82	91.55	82.98	91.18
	2nd	86.96	95.45	81.69	89.8	84.85	91.38	82.46	86.36	87.32	82.98	89.71
	Both	81.74	93.18	74.65	87.76	77.27	84.48	78.95	77.27	84.51	74.47	86.76
Gum	1st	95.65	93.18	97.18	93.88	96.97	98.28	92.98	97.73	94.37	97.87	94.12
	2nd	93.91	88.64	97.18	89.8	96.97	96.55	91.23	95.45	92.96	100.00	89.71
	Both	93.04	86.36	97.18	87.76	96.97	96.55	89.47	95.45	91.55	97.87	89.71

Toast with margarine (T+M), toast without margarine (T-M) and chewing gum (gum)

Discussion

The production of sufficient saliva is indispensable for good chewing. The important role of saliva for chewing and swallowing is demonstrated by the finding that the number of chewing strokes, hence time in the mouth, needed for swallowing significantly increases after experimentally induced oral dryness (Liedberg & Owall, 1991).

In relation to U-SFR, the mean values found in this study (0.70 mL/min) are comparable to Bretz et al (2001-site 7, Rio de Janeiro), Dezan et al (2002) and Negoro et al (2000), and are higher than those found by other studies (Watanabe & Dawes, 1990; Watanabe et al, 1995). This difference in flow may be due to differences in ages of samples, origin of children, site of study and techniques used for saliva collection. In relation to S-SFR, the mean 1.26 mL/min (Table 1) is similar to that of Torres et al (2006) and Alamoudi et al (2004).

Boys had significantly higher SFR than girls (Table 1), which is in accordance to previous studies (Söderling et al, 1993; Tukia-Kulmala & Tenovuo, 1993), but in contrast with other studies with Brazilian children (Torres et al, 2006; Dezan et al, 2002), and from other countries (Alamoudi et al, 2004; Watanabe et al, 1995; Watanabe & Dawes, 1990). The difference between genders in salivary flow rates may be partly due to different developing gland sizes, as noted in adults (Ono et al, 2007), although this difference has not been reported in children so far.

In the present study, no significant difference was found between genders in total protein content, agreeing with previous studies in children (Ben-Aryeh et al, 1990; Dezan et al, 2002). The content of protein was higher in the stimulated saliva which is in contrast with Engelen et al (2007) who found that protein concentration was highest in unstimulated saliva, followed by saliva stimulated by odour, chewing, and citric acid. The contrasting results might be due to some technique factors such as different sample ages.

The values of total protein content for both unstimulated and stimulated saliva are lower compared to those found by Ben-Aryeh et al (1984) and Tenovuo et al (1986), higher than that of Hyyppã et al (1989) and comparable to those of Dezan et al (2002) in children from 18 to 42 months. Hypothetically, the differences in values might be due to the slight differences in techniques, time of freezing of the samples and, as stated by Dezan

et al (2002) age, geographic location, and nutritional habits of the individuals, and the method of saliva collection used.

The number of chewing cycles and chewing frequency for soft foods (banana and cheese) were similar and lower than for hard foods (carrot and both toasts). These results are in agreement with previous findings on the relationship between chewing rate and food hardness (van der Bilt et al, 2006; Gavião et al, 2004; Hiiemae & Palmer, 1999). The children chew the softer foods slower, with lesser number of chewing cycles and therefore the chewing time was lower, which in accordance with the study of Gambareli et al (2009) and Hiiemae & Palmer (1999).

There was a significant difference in chewing time and number of chewing cycles between toast with and without margarine. Toast with margarine was chewed for less time and it was similar to carrot, agreeing with Gavião et al (2004) who found that buttering toast decreases significantly the chewing time and number of chewing cycles. With the addition of margarine, the toast became easier to be chewed, compared to carrot, which is 90% water (Ottenhoff et al, 1992). However, the chewing frequency of both toasts did not differ ($p>0.05$). This means that despite the longer time to chew toast without margarine (hard and dry), the chewing rate was similar between toasts, since the chewing time is compensated by the number of chewing cycles.

Chewing gum was chosen as a test food because it forms a coherent bolus and the mastication could be considered more uniform (Gambareli et al, 2009). The chewing frequency of gum was similar to hard foods when considering genders separately, but different from all other foods when considered the total sample. This might be explained by the springy nature of the gum.

As it was expected, SFR correlated negatively with chewing time and number of chewing cycles for carrot, cheese and both toasts (Table 3), meaning that the higher the flow rate, the lower the time and cycles before the first swallow. Also, SFR was positively related to chewing frequency of carrot and both toasts, meaning that children with higher salivary flow rates chewed the food faster. This was expected since children with low flow rates would need a longer time to add saliva into the food to form a coerent bolus suitable for swallowing. Nevertheless, this result is in contrast to that found by Gavião et al (2004)

and Ottenhoff et al (1992) who stated that a subject with a relative large salivary flow does not necessarily swallow the food after a relative smaller number of chewing cycles. In addition, despite the high percentage of water in carrot, the chewing time and number of chewing cycles of this food also correlated negatively with SFR, although the correlations were weak (Table 2).

Proteins can also influence lubrication (Erickson, 1992), which could have occurred in the mastication of toast with margarine in boys (Table 3) leading them to chew with a higher number of cycles in order to the proteins better lubrificate the food. Nevertheless, this correlation did not occur in girls or in the case of toast without margarine. Moreover, in this study the concentration of proteins influenced the chewing of carrot (a highly wet product). Therefore, this correlation should be verified in future studies.

There was a significant negative correlation of protein content and flow for stimulated saliva (Table 4), as found by Kedjarune et al (1997). Probably, saliva dilutes the proteins, so the higher the flow rate, the lower the protein content. Nevertheless, no correlation between protein content and flow in the unstimulated saliva was found in this study (Table 4), as found by Santos et al (2007) in patients with cerebral palsy. This absence of correlation in the unstimulated saliva might be related to the sample age in the present study and the sample conditions in the study of Santos et al (2007).

It has been speculated that changes in the quantity and quality of saliva affect taste sensitivity during the initial processes of taste stimulation, as well as the health and integrity of the taste cells (Matsuo, 2000). In relation to this variable, a linear correlation could not be determined, since this attribute is abstract. Therefore, we compared the number of children who rated the foods correctly. It can be seen on Table 5 that SFR might have an influence on choosing the correct flavor. It has been demonstrated that short-term reductions in salivary flow have very little impact on taste perception (Christensen et al, 1984), while long-term deficiency of saliva resulted in lower taste sensitivity and altered preferences (Galili et al, 1981).

Nevertheless, this attribute is learned throughout life and as it is known, girls develop earlier than boys, this might explain why girls had more correct answers than boys.

In the study of Laureati et al (2008) females were superior to males in absolute memory tests of taste, texture and aroma. The authors also showed that liker-status had an impact on absolute memory, in our case, the liker-status could have interfered with choosing the flavor, since the two foods that had the least correct answers (carrot and cheese) were the foods that children liked the least (observed by the examiner, data not shown).

Children with lower content of protein chose the right flavor more times than children with higher contents of protein for all foods, except cheese in the stimulated saliva and chewing gum, on both types of saliva. This result is in agreement with the findings of Engelen et al (2007) who found that greater concentrations of proteins were correlated with lower flavor perception. Therefore, in light of our study, we support the conjecture pointed by those authors that proteins bind the flavor compounds and in that manner decrease flavor release. Nevertheless, the opposite happened for chewing gum in both types of saliva and cheese (unstimulated saliva). In the case of chewing gum, this result might be related to its springy nature, and its astringency, since proteins may play a role in taste chemoreception and in the perception of astringency, viscosity, and other mouth feel attributes (Guinard & Mazzucchelli, 1996).

In relation to cheese in the stimulated saliva, it might be related to its fat content. Previous studies have hypothesized that increased fat content results in increased flavor release of the fat-soluble flavors (de Wijk et al, 2004). Saliva acts as a solvent of tastants in the initial process of taste transduction, and in this respect, proteins can influence taste perception of food with high fat content.

Conclusion

It was concluded that a higher salivary flow rate facilitates the chewing of foods, and it is also important for taste sensitivity, which means that saliva is indeed important for the efficient mastication and adequate food perception and fragmentation.

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IV – CONCLUSÕES GERAIS

Considerando os resultados encontrados, concluiu-se que:

1. A ultrasonografia permanece como uma técnica promissora para o estudo dos músculos da mastigação;
2. Houve aumento da FM e espessura do masseter após a reabilitação e também relação entre FM e espessura do temporal, o que sugere que a reabilitação bucal influencia os aspectos morfológicos e funcionais dos músculos da mastigação.
3. Houve relação entre FM e FS em crianças com oclusão normal, mas não em crianças com maloclusão.
4. Em crianças de 7-10 anos de idade com ausência de maloclusão a maior FM apresentou-se relacionada à maior freqüência mastigatória, o que indica facilidade de mastigação.
5. A maior taxa de fluxo salivar facilitou a mastigação de alimentos e foi relevante para a sensibilidade ao sabor, o que indica que a saliva é, de fato, importante para a mastigação eficiente e para a percepção e fragmentação apropriada do alimento.

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ANEXOS

Anexo 1 - Resolução CCPG/002/06 a qual dispõe a respeito do formato das teses de mestrado e doutorado aprovados pela UNICAMP (Parte I)

INFORMAÇÃO CCPG/OO2/06[6]

Tendo em vista a necessidade de revisão da regulamentação das normas sobre o formato e a impressão das dissertações de mestrado e teses de doutorado e com base no entendimento exarado no Parecer PG n° 1985/96, que trata da possibilidade do formato alternativo ao já estabelecido, a CCPG resolve:

Artigo 1º - O formato padrão das dissertações e teses de mestrado e doutorado da UNICAMP deverão obrigatoriamente conter:

I. Capa com formato único ou em formato alternativo que deverá conter informações relativas ao nível (mestrado ou doutorado) e à Unidade de defesa, fazendo referência à Universidade Estadual de Campinas, sendo o projeto gráfico das capas definido pela PRPG.
II. Primeira folha interna dando visibilidade à Universidade, a Unidade de defesa, ao nome do autor, ao título do trabalho, ao número de volumes (quando houver mais de um), ao nível (mestrado ou doutorado), a área de concentração, ao nome do orientador e co-orientador, ao local (cidade) e ao ano de depósito. No seu verso deve constar a ficha catalográfica.
III. Folha de aprovação, dando visibilidade à Comissão Julgadora com as respectivas assinaturas.
IV. Resumo em português e em inglês (ambos com no máximo 500 palavras).
V. Sumário.
VI. Corpo da dissertação ou tese dividido em tópicos estruturados de modo característico à área de conhecimento.
VII. Referências, formatadas segundo normas de referenciamento definidas pela CPG da Unidade ou por critério do orientador.
VIII. Todas as páginas deverão, obrigatoriamente, ser numeradas, inclusive páginas iniciais, divisões de capítulos, encartes, anexos, etc... As páginas iniciais poderão ser numeradas utilizando-se algarismos romanos em sua forma minúscula.
IX. Todas as páginas com numeração "impar" serão impressas como "frente" e todas as páginas com numeração "par" serão impressas como "verso".

§ 1º - A critério do autor e do orientador poderão ser incluídos: dedicatória; agradecimento; epígrafe; lista de: ilustrações, tabelas, abreviaturas e siglas, símbolos; glossário; apêndice; anexos.

§ 2º - A dissertação ou tese deverá ser apresentada na língua portuguesa, com exceção da possibilidade permitida no artigo 2° desta Informação.

§ 3º - As dissertações e teses cujo conteúdo versar sobre pesquisa envolvendo seres humanos, animais ou biossegurança, deverão apresentar anexos os respectivos documentos de aprovação.

Artigo 2º - A critério do orientador e com aprovação da CPG da Unidade, os capítulos e os apêndices poderão conter cópias de artigos de autoria ou de co-autoria do candidato, já publicados ou submetidos para publicação em revistas científicas ou anais de congressos sujeitos a arbitragem, escritos no idioma exigido pelo veículo de divulgação.

Anexo 1 - Resolução CCPG/002/06 a qual dispõe a respeito do formato das teses de mestrado e doutorado aprovados pela UNICAMP (Parte II)

§ único - O orientador e o candidato deverão verificar junto às editoras a possibilidade de inclusão dos artigos na dissertação ou tese, em atendimento à legislação que rege o direito autoral, obtendo, se necessária, a competente autorização, deverão assinar declaração de que não estão infringindo o direito autoral transferido à editora.

Artigo 3º - Dependendo da área do conhecimento, a critério do orientador e com aprovação da CPG da Unidade, a dissertação ou tese poderá ser apresentada em formato alternativo, desde que observados os incisos I, II, III IV, V e VII do artigo 1º.

Artigo 4º - Para impressão, na gráfica da Unicamp, dos exemplares definitivos de dissertações e teses defendidas, deverão ser adotados os seguintes procedimentos:

§ 1º - A solicitação para impressão dos exemplares de dissertações e teses poderá ser encaminhada à gráfica da Unicamp pelas Unidades, que se responsabilizarão pelo pagamento correspondente.

§ 2º - Um original da dissertação ou tese, em versão definitiva, impresso em folha tamanho carta, em uma só face, deve ser encaminhado à gráfica da Unicamp acompanhado do formulário "Requisição de Serviços Gráficos", onde conste o número de exemplares solicitados.

§ 3º - A gráfica da Unicamp imprimirá os exemplares solicitados com capa padrão. Os exemplares solicitados serão encaminhados à Unidade em, no máximo, cinco dias úteis.

§ 4º - No formulário "Requisição de Serviços Gráficos" deverão estar indicadas as páginas cuja reprodução deva ser feita no padrão "cores" ou "foto", ficando entendido que as demais páginas devam ser reproduzidas no padrão preto/branco comum.

§ 5º - As dissertações e teses serão reproduzidas no padrão frente e verso, exceção feita às páginas iniciais e divisões de capítulos; dissertações e teses com até 100 páginas serão reproduzidas no padrão apenas frente, exceção feita à página que contém a ficha catalográfica.

§ 6º - As páginas fornecidas para inserção deverão ser impressas em sua forma definitiva, ou seja, apenas frente ou frente/verso.

§ 7º - O custo, em reais, de cada exemplar produzido pela gráfica será definido pela Administração Superior da Universidade.

Artigo 5º - É obrigatória a entrega de dois exemplares para homologação.

Artigo 6º - Esta Informação entrará em vigor na data de sua publicação, ficando revogadas as disposições em contrário, principalmente as Informações CCPG 001 e 002/98 e CCPG/001/00.

Campinas, 13 de setembro de 2006

Profa. Dra. Teresa Dib Zambon Atvars
Presidente
Comissão Central de Pós-Graduação

Anexo 2 – Certificado do Comitê de Ética em Pesquisa

COMITÊ DE ÉTICA EM PESQUISA
FACULDADE DE ODONTOLOGIA DE PIRACICABA
UNIVERSIDADE ESTADUAL DE CAMPINAS

CERTIFICADO

O Comitê de Ética em Pesquisa da FOP-UNICAMP certifica que o projeto de pesquisa "**Avaliação fisiológica e morfológica do sistema mastigatório em crianças**", protocolo nº **033/2006**, dos pesquisadores **MARIA BEATRIZ DUARTE GAVIÃO e MARCIA DIAZ SERRA**, satisfaz as exigências do Conselho Nacional de Saúde – Ministério da Saúde para as pesquisas em seres humanos e foi aprovado por este comitê em 12/04/2006.

The Research Ethics Committee of the School of Dentistry of Piracicaba - State University of Campinas, certify that project "**Physiologic and morphologic evaluation of the masticatory system in children**", register number **033/2006**, of **MARIA BEATRIZ DUARTE GAVIÃO and MARCIA DIAZ SERRA**, comply with the recommendations of the National Health Council – Ministry of Health of Brazil for researching in human subjects and was approved by this committee at 12/04/2006.

Profa. Cecília Gatti Guirado
Secretária
CEP/FOP/UNICAMP

Prof. Jacks Jorge Júnior
Coordenador
CEP/FOP/UNICAMP

Nota: O título do protocolo aparece como fornecido pelos pesquisadores, sem qualquer edição.
Notice: The title of the project appears as provided by the authors, without editing.

Anexo 3 – Ilustrações Metodologia

Figura 1 – Voluntário com ausência de dentes (capítulo 2)

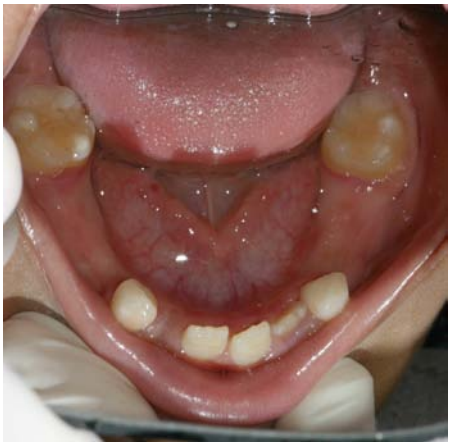

Figura 2 – Voluntário após colocação do mantenedor de espaço funcional (capítulo 2)

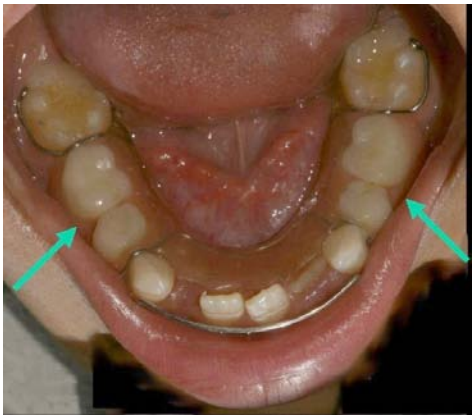

Figura 3 - Transdutor de força de mordida (capítulos 2 e 3)

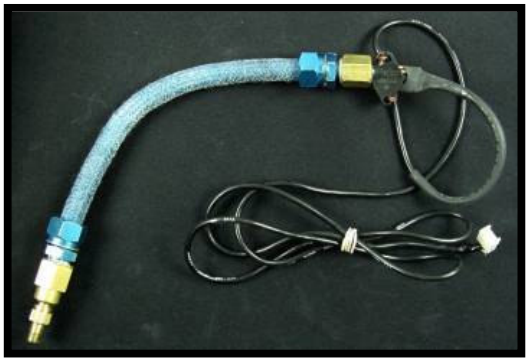

Figura 4 – Voluntário com transdutor de força de mordida posicionado (capítulos 2 e 3)

Figura 5 – Equipamento de Ultrassom digital Just Vision 200 (capítulo 2)

Figura 6 – Transdutor linear de 56 mm (capítulo 2)

Figura 7 – Palpação músculo masseter (capíutlo 2)

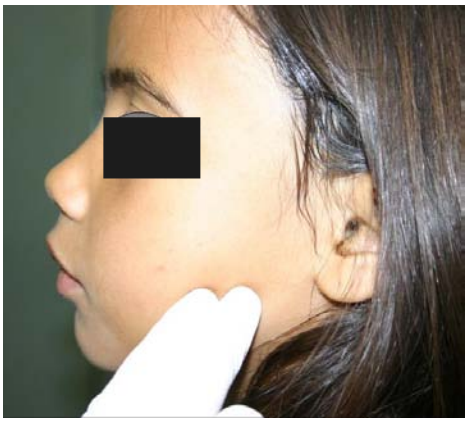

Figura 8 – Posicionamento do transdutor sobre músculo masseter (capítulo 2)

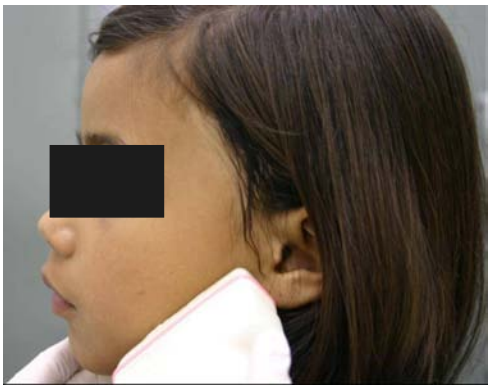

Figura 9 – Palpação do músculo temporal (capítulo 2)

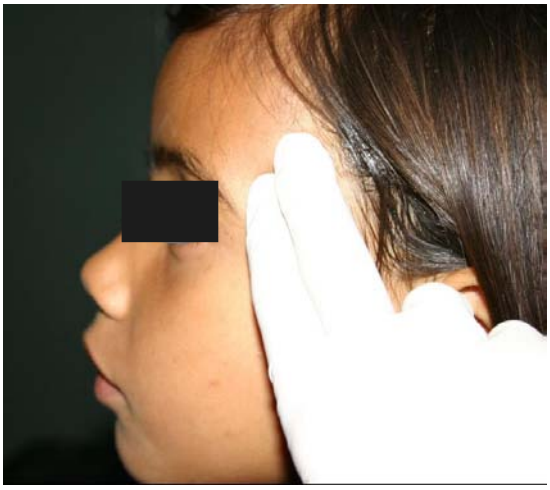

Figura 10 – Posicionamento do transdutor sobre o músculo temporal (capítulo 2)

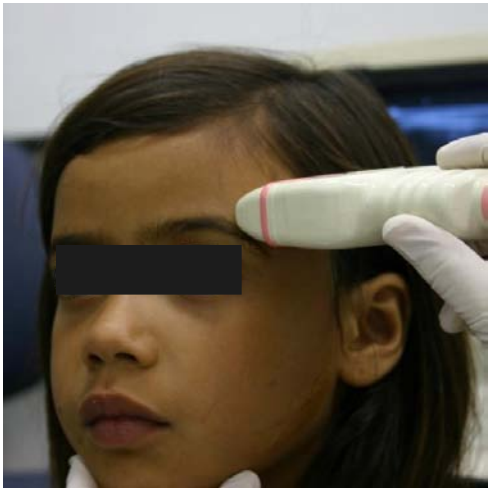

Figura 11 – Balança utilizada para pesagem do recippiente de coleta da saliva (capítulos 3 e 5)

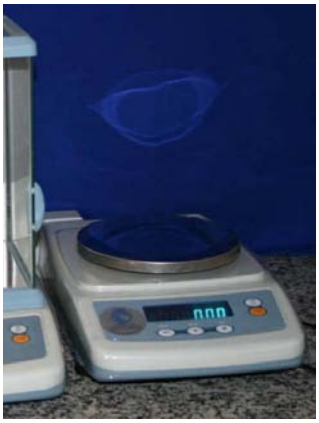

Figura 12 – Recipiente descartável e estéril utilizado para coleta da saliva (capítulos 3 e 5)

Figura 13 - Parafilm utilizado para estimulação da saliva (capítulo 3 e 5**)**

Figura 14 – Ilustração dos alimentos utilizados para avaliação dos parâmetros de mastigação, percepção de sabor e textura (capítulos 4 e 5)

Figura 15 - Figuras de alimentos utilizados para ilustrar sabor amargo (capítulo 5)

Figura 16 – Figuras de alimentos utilizados para ilustrar sabor azedo (capítulo 5)

Figura 17 – Figura de alimentos utilizados para ilustrar sabor salgado (capítulo 5)

Figura 18 – Figuras de alimentos utilizados para ilustrar sabor doce (capítulo 5)