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SAMUEL DE CARVALHO CHAVES JUNIOR

AVALIAÇÃO DO COMPORTAMENTO ALIMENTAR, ASPECTOS NUTRICIONAIS, CONDIÇÕES BUCAIS E BIOMARCADORES SALIVARES NA PRIMEIRA INFÂNCIA

EVALUATION OF FOOD BEHAVIOR, NUTRITIONAL ASPECTS, ORAL CONDITIONS AND SALIVARY BIOMARKERS IN EARLY CHILDHOOD

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Tese apresentada à Faculdade de Odontologia de Piracicaba da Universidade Estadual de Campinas como parte dos requisitos exigidos para a obtenção do título de Doutor em Odontologia, na Área de Odontopediatria.

Thesis presented to the Piracicaba Dental School of the University of Campinas in partial fulfillment of the requirements for the degree of Doctor in Dentistry, in Pediatric Dentristry área.

Orientadora: Profa. Dra. Maria Beatriz Duarte Gavião

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RESUMO

A nutrição é um pilar do desenvolvimento humano. Assim, deve-se entender o papel da nutrição na saúde das pessoas, principalmente em crianças que estão na fase de crescimento e desenvolvimento. O objetivo deste estudo foi avaliar o comportamento alimentar, as condições bucais, quantificar os biomarcadores salivares de apetite (leptina e grelina) e relacionar com o estado nutricional em crianças na primeira infância. Foram selecionadas 160 crianças de 3 a 5 anos e seus responsáveis. O estado nutricional foi determinado pelo Índice de Massa Corporal (IMC), de acordo com a Organização Mundial de Saúde, sendo que para as crianças consideou-se as curvas de crescimento para a idade e os escores-Z e para as mães os valores nutricionais de referência. O comportamento alimentar foi avaliado com a versão em português do questionário *Children's Eating Behavior Questionnaire* (CEBQ). Para as condições bucais, foi realizado exame clínico odontológico para verificar a saúde bucal pelo índice ceo-d, presença de gengivite, características de oclusão e análise das funções orais. Para os biomarcadores salivares, leptina e grelina, foram realizadas coletas de saliva estimulada, utilizando *salivettes* e rolo de algodão. A dosagem foi realizada pelo método ELISA. Os dados foram analisados por estatística descritiva, testes de comparações, correlações, modelos de regressão e teste alfa de Cronbach. O nível de significância considerado foi de 5%. Observou-se que 21% das crianças e 64% das mães estavam acima do peso e com obesidade. Sessenta porcento das mães apresentaram bom nível de educação. Quanto ao questionário CEBQ, não houve diferença estatística entre os escores dos domínio com relação ao estado nutricional, porém o IMC foi significativamente correlacionado aos domínios 'prazer em comer', 'resposta a saciedade', IMC da mãe e idade das crianças. Com relação a saúde bucal, 66 % das crianças eram livres de lesões de cárie cavitadas, com valor médio do ceo-d de 1.2. A avaliação das funções orais mostrou que a mastigação bilateral foi a mais prevalente (84% das crianças) e a deglutição atípica presente em 32% das crianças. Com relação as análises de oclusão, na relação vestíbulo-lingual de molares, cerca de 94% das crianças foi normal. Na relação de incisivos, a mordida aberta estava presente em 33% e a sobremordida acentuada em 13%. No modelo de regressão logística multivariada, a variável que teve influência no estado nutricional das crianças, foi o estado nutricional da mãe. Crianças cujas mães estavam com sobrepeso e obesas, tiveram chance três vezes maior de ter a mesma condição nutricional. Os valores da leptina salivar não apresentaram diferenças significativas entre os diferentes estados nutricionais, enquanto que para a grelina os valores foram significativamente menores em crianças com sobrepeso/obesidade. Os valores da leptina salivar correlacionaram-se positivamente com os valores da grelina salivar. Ambos os hormônios correlacionaram-se

negativamente com o IMC e a grelina apresentou correlação negativa com o domínio do CEBQ 'Prazer em comer'. Concluiu-se que os aspectos da alimentação relacionados ao apetite e a tendência a comer mais, bem como o estado nutricional materno, influenciaram o peso das crianças estudadas. As condições bucais, características de oclusão, funções de mastigação e deglutição bucais não mostraram influência no estado nutricional das crianças. Os valores significativamente menores de grelina salivar para crianças com sobrepeso/obesidade e a correlação negativa entre o IMC e com o domínio do CEBQ "Prazer em comer" sugerem tendência de regulação alterada do apetite em idades precoces.

Palavras chave – Comportamento alimentar, índice de massa corporal, grelina, leptina, saliva.

ABSTRACT

Nutrition is a pillar of human development. Thus, one should understand the role of nutrition in people's health, especially in children who are in the growth and development stage. The present study is a cross-sectional analytical, which aimed to evaluate eating behavior, oral conditions, quantify salivary biomarkers of appetite (leptin and ghrelin) and relate them to the weight status in children in early childhood. A total of 160 children from 3 to 5 years and their parents/guardians were selected. Anthropometric evaluation was performed to analyze the nutritional status of children, determining body mass index (BMI) and using the reference values of nutritional status by growth curves for age according to World Health Organization (WHO). For the mothers, weight and height were self-declared and the nutritional status was determined using WHO reference values for BMI. The eating behavior was evaluated using the Portuguese (Brazilian) version of the Children's Eating Behavior Questionnaire (CEBQ). For oral conditions, a dental clinical examination was performed to verify oral and oral health characteristics by dmft index and occlusion analysis. For salivary biomarkers, leptin and ghrelin, stimulated saliva collections were collected using salivettes and cotton roller. The dosage was performed by the ELISA method. The data were analyzed using descriptive statistics, tests of comparisons, correlations, regression models, and Cronbach's alpha test. The significance level considered was 5%. It was observed that 21% of the children and 64% of the mothers were overweight and obese. Sixty percent of mothers had a good level of education. Regarding the CEBQ questionnaire, there was no statistical difference between the scores of the domains in relation to the different weight status, but the BMI of children was significantly correlated with the domains 'Enjoyment of food', 'Satiety responsiveness', BMI of the mothers and children age. For oral health evaluation, 66% of the children were caries-free. The average value of dmft was 1.2. The severe dmft pattern was seen in 8 children (5%). Regarding oral functions, bilateral chewing was more prevalent, 84%. Atypical swallowing was frequent in 32% of children. The occlusion analyzes showed that about 94% of the children presented normal buccal-lingual molar relationship. For the incisor relationship, the open bite was present in 33% and the overbite was accentuated in 13%. According to the multivariate logistic regression model, the variable that had influence on the weight status of the children was the nutritional status of the mothers. Children whose mothers were overweight/obese, were three times more likely to have the same weight condition. The values of salivary leptin did not differ between different wight status, but the values of salivary ghrelin were significantly higher for overweight/obese children. Both hormones correlated negatively with children's BMI and salivary ghrelin was negatively correlated to the BMI and domain 'Enjoyment of food'

of CEBQ. The salivary leptin and ghrelin were positively correlated. The BMI was negatively correlated with the dmft and the subscale 'Satiety responsiveness' of CEBQ. Aspects of food related to appetite and the tendency to eat more, environmental aspects, such as weight status mother, influenced the weight of the children studied. Oral conditions, occlusion characteristics, oral chewing and swallowing functions did not influence the weight status of the sample. Higher values of salivary ghrelin for overweight/obese children and its inverse relationship with BMI and the domain ‘Enjoyment of food’ can infer the tendency for disturbed appetite regulation in preschoolers.

Keywords – Feeding behavior, body mass index, ghrelin, leptin, saliva.

SUMÁRIO

1 INTRODUÇÃO

Hábitos alimentares e preferências por alimentos são adquiridos precocemente na infância, representando características comportamentais que podem mudar ao longo do tempo, influenciadas por experiências individuais (Carnell e Wardle, 2008). Além das influências sociais que determinam impacto na dieta, é aceito que influências familiares ambientais e genéticas têm papel preponderante no padrão de ingestão de alimentos, no comportamento dietético e no estado nutricional (Dubois et al., 2007; Miglioli et al., 2015). O estado nutricional expressa o grau no qual as necessidades fisiológicas por nutrientes são alcançadas para manter a composição e as funções adequadas do organismo, resultando no equilíbrio entre ingestão e necessidade de nutrientes (DeHoog, 1998).

O índice de massa corporal (IMC) tem sido o método antropométrico mais utilizado para avaliação do estado nutricional, por ser uma medida simples e de baixo custo com adequada concordância entre os indicadores de adiposidade no diagnóstico de sobrepeso e obesidade em crianças (Kesim et al., 2016). Alguns autores sugerem que a indicação imprecisa de gordura corporal fornecido pelo índice de massa corporal (IMC), pode muito bem ser a raiz do suposto paradoxo da obesidade (ABESO, 2016). Neste contexto, Jensen et al. (2015) consideraram a importância da determinação do IMC em crianças jovens para compreender efetivamente o desenvolvimento do sobrepeso e da obesidade na infância. Nesta população, em função das alterações de peso e altura durante o crescimento e desenvolvimento, recomenda-se a utilização de referenciais específicos para a idade e gênero, como o diagnóstico de sobrepeso e obesidade proposto pela Organização Mundial da Saúde, que se baseia na distribuição do escore-Z de peso para altura, sendo a relação entre o peso encontrado e o peso ideal para a altura (WHO, 1995).

Além disso, a obesidade é um fator determinante e um importante fator de risco para outras doenças, tais como diabetes mellitus não insulina dependente, doenças cardiovasculares, incluindo hipertensão e certos tipos de câncer, a consequência mais significativa da obesidade na infância, a longo prazo, é que predispõe à obesidade mais tarde e, portanto, aumenta o risco a estas doenças e morte prematura na idade adulta (Craig et al., 2016).

O apetite, o prazer em comer, a sensibilidade a fatores externos associados aos alimentos (estímulos sensoriais como o gosto e o aroma, por exemplo) e os aspectos emocionais são considerados determinantes na nutrição, seleção e estilo alimentar relacionados ao incremento da prevalência da obesidade (Tepper e Barbarossa, 2020). Existem parâmetros utilizados para avaliar comportamentos alimentares em crianças e adultos que podem predizer o risco de distúrbios alimentares e problemas relacionados ao peso corporal, os chamados instrumentos psicométricos (Gallant et al., 2010). O *Children's Eating Behavior Questionnaire* (CEBQ) tem

sido considerado um instrumento mais confiável para avaliação do comportamento alimentar em crianças. Este questionário foi desenvolvido e validado na Inglaterra (Wardle et al., 2001) e também traduzido em outros países da Europa, como a Holanda (Sleddens et al., 2008) e Portugal (Viana e Sinde, 2008). O CEBQ foi criado tendo como suporte teórico o conhecimento atual sobre as causas alimentares da obesidade privilegiando, entre estas, os determinantes comportamentais (Viana e Sinde, 2008) e emocionais (Pan et al., 2017)

O CEBQ foi desenvolvido especificamente para investigar o comportamento alimentar em crianças e jovens, por meio das respostas fornecidas pelos seus cuidadores. O instrumento é composto por 35 itens e tem por objetivo avaliar oito itens ou domínios: (1) Resposta à comida, (2) Prazer em comer (ambos avaliam o interesse pela comida); (3) Sobre ingestão emocional (perante a ação de fatores emocionais o sujeito tende a comer mais do que o habitual); (4) Desejo de Beber (desejo por bebidas açucaradas e refrigerantes); (5) Resposta à Saciedade (avalia a capacidade de regulação do apetite), (6) Seletividade Alimentar (inclui falta de apetite e preferência por um grupo muito restrito de alimentos); Itens, que avaliam evitar a comida: (7) Sub-ingestão Emocional (diante de fatores emocionais o sujeito perde o apetite e come menos do que o habitual) e (8) Ingestão Lenta (relacionada com a falta de interesse pela comida).

Além desses fatores, estudos sugerem que a condição socioeconômica pode interferir nos valores do IMC (Camargos et al., 2019). Estudos sinalizam que o estado nutricional tende a ser inversamente proporcional ao fator socioeconômico (McLaren, 2007; Chung et al., 2016), uma vez que o ambiente social e as condições econômicas podem determinar ou limitar o poder de compra, especialmente de alimentos (Black et al., 2008). O nível educacional dos pais, especialmente da mãe, e o estado nutricional materno, foram associados à obesidade infantil (Burke et al., 2001; Madden et al., 2017). Assim, torna-se de importância conhecer o ambiente familiar da criança, levando em conta que a mãe pode estabelecer o respectivo elo.

Considerando que o processo digestório inicia na cavidade bucal, presume-se que as características do sistema mastigatório e a respectiva função podem ter influência no estado nutricional das pessoas, tornando-se de interesse a abordagem em crianças. Um estado nutricional adverso, pode prejudicar a saúde oral, da mesma forma que a saúde oral precária, pode influenciar a ingestão de alimentos e a nutrição (Gondivkar et al., 2018). A preferência por alimentos, a dieta e a nutrição podem ser afetadas pelas condições dentárias, sendo que a dificuldade na mastigação é o mecanismo mais provável pelo qual a saúde bucal comprometida pode afetar a ingestão de alimentos (Peyron et al., 2018). Sabe-se que a performance mastigatória diminui com a perda de dentes e com alterações bucais em geral. Quando as

superfícies oclusais estão comprometidas por lesões cariosas ou restaurações inadequadas, por exemplo, os pacientes tendem a mastigar de maneira não adequada, ao invés disso, deglutem partículas maiores e, também a força mastigatória é prejudicada (Subramaniam et al., 2016; Gondivkar et al., 2018). Podem alterar a dieta dando preferência a alimentos macios, mais fáceis de mastigar, como os industrializados, em detrimento dos ricos em fibras e nutrientes, que podem apresentar conteúdo nutritivo inadequado (Hujoel et al., 2017). Isto pode resultar no decréscimo da absorção de nutrientes, pois o fenômeno físico-químico do processo digestório se inicia na cavidade bucal, como já comentado.

A mastigação é interligada fisiologicamente à deglutição, envolvendo sincronia sensório-motora. Os alimentos precisam ser triturados e deglutidos de maneira adequada, para que então sejam levados ao estômago. Quando ocorre uma alteração nessas funções, como a perda dos dentes, por exemplo, pode haver alteração no padrão de deglutição (Silva et al., 2019). Como resultado, a dieta prejudicada pode aumentar o risco de distúrbios gastrointestinais e de doenças relacionadas aos distúrbios nutricionais (Pace et al., 2016; Manohar et al., 2019). Quando ocorre um incorreto posicionamento da língua, no ato da deglutição, é chamado de deglutição atípica, um problema miofuncional, de etiologia multifatorial e tem conexão recorrente com a presença de más oclusões (Maspero et al., 2014).

A mordida aberta, uma das más oclusões mais prevalente em crianças abaixo de 5 anos, tem prevalência de 12,1%, segundo o último levantamento nacional (SBBrasil, 2012). Pacientes com mordida aberta, os músculos mastigatórios tendem a gerar uma pequena força máxima de mordida, apresentam padrão mastigatório mais estreito, ciclos mastigatórios de menor duração e menor ativação muscular, exibindo menor eficiência mastigatória (Piancino et al., 2012; Corrêa et al., 2018). Com relação a mordida cruzada posterior, pode haver diminuição na força de mordida e função muscular assimétrica quando compara-se com crianças sem mordida cruzada (Castelo et al., 2007; Castelo et al., 2008; Andrade et al., 2009). Quando às funções de mastigação e deglutição são comprometidas, assim como as condições bucais precárias e a presença de más oclusões, podem impactar negativamente na ingestão de alimentos e, consequentemente, no estado nutricional (Peyron et al., 2018).

Os estudos utilizando saliva tem sido sempre um dado interessante e os biomarcadores, leptina e grelina, têm despertado interesse como eventual ligação entre apetite-saciedade, peso corporal, além de desempenhar um papel importante na regulação da ingestão alimentar (Klok et al., 2007; Singh et al., 2015), são secretados por diversos tecidos, incluindo as glândulas salivares (Önnerfält et al., 2018; Pîrsean et al., 2019).

A leptina é o hormônio da saciedade, diminuindo a ingestão de alimentos, porque atua na regulação dos neuropeptídeos associados à ingestão de alimentos, cuja ação ocorre nas regiões hipotalâmicas do cérebro que controlam o comportamento alimentar, desempenhando um papel importante na manutenção do metabolismo do corpo (Wasim et al., 2016; Guzmán et al., 2019), em pessoas obesas, foi observado um nível elevado de leptina (Mehrdad et al., 2020), porém essas pessoas têm mais resistência a esse hormônio (Tanakun et al., 2014). Segundo Rolls (2011), os estímulos sensoriais produzidos pelo gosto, cheiro, textura e aparência dos alimentos interagem com os sinais de saciedade produzidos pela dilatação do estômago e pela liberação de hormônios da saciedade determinando o prazer e o sabor do alimento, influenciando na escolha do tipo e da quantidade do alimento a ser ingerido. Neste sentido, Pîrsean et al. (2019) observaram que o IMC foi o principal indicador dos níveis de leptina, pois os valores em crianças com obesidade foram três vezes maiores em comparação aos outros estados nutricionais.

Ao contrário da leptina, a grelina estimula a ingestão de alimentos e aumenta o IMC, promovendo ganho de peso corporal e adiposidade, estimulando a ingestão de alimentos, enquanto diminui o gasto energético e a aumenta gordura corporal (Miljković, 2017). Li et al. (2011) encontraram correlação positiva significativa entre níveis de grelina plasmática e grelina salivar, que por sua vez foram correlacionadas negativamente com o IMC em crianças e adolescentes obesos, sugerindo que a mensuração de grelina na saliva pode ser um método alternativo eficaz de quantificação associada ao desenvolvimento da obesidade nesta população. Sabe-se que esse hormônio pode estar em menor quantidade na obesidade, porém indivíduos obesos são mais sensíveis à grelina (Makris et al., 2017). Como os níveis de leptina e grelina podem estar alterados na obesidade, a avaliação destes hormônios é importante para o estabelecimento de novas estratégias diagnósticas e, possivelmente, terapêuticas (Klok et al., 2007).

Desta forma, este estudo teve por objetivo investigar o comportamento alimentar, as condições bucais, socioeconômicos e a dosagem dos biomarcadores leptina e grelina salivar e associá-los com os aspectos nutricionais em crianças de 3 a 5 anos.

2 ARTIGO

Weight status and its relationship with food behavior, oral characteristics and functions, salivary leptin and ghrelin in preschoolers

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Abstract

Aim: The aim was to assess the eating behavior, oral conditions, quantify salivary biomarkers, leptin and ghrelin and relate them to the weight status in early childhood.

Methods: A total of 160 children from 3 to 5 years and their parents/guardians were selected. The weight status was determined by the Body Mass Index (BMI) and the growth curves were used for reference values. Mothers' BMI was also determined. The eating behavior was evaluated using the Children's Eating Behavior Questionnaire (CEBQ). The dmft index, dental occlusion, mastication and swallowing were assessed. Salivary leptin and ghrelin levels were quantified using stimulated saliva and the ELISA method. Descriptive statistics, comparison tests, correlations, ANOVA, and regression models were applied and significance level set at 5%.

Results: Regarding weight status, 21% of children and 64% of mothers were overweight/obese. There was no statistical difference between domain scores of the CEBQ questionnaire among different weight status, but BMI was correlated with the domains 'Enjoyment of food', 'Satiety responsiveness', mothers' BMI and children's age. In the multivariate logistic regression model, the variable that influenced the condition of the children's weight was the mother's weight status (OR=2.75). Oral conditions and functions were not influencing factors for weight status. Overweight/obese children presented significant higher levels of salivary ghrelin than children with normal weight; salivary leptin correlated significantly with salivary ghrelin levels. Both correlated negatively with BMI and ghrelin showed an inverse relationship with 'Enjoyement of food'.

Conclusion: Aspects of food related to appetite and the tendency to eat more and maternal nutritional condition, influenced the children's weight status. Oral conditions, occlusion characteristics, and chewing and swallowing had no influence. Higher values of salivary ghrelin for overweight/obese children and its inverse relationship with BMI and the domain 'Enjoyment of food' can infer the tendency for disturbed appetite regulation in preschoolers.

Keywords: Feeding Behavior. Body Mass Index. Ghrelin. Leptin. Saliva

Introduction

Nutrition is a very important parameter for an individual's health and a pillar of human development (Gondivkar et al., 2019). It is known that nutritional status is the balance between the intake of nutrients and the expenditure of these in the processes of growth, reproduction, and health maintenance (WHO, 2020); consequently, overweight and obesity is caused by an energy imbalance between calories consumed and calories spent. In this context, overweight/obesity, considered a public health problem, is very important, especially in children, as this population is in a stage of growth and development and can acquire comorbidities early (Craig et al., 2016).

The characteristics of contemporary societies and family lifestyle behaviors play a major role in the pattern of food intake, dietary behavior and nutritional status of the children, in addition to the genetic influence (Dubois et al., 2007; Hetherington and Boyland 2007). Appetite, pleasure in eating, sensitivity to external factors associated with food (sensory stimuli, such as taste and aroma) and emotional aspects can also influence nutrition, food selection and eating style and may be related to obesity (Wardle et al., 2001; Pan et al., 2017; Tepper and Barbarossa, 2020). Moreover, family lifestyle behaviors can shape children's nutritional status (Dubois et al., 2007; Litchford et al., 2020; Pesch et al., 2020) and depend on the characteristics of promoting or preventing health nutritional habits (Madden et al., 2017; Keller et al., 2019). It has been also considered that overweight and obesity may have an inverse relationship with low levels of income and parental education in young children (Manohar et al., 2019).

It has been considered that nutrition and oral health may be associated with many interrelated factors. According to Gondivkar et al. (2019), poor oral health can influence an individual's dietary intake, compromising nutrition. Thus, identifying and managing oral morphologic and functional oral conditions and issues related to nutrition are important for improving health (Gondivkar et al., 2019).

In this sense, is important to highlight that the excess of carbohydrate consumption is a risk factor for both obesity and dental caries (Manohar et al., 2019). A recent systematic review noted that overweight and obese children have a greater experience of dental caries compared to children with normal weight (Manohar et al., 2019), but this is a controversial issue in the literature. It is noteworthy that dental caries, tooth lost, deficient restorations and other impaired morphologic and functional oral conditions, such as malocclusions, chewing, and swallowing, can influence oral food processing. For example, impaired chewing can generate large chewed particles, which can have an impact on the digestion process (Papas et al., 1998; Budtz-

Jorgensen et al., 2000). Impaired oral conditions can also influence eating behavior, that is, the individual can choose soft foods that are easier to chew, but calorie-rich and high-fat contents, which in turn can influence nutritional aspects (Nowjack-Raymer and Sheiham, 2007). Swallowing is linked to chewing and consists of a physiological mechanism that allows the transport of boluses from the oral cavity to the stomach, being important for the correct development of oral structures (Farronato et al., 2012). When chronic oral dysfunctions and orofacial myofunctional disorders occur, such as atypical swallowing, they can result in malocclusions (D'Onofrio, 2019), which in turn can cause changes in the development of jaws, can influence behavioral factors in feeding, such as food preferences (Carvalho et al., 2011; Maćkowiak et al., 2016) and can interfere in proper chewing function (Bourdiol et al., 2017). Compromised chewing and swallowing functions, precarious dental oral conditions and malocclusions can have an impact on food intake and consequently on nutritional status (Peyron et al., 2018).

Other oral component that can contribute for nutritional studies is saliva, since biomarkers such as leptin and ghrelin secreted by salivary glands, have aroused interest in their possible link between satiety and appetite, body weight, besides playing an important role in regulating food intake (Klok et al., 2007). Ghrelin, a peptide of 28 amino acids, is mainly produced in the stomach from a group of endocrine cells. Unlike leptin, it stimulates food intake and increases BMI, promoting body weight gain and adiposity, stimulating food intake, reducing energy expenditure and increasing body fat (Miljković et al., 2017). Leptin regulates body weight by modifying energy levels and increasing metabolic rate and decreasing food intake. Most overweight and obese people show resistance to leptin at the recipient level and therefore have higher levels of leptin than overweight individuals (Thanakun et al., 2014).

The mentioned aspects emphasize the needs to understand the role of nutrition for people's health, especially in young children, who are in stage of growth and development. For this, this study aimed to verify the possible influences of eating behavior, socio-environmental aspects, morphological and functional oral characteristics, and salivary biomarkers on the weight status of preschoolers.

Methods

This is an observational, cross-sectional study with a quantitative approach, which was approved by the Research Ethics Committee of Piracicaba Dental School, University of Campinas, protocol number 50586615.4.0000.5418. Pre-school children who attend municipal daycare centers in Piracicaba, São Paulo, Brazil, and their mothers or guardians participated. They were informed about the procedures, possible discomforts or risks and the possible benefits of the study.

To calculate the sample size, data from the Obesity map of ABESO were considered (Brazilian Association for the Study of Obesity and Metabolic Syndrome- ABESO- http://www.abeso.org.br/atitude-saudavel/mapa-obesidade), considering the prevalence of obesity in childhood of 38.8%, in the Southeast Region of Brazil. The prevalence was considered for any outcome of 50%, significance level of 5% and test power of 80%, obtaining the sample size of 118. To this number, 20% was added, in view of the possibility of loss of the sample, totaling a minimum of 142 children.

Data collection

Three daycare centers in the municipality of Piracicaba, São Paulo, Brazil, were randomly selected. The inclusion criteria for sample selection were children of both sexes aged 3 to 5 years, whose parents or guardians agreed to participate, signing the informed consent. The exclusion criteria consisted of children with systemic disorders that could compromise the masticatory system, such as neurological disorders and chronic diseases, and non-cooperative children. Data were collected from June 2017 to June 2019. All examinations took place at daycare centers using daylight.

Anamnesis

The anamnesis was carried out using a pre-structured questionnaire delivered to the parents, verifying prenatal, natal and post-natal medical history, such as the use of medications and presence of underlying pathologies, and childhood diseases. Questions about individual biological characteristics of the children, such as gender, age, self-declared color/race, were addressed. Moreover, height and weight of parents were asked. Socioeconomic factors, parents' education, marital status, employment status and family income were included (Demir et al., 2017). The 2019 version of the Brazil Economic Classification Criterion (CCEB) was used. The economic classes (A, B1, B2, C1, C2, D, E) are defined based on monthly family income,

from US$ 6,217.60 to US$ 175.14). Parents had to answer about monthly family income. Dental history consisted of information about oral hygiene, presence of harmful oral habits, and past dental treatments.

Weight status

Body Mass Index of Children

Weight status was taken into account in accordance with the guidelines of the WHO. The z-score weight-for-length/height indicator, adjusted for age and sex, according to the WHO Child Growth Standards (WHO Multicentre Growth Reference Study Group, 2006) was determined for each child.

Weight was measured to the nearest 0.1 kg using a portable electronic scale. A tape measure was attached to the wall to assess height (m). The BMI was calculated as the body weight (kg) divided by the height squared (m^2). Data on anthropometry were classified by WHO Anthro version 3.2.2.

Body Mass Index of Mothers

Weight (Kg) and height (m) were obtained by self-report. The BMI interpretation was based on weight status categories: Thinness (Below 18.5); Normal (18.5 – 24.9); Overweight (25.0 – 29.9); Obese (30.0 and above), according to the WHO.

Clinical examination

For oral examination, clinical instruments were used, such as dental mirror, gingival probe with round-ended and protective supplies. One calibrated examiner (S.C.C.J.) (gold standard M.B.D.G.) verified the conditions of oral structures and the morphologic occlusion, according to guidelines of World Health Organization (WHO), and oral functions. Kappa test was applied to verify he inter-examiner agreement, showing a value of 0.8.

Oral health assessment

Oral health assessment consisted of clinical examination of dental conditions and determination of decayed, missing and filled primary teeth index (dmft). The severity of dental caries experience was categorized as caries free (dmft = 0), low severity (dmft <6) and high severity (dmft ≥ 6), according to Abanto et al. (2011). For evaluation of gingival condition, the

presence of gingivitis was confirmed by changes in the contour and color of the gums in upper incisors, according to Silness and Loe (1964) and Alaluusua and Malmivirta (1994).

The morphologic characteristics of the occlusion was assessed by direct inspection. The child was required to keep dental arches in maximum habitual intercuspation or in centric relation when indicated. The following aspects were observed (Lopes-Freire et al., 2015):

- Posterior crossbite; The unilateral or bilateral posterior crossbite was considered as the transversal and reverse interrelation of one or more posterior teeth in right or left side or in both.
- Anterior crossbite: The anterior crossbite was considered when maxillary incisors were in lingual position in relation to the mandibular incisors.
- Vertical anterior relationship:
    - Anterior open bite: Open bite was considered when there was no overlap between the upper and lower incisors.
    - Overbite: The vertical distance between the upper and lower central incisor edges was measured. Values greater than 3 mm were considered increased overbite.

Oral functions

Mastication

For evaluation of mastication, a chocolate-flavored stuffed Bono cookie (Nestlé, São Paulo, SP, Brasil) was used. A half part of the cookie was previously prepared, and the child was instructed to put it inside the mouth and to chew in his/her habitual manner, following an adapted protocol of de Felício et al. (2010). After swallowing, the occlusal surfaces were observed for determination of unilateral or bilateral mastication side (Siqueira, 2016). If a side retained greater number of chewed particles over the occlusal surfaces than the other side, the mastication was determined as unilateral. If the amount of retained chewed particles were the same for both sides, the mastication was considered bilateral.

After this task, the child brushed his/her teeth. The child's familiarity with the cookie was guaranteed as well as absence of allergic manifestation.

Swallowing

For evaluation swallowing, a cup of 200 ml containing 100 ml of water was offered for the child, who was instructed to drink some portion without swallowing. Immediately, the examiner discreetly lowered the child's lower lip and asked the swallow in a slowly manner. The effort of the perioral muscles and the position of the tongue at the time of swallowing were

observed. The test was repeated 3 times. The swallowing was classified as atypical or adapted when malocclusion was present.

CEBQ application

The CEBQ is a questionnaire developed specifically to investigate eating behavior in children and young people, through the answers provided by their caregivers (Wardle et al., 2001). The CEBQ was translated for Portuguese (Portugal) language by Viana et al. (2008) and adapted for Portuguese (Brazilian) language by our research group. The instrument consists of 35 items composed of eight domains, defined as follows (Wardle et al., 2001; Demir et al., 2017) (Attachment nº 3):

(1) Food responsiveness: measures whether children consume more than normal under the effects of external stimuli (taste, smell, appearance, etc.) or because of eating cues.
(2) Enjoyment of food: includes hunger, desire to eat, and taking pleasure in eating. It indicates a general interest in all foods.
(3) Emotional over-eating: defined as an increase in eating in response to negative feelings such as anger and anxiety.
(4) Desire to drink: includes the increased desire for sweetened beverages.
(5) Satiety responsiveness: represents children's ability to reduce food intake after eating. It provides the child with cues on the more sensitive internal response to satiety.
(6) Food fussiness: when a child's refusal to eat significant amounts of foods generally served by parents, and to consume an insufficient amount of certain foods.
(7) Emotional under-eating; defined as a decrease in eating in response to negative feelings such as anger and anxiety.
(8) Slowness in eating: indicates a substantially low interest in eating.

Five items were reversed, one in “Satiety responsiveness” domain, one in “Slowness in eating” domain, and three in “Food fussiness” domain.
The CEBQ was delivered to the parents and returned to the researcher on the day of the clinical examination.

Saliva collection - Leptin and Ghrelin Biomarkers

To quantify salivary biomarkers, half of the total sample was selected (n = 82, 42 boys and 40 girls). Overweight/obese children (n=29) were first selected by convenience and the others were randomized.

The saliva collection was managed by the researcher in the day of the clinical examination. All samples were collected at the daycare center. Stimulated saliva was collected using coded salivettes (Salivette®, Sarstedt, Germany) containing a cotton roll, which was placed on the tongue, was kept and moved inside the child's mouth for one to two minutes, until it was soaked with saliva. After that, the roll was inserted into the salivette and immediately stored on ice to be transported to the laboratory. Saliva collections were carried out in the morning and in the afternoon, with an hour interval between the last meal and the collection. The child could not have brushed his/her teeth before collection. In the laboratory, the salivettes were centrifuged at 3000 rpm for 15 minutes and the debris was discarded, only the supernatant was used, in 2 microcentrifuge tube, one for leptin and one for ghrelin. The samples were stored at -80 ° C, until the quantification of the biomarkers was performed. For the dosages of the biomarkers, the samples were dosed in duplicates, in such a way that the samples from the same individual were dosed in the same test. On the day of the test, the samples were defrosted in the refrigerator and centrifuged again, at 3000 rpm for 10 minutes. Saliva was measured using Elisa kits, Human LEP(Leptin) ELISA Kit and Human GHR(Ghrelin) ELISA Kit (Elabscience®), according to the manufacturer's instructions. The results were expressed in pg/mL.

Statistical analysis

Descriptive statistics, consisting of frequencies, means, standard deviations, medians, quartiles and ranges were considered. The D'Agostino-Pearson test was applied to verify the distribution of the data in order to direct the parametric or non-parametric tests. After that, Kruskall-Wallis, Mann-Whitney tests, and Spearman correlations were carried out. Logistic regression models were built, considering the dependent variable the weight status. The independent variables were oral characteristics (caries, caries-free = 0, caries = 1; early tooth loss, without loss = 0, early loss = 1; total dmft; open bite, without = 0, with = 1; posterior crossbite, without = 0, with = 1; anterior crossbite, without = 0, with = 1; gingivitis, absent = 0, present = 1; overbite, up to 3mm = 0, increased overbite = 1), oral functions (Mastication, unilateral = 0, bilateral = 1; swallowing, normal = 0, atypical or adapted swallowing = 1). First, a univariate logistic regression was applied and the variables with $P<0.25$ were inserted in the multiple model. The significance level was considered as $\alpha = 0.05$.

Results

The results in Table 1 about socio-demographic characteristics show that 21% of the children were overweight/obesity. The mothers' level of education showed that 9% had no education, while 46% had a high school education.

Table 1. Socio-demographic characteristics according to children sex

		Male n (%)	Female n (%)	Total n (%)
Weight status (children)	Thinness	5 (6)	8 (10)	13 (8)
	Normal	60 (72)	53 (69)	113 (71)
	Overweight	7 (8)	11 (14)	18 (11)
	Obesity	11 (13)	5 (6)	16 (10)
	Total	83 (52)	77 (48)	160 (100)
Weight status Mother [n (%)]	Thinness	1 (1)	1 (1)	2 (1)
	Normal	26 (31)	30 (39)	56 (35)
	Overweight	31 (37)	22 (29)	53 (33)
	Obesity	25 (30)	24 (31)	49 (31)
Mother's level of education [n (%)]	No schooling	9 (11)	5 (6)	14 (9)
	Basic education	28 (34)	21 (27)	49 (31)
	High school	31 (37)	43 (56)	74 (46)
	University	15 (18)	8 (10)	23 (14)
Social Economic Stratum [n (%)] Mean±SD (US$)	DE	10 (12)	11 (14)	21 (13)
		85.72±32.80	97.83±49.74	91.78±11.98
	C2	37 (45)	32 (42)	69 (43)
		294.54±60.73	277.31±57.31	285.93±2.42
	C1	29 (35)	26 (34)	55 (34)
		564.61±112.01	559.14±98.08	561.88±9.85
	B2	7 (8)	8 (10)	15 (9)
		1.268.68±552.41	961.07±130.70	1.114.88±298.19

B2 = up to US$ 2.744.31; C1 = up to US$ 750.73; C2 = up to US$ 425.45; DE = up to US$ 175.14 (monthly)

The descriptive data of the domain scores according to the weight status of the CEBQ and its internal reliability are demonstrated in Table 2. There were no significant statistical differences among the three weight status (ANOVA one way or Kruskal-Wallis test when indicated, $P>0.05$). The internal reliability of the CEBQ was considered good, since Cronbach's

alpha was greater than 0.70. For the domains, the respective values ranged from 0.57 to 0.78, demonstrating moderate to good internal reliability.

Table 2 - Descriptive data of CEBQ domains according to weight status and internal reliability of the CEBQ

		Thinness N= 13	Normal N= 113	Overweight/Obesity N= 34	*P*-value	Cronbach's alpha
CEBQ total						0.71
Enjoyment of food (EF)	Mean ± SD	4.02 ± 0.23	3.62 ± 0.16	3.93 ± 0.33	0.1	0.78
	Median	5	4	4		
	25% - 75%	2.75 - 5.00	2.75 - 5.00	2.75 - 5.00		
Food responsiveness (FR)	Mean ± SD	2.74 ± 0.09	2.24 ± 0.13	2.99 ± 0.15	0.57	0.78
	Median	2	3	3		
	25% - 75%	1.00 - 3.00	1.00 - 5.00	1.06 - 5.00		
Satiety responsiveness (SR)	Mean ± SD	2.72 ± 0.23	2.98 ± 0.01	2.68 ± 0.17	0.21	0.71
	Median	3	3	3		
	25% - 75%	2.00 - 3.00	2.00 - 4.00	1.25 - 3.75		
Slowness in eating (SE)	Mean ± SD	3.00 ± 0.17	3.19 ± 0.08	2.92 ± 0.16	0.41	0.57
	Median	3	3	3		
	25% - 75%	1.75 - 4.00	1.75 - 5.00	1.00 - 4.25		
Food fussiness (FF)	Mean ± SD	2.94 ± 0.20	2.78 ± 0.06	2.70 ± 0.19	0.74	0.73
	Median	3	3	3		
	25% - 75%	2.00 - 4.00	1.00 - 4.00	1.06 - 3.56		
Emotional over-eating (EOE)	Mean ± SD	1.83 ± 0.30	1.86 ± 0.11	2.18 ± 0.11	0.1	0.65
	Median	1	1	1		
	25% - 75%	1.00 - 2.25	1.00 - 3.00	1.00 - 3.00		
Emotional under-eating (EUE)	Mean ± SD	2.35 ± 0.24	2.44 ± 0.21	2.47 ± 0.22	0.9	0.6
	Median	2.5	2.5	3		
	25% - 75%	1.00 - 3.25	1.00 - 3.50	1.00 - 4.00		
Desire to drink (DD)	Mean ± SD	3.72 ± 0.38	3.38 ± 0.19	3.34 ± 0.21	0.55	0.79
	Median	4	3	3		
	25% - 75%	2.50 - 5.00	2.00 - 5.00	2.00 - 4.81		

SD - Standard deviation

In Table 3, the children's oral condition and functions and occlusal characteristics are demonstrated. There were no significant differences between thinness, normal and overweigh/obesity for each variable (Fisher exact or chi-squared when indicates (P>0.05). The average dmft in preschoolers was 1.2. Thirty-three percent of the children had at least one decayed tooth, differing significantly of those with filling and missing teeth (P<0.05). Gingivitis was present in 19% of variable. Bilateral chewing was more prevalent (P<0.05). The proportion of caries free children was significantly higher than children with caries (P<0.05), but no differences was found between the degrees of severity (P>0.05). The proportion of children with normal gums was higher than those with gingivitis (P<0.05). Bilateral mastication was significantly predominant, as well as normal swallowing (P<0.05). Crossbite was present in 14% of the samples. The open bite was present in 33% of the children.

Table 3 – Oral conditions and occlusal characteristics according to weight status

	Thinness (N=13)	Normal (N=113)	Overweight/ Obesity (N=34)	Total (N=160)
dmft (mean±SD)	1.00±2.00[a]	1.27±2.48[a]	1.03±1.87[a]	1.2±2.32
Decayed teeth [n (%)]	4 (31)	38 (34)	10 (29)	52 (33)[A]
Missing teeth [n (%)]	-	4 (4)	-	4 (3)[B]
Filling teeth [n (%)]	3 (23)[a]	8 (7)[b]	2 (6)[b]	13 (8)[B]
Caries Free [n (%)]	8 (62)	74 (65)	23 (68)	105 (66)[A]
dmft Low severity [n (%)]	4 (31)	33 (29)	10 (29)	47 (29)[B]
dmft High severity [n (%)]	1 (8)	6 (5)	1 (3)	8 (5)[C]
Gingivitis				
Presence	2 (15)	24 (21)	5 (15)	31 (19)[A]
Absence	11 (85)	89 (79)	29 (85)	129 (81)[B]
Mastication				
Unilateral right [n (%)]	1 (8)	8 (7)	1 (3)	10 (6)[A]
Unilateral left [n (%)]	1 (8)	13 (12)	1 (3)	15 (9)[A]
Bilateral [n (%)]	11 (85)	92 (81)	32 (94)	135 (84)[B]
Swallowing				
Atypical [n (%)]	7 (54)	35 (31)	9 (26)	51 (32)[A]
Adapted [n (%)]	1 (8)	20 (18)	8 (24)	29 (18)[A]
Normal [n (%)]	5 (38)	53 (47)	17 (50)	75 (46)[B]
Buccolingual molar relationship				
Normal Right	11 (85)	106 (94)	30 (88)	147(92)[A]
Normal Left	11 (85)	108 (96)	32 (94)	151 (94)[A]
Cross Right	2 (15)	7 (6)	4 (12)	13 (8)[B]
Cross Left	2 (15)	5 (4)	2 (6)	9 (6)[B]
Incisor relationship				
Normal	7 (54)	54 (48)	14 (41)	75 (59)[A]
Open bite	5 (38)	33 (29)	15 (44)	53 (33)[B]
Cross bite	1 (8)	2 (2)	-	3 (2)[C]
End-to-End bite	-	7 (6)	2 (6)	9 (6)[C]
Deep Overbite	-	17 (15)	3 (9)	20 (13)[C]

Different capital letters mean statistical differences among the characteristics for each variable
Different small letters mean statistical differences by weight status; dmft – decayed, missing, filled tooth

Table 4 shows the significant Spearman's correlations between children's weight status and CEBQ domains scores, mother's weight condition, dmft and age. There was a positive correlation between children's BMI and the 'Enjoyment of Food', 'Emotional over-eating' domains and between the children's BMI, the mother' BMI and age. There was a negative correlation between children's BMI and the 'Satiety responsiveness' domain.

Table 4. Spearman's correlation between children's weight status and CEBQ domains, mother's weight status, dmft and children age

		r	*P*-value
BMI	Enjoyment of food	0.18	0.021
	Food responsiveness	0.09	0.265
	Satiety responsiveness	-0.21	0.008
	Slowness in eating	-0.13	0.114
	Food fussiness	-0.01	0.915
	Emotional over-eating	0.16	0.038
	Emotional under-eating	0.09	0.274
	Desire to drink	-0.04	0.630
	BMI mother	0.21	0.007
	dmft	-0.10	0.202
	Age	0.40	<0.001

r – Spearman's coefficient

BMI – Body Mass Index

The results of univariate and multiple logistic regression models, with the dependent variable being the children's weight status, are demonstrated in Table 5. Oral conditions and functions, occlusal characteristics, mother's weight status and maternal education were the independent variables. Only those variables with *P*-value less than 0.25 found in univariate logistic regression were inserted into the multiple regression model. It was observed that oral conditions and functions, occlusal characteristics and maternal education did not influence the

children's weight status. On the other hand, the mothers' weight status was positively associated with the children's weight status. These findings mean that the chance of a child being overweight/obese when the mother is overweight/obese is about three times.

Table 5 - Univariate and multiple logistic regression models considering children's weight as dependent variable

Univariate logistic regression					
Dependent variable: Weight status					
	Independent variables	Coefficient	*P*-value	OR	IC 95%
Oral conditions	Decayed tooth	-0.18	0.66	0.83	0.37-1.90
	Total dmft	-0.04	0.63	0.96	0.8-1.14
	Gingivitis	-0.41	0.44	0.66	0.23-1.88
Occlusal characteristics	Posterior crossbite	0.32	0.56	1.38	0.46-4.14
	Anterior crossbite	-0.32	0.69	0.73	0.15-3.48
	Open bite	0.60	0.12	1.83	0.84-3.97
	Overbite	-0.36	0.50	0.70	0.24-1.98
Oral functions	Mastication	1.27	0.09	3.57	0.8-15.99
	Swallowing	0.00	1.00	1.00	0.47-2.13
	Mother's level of education	0.38	0.34	1.47	0.66-3.27
	Mother's weight status	0.96	0.03	2.62	1.06-6.48
Multiple logistic regression					
Dependent variable: Weight status				*(Model chi-square – 10.95 P=0.01)*	
	Independent variables	Coefficient	*P*-value	OR	IC 95%
	Mastication	1.42	0.06	4.13	0.91-18.87
	Open bite	0.48	0.24	1.61	0.72-3.58
	Mother's weight status	1.01	0.03	2.75	1.09-6.91

dmft – decayed, missing, filled tooth OR – Odds ratio

Table 6 shows the descriptive analysis of the dosages of the salivary leptin and ghrelin in the subsample, according to the weight status. Overweight / obese children had significantly lower ghrelin values than children with normal weight. For leptin, there was no significant difference between groups.

Table 6. Median, first quartile, third quartile, mean and standard deviation of the dosages of the salivary leptin and ghrelin in the subsample according to weight status

	Leptin (pg/mL)		Ghrelin (pg/mL)	
	Normal weight (n=53)	Overweight/Obesity (n=29)	Normal weight (n=53)	Overweight/Obesity (n=29)
Mean (SD)	0.37 (0.37)	0.23 (0.29)	2.1 (0.46)*	1.82 (0.48)*
Median	0.22	0.09	2.21	1.76
First Quartile (25%)	0.08	0.07	1.79	1.46
Third Quartile (75%)	0.53	0.24	2.51	1.92
Range	0.05-1.46	0.05-1.01	0.77-3.13	1.00-2.81

* Mann-Whitney $P < 0.05$

SD – Standard deviation

Significant correlations were observed between leptin and ghrelin levels, which in turn correlated negatively with BMI. In addition, ghrelin levels were negatively correlated with the domain of the CEBQ "Food enjoyment". The other domains did not correlate significantly with salivary biomarkers (Table 7).

Table 7. Spearman correlations of leptin and ghrelin dosages with BMI and CEBQ domains in the subsample (n=82)

	Leptin		Ghrelin	
	r	*P-value*	r	*P-value*
Ghrelin	0.69	<0.001	-	-
BMI	-0.24	0.027	-0.30	0.007
CEBQ domains				
Enjoyment of food	-0.17	0.122	-0.30	0.007
Food responsiveness	-0.12	0.292	-0.20	0.069
Satiety responsiveness	0.03	0.797	0.06	0.619
Slowness in eating	0.18	0.111	0.16	0.144
Food fussiness	-0.01	0.898	-0.09	0.436
Emotional over-eating	0.06	0.569	0.04	0.747
Emotional under-eating	0.11	0.347	0.12	0.302
Desire to drink	-0.08	0.488	-0.20	0.074

r – Spearman's coefficient
BMI – body mass index
CEBQ – children eating behavior questionnaire

Discussion

This study was developed to verify some factors involved in the eating behavior of young children that can influence weight status, taking into account the occlusal characteristics, oral conditions and functions and the possible role of salivary leptin and ghrelin. Weight status is a complex condition with multiple factors involved, mainly overweight/obesity that has been considered a serious global public health problem (Swinburn et al., 2011; WHO, 2020), which turns important the studies in this field targeting young children to understand the mechanism, the factors involved and provide further preventive strategies (Craig et al. 2016).

In this study, it was observed a frequency of 21% of children with overweight/obesity. In such a young population, this value is considered of extreme importance and it agrees with

Kumar et al. (2017) who verified a prevalence about 22% of overweight or obesity in preschool children in USA. Nevertheless, there are a great variability in prevalence of overweight/obesity according to age and measurement parameters adopted (Jesus et al., 2010; Moreira et al., 2012; Müller et al., 2014).

There is considerable evidence that weight status, both for children and adults, can be affected by the socioeconomic factor, since obesity tends to be inversely proportional to the socioeconomic level (McLaren, 2007; Chung et al., 2016). In the present study most of the children were from families of low to medium income and the frequency of overweight and obesity was great and similar with preceding studies (Saldiva et al., 2004; Naghettini et al., 2010; Manios et al., 2019). Previously, overweight and obesity were considered a problem that mainly affected developed high-income countries, but today their impact is evident in low- and middle-income countries (LMIC), particularly in urban environments (Swinburn et al., 2011), as noted in Brazil.

Furthermore, maternal education has an impact on children's weight status, once dietary decisions are made by the primary caregiver, usually the mother, thereby generating a more significant impact on childhood obesity (Madden et al., 2017). Most mothers of the sample surveyed had primary and secondary education, guarantying sample homogeneity. In this context, it is worth mentioning that the weight status can be determined by a combination of factors at the individual, family or community level (Reilly, 2005; Swinburn et al., 2011).

Due to the increasing prevalence of childhood obesity, there is a growing interest in researching children's eating behaviors. Thus, the CEBQ instrument was created to investigate the eating behaviors for determining possible factor implicated in weight status. In the present research, the internal consistency of the instrument measured by Cronbach's alpha was 0.71, demonstrating a substantial internal reliability, agreeing with previous studies (Viana and Sinde, 2008; Kumar et al., 2017), thus showing the reliability of the respective findings in the present study.

One unexpected result was the lack of difference of the CEBQ domains among the different weight status. The domains food responsiveness, enjoyment of food, and emotional overeating indicate having an appetite and the tendency to eat (Wardle et al., 2001; Demir et al., 2017), so greater values were expected for overweight and obese children, as found by Demir et al., 2017. However, a positive correlation was observed between BMI and the domain ‘Enjoyment of food’; in addition, ‘Emotional over-eating” was also positively correlated with BMI, inferring more appetite and feeding in heavier children. These findings corroborate previous studies (Wardle et al., 2001; Viana and Sinde, 2008; Demir et al., 2017), who considered that values of the domain

‘Emotional over-eating’ increases in functions of weight groups, confirmed by the fact that children of low weight decrease the food ingestion due to emotional stress, but also depends on maternal feeding practices (Ainuki and Akamatsu, 2011). In line with the last consideration, the domain ‘Emotional under-eating’ did not relate with the weight status, maybe due to the low frequency of thinness children in the sample. Wardle et al. (2001) considered that ‘Emotional under-eating’ can decrease with age and could be a development pattern. However, Carnell and Wardle (2008) and Jansen et al. (2012) found that ‘Emotional over-eating’ did not significantly affect children's weight status. The controversial results may be due to differences in eating habits of different countries.

The scores on the ‘Satiety responsiveness’ and ‘Food fussiness’ indicate lack of appetite, so it was expected smaller values for overweight and obese children. However, BMI was negatively related to the ‘Satiety responsiveness’ domain and age, inferring that obese and older children need more time to reach the satiety, consequently eating more, as previously reported (do Passos et al., 2015; Demir et al., 2017). The comparisons of other CEBQ domains among the children with different weight status did not demonstrate significant differences or correlations. Possibly, the similar values on comparing CEBQ domains in the present study may be attributed to number of children in each category. Besides, the significant coefficient correlations can be considered weak, but is a common finding in qualitative data.

Considering that the digestive process starts in the mouth, it is assumed that the characteristics of the masticatory system and its function can influence people's weight status, especially in children. In this context, one of oral conditions of our study was analyzed by dmft, an index recommended by WHO. The average of dmft was 1.2, with 44% of children presenting caries experience. A 10-year follow-up survey in the State of São Paulo showed a decrease of dmft value from 1.88 to 0.99 (Garbin et al., 2019). In the last national oral health survey, the average dmft was 2.43 (SBBrazil, 2012). In addition to 66% of the children being free of caries, the dmft values of the present sample can be considered low, and the high severity was observed in only eight children. Furthermore, gingivitis was present in 19% of children. Despite the low frequency, these findings are relevant to the general context of oral health, considering the age of the sample studied.

The mastication is the most likely mechanism by which compromised oral health can affect food intake (Papas et al., 1989). Bilateral mastication is required to allow a proper growth and development of maxilla and mandible (Balcioglu et al., 2009; Zou et al., 2018). Eighty-five percent of the children in the present study had bilateral chewing, according to the method used.

Despite this good result, unilateral mastication cannot be neglected due to the harmful consequences for the stomatognathic system (Farronato et al., 2012). Moreover, the side of the mastication observed in this study cannot be considered as preferred, because the number of chewing cycles was not quantified, thus the results should be interpreted with caution.

Swallowing is a progressive physiological mechanism that allows the transport of boluses, saliva and fluids from the oral cavity to the stomach (Farronato et al., 2012), being important for an adequate development of oral structures and functions. Adapted or atypical swallowing was found in more than half of the sample, which can be attributed to several factors, such as non-nutritive sucking habits, malocclusion, mouth breathing and other problems related to the upper respiratory tract (Bertolini et al., 2001; Maspero et al., 2014). The age of the sample may have been determinant for the high frequency of adapted or atypical swallowing found, due to the non-nutritive sucking habits common in preschoolers. Atypical swallowing needs to be corrected early so that, together with other harmful oral habits in childhood, it cannot compromise dental-skeletal development (Farronato et al., 2012; Maspero et al., 2014). Consequently, the assessment of oral habits must be taken into account to support the findings, which will serve as a basis for our further studies.

Since the characteristics of the occlusion can be related to the chewing pattern, inferring that chewing behavior can be also affected, those characteristics were checked in the present study. The frequency of posterior crossbite was low, agreeing with da Silva-Filho et al. (2007) but lower than found by Bauman et al. (2018). The divergence may be due to different geographic regions where the studies were carried out, sample size and diagnostic criteria, in addition to the lack of a universally accepted index (Lopes-Freire et al., 2015). The factors mentioned imply the interaction of genetic and environmental factors (Perillo et al., 2015). In relation to open bite, about one third of the sample presented this type of malocclusion that can be explained by the sample age, as commented above. That value was slightly higher than that found by Corrêa-Faria et al. (2014), who verified bottle feeding and harmful oral habits as determinants of malocclusion in young children. Nonetheless, at present, there is no consensus related to the association between feeding habits and malocclusion (Lopes-Freire et al., 2015). In this context, an association about harmful oral habits and malocclusion in preschoolers should be provided as carried out by others and can be considered as a limitation of the current study.

As mentioned already, oral conditions and occlusion can cause changes in the development of the jaws, which can result in functional, aesthetic and psychosocial implication, in addition to being related to anthropometric deficits, which in turn, can affect weight status

(Thomaz and Valença, 2009). Thus, we verified whether the weight status of the sample could be affected by altered oral characteristics and functions. Besides, maternal weight status deserve attention regarding the nutritional conditions of children. Thus, it was observed that mastication, open bite and mother's weight status could be factors individually associated to children's weight status. When those factors were analyzed together, only mother's weight status were associated with nutritional condition of the sample. The main factor was the mother's weight status, since children of mothers with higher weights were about three times more likely to have the same condition. In this way, we agree with Reilly (2005) and Swinburn et al. (2011) about the influence of individual and household factors on children weight status.

Leptin levels in normal weight and obese children of the present sample were similar. This is a controversial result, since some researches have demonstrated high levels of leptin in obese individuals (Goodson et al., 2014; Flank et al. 2016) and BMI (Rodrigues et al., 2017). Nevertheless, the similar leptin levels found are in line with Pîrsean et al. (2019) who considered that salivary leptin level was highly variable in case of obese children, with similar levels between some obese children and control ones. In this way, leptin resistance is variable among obese individuals and may depend on the nutrients consumed from the diet (e.g., high-fat foods and omega-3 supplementation) (Sáinz et al., 2015) and cannot be correlated with Metabolic Syndrome (Thanakun et al. 2014). The lack of difference in the present study corroborates the fact that leptin was not correlated with CEBQ domains, inferring that the chewing behavior was not a determinant factor for this variable. The negative significant correlation of leptin with BMI generated doubt, despite the weak coefficient. Maybe the sample size and the children age may be influencing factors, summed to the fact that children were from a general population, i.e., not looking for a treatment and not diagnosed with metabolic syndrome. Those findings require further analysis and evaluation about diet and its nutrients (Sáinz et al. 2015) and other determinants of metabolic syndrome, as well.

As expected, the ghrelin values were significantly lower for overweight/obese children than normal weight ones, corroborating previous studies, which verified the decrease of ghrelin levels due to increased caloric intake in patients with obesity (Nakazato et al. 2001; Miljković et al., 2017). Moreover, a significant inverse relation between BMI and ghrelin levels was found. This finding could suggest the trend for disturbed appetite regulation in early ages, as verified by Önnerfält et al. (2018) by quantification of serum fasting ghrelin levels. They found also a significant inverse relation between body mass index and fasting ghrelin levels, similar to our results. In addition, the domain of CEBQ 'Enjoyment of food' was negatively correlated with

salivary ghrelin levels, reinforcing the differences in the respective values between children with normal weight and overweight/obese ones. This dimension of the CEBQ highlights the "food approach" (Carnell and Wardle 2008), including hunger, desire to eat, and taking pleasure in eating. It indicates a general interest in all foods (Demir et al. 2017) justifying the found results.

The importance of all the variables analyzed is based on the fact that the nutritional aspect in young children is considered an indirect risk factor for further comorbidities, especially in adolescence, such as cardiometabolic risk via several pathways that involved BMI and appetite hormones markers of inflammation, and insulin resistance (Martinez et al., 2019).

Concluding, the aspects of eating related to have a large appetite and the tendency to eat more, the environmental aspects, such as maternal nutritional condition, had an influence on the weight status of the studied children. The oral conditions, occlusion characteristics, oral chewing and swallowing functions, had no influence on the weight status in the sample. Higher values of ghrelin for overweight/obese children and its inverse relationship with BMI and with the domain 'Enjoyment of food' can infer the tendency for disturbed appetite regulation in preschoolers.

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3 CONCLUSÃO

Pode-se concluir que:

- Os aspectos da alimentação relacionados ao apetite e a tendência a comer mais, os aspectos ambientais, como a condição nutricional materna, influenciaram o peso das crianças estudadas.
- As condições bucais, características de oclusão, funções de mastigação e deglutição bucais não influenciaram o estado nutricional das crianças avaliadas.
- Valores significativamente mais altos de grelina encontrados em crianças com sobrepeso/obesidade, a relação inversa desse hormônio com o IMC e com o domínio do CEBQ “Prazer em comer” podem inferir em tendência à regulação alterada do apetite em pré-escolares.

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Anexo 1 - Relatório de Verificação de Originalidade e Prevenção Plágio

Avaliação do comportamento alimentar, aspectos nutricionais, condições bucais e biomarcadores salivares na primeira infância

RELATÓRIO DE ORIGINALIDADE

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Anexo 2 – Comprovante de submissão do artigo

Manuscript Details

Manuscript number	APPETITE_2020_252
Title	Weight status and its relationship with food behavior, oral characteristics and functions, salivary leptin and ghrelin in preschoolers
Article type	Full Length Article

Abstract

The aim was to assess the eating behavior, oral conditions, quantify salivary biomarkers, leptin and ghrelin and relate them to the weight status in early childhood. A total of 160 children from 3 to 5 years and their guardians were selected. The eating behavior was evaluated using the Children's Eating Behavior Questionnaire (CEBQ). The weight status was determined by the Body Mass Index (BMI) and the reference values using growth curves. Mothers' BMI was also determined. The dmft index, dental occlusion, mastication and swallowing were assessed. Salivary leptin and ghrelin levels were quantified using stimulated saliva and the ELISA method. Descriptive statistics, comparison tests, correlations, ANOVA, and regression models were applied and significance level set at 5%. Regarding weight status, 21% of children and 64% of mothers were overweight/obese. There was no statistical difference between domain scores of the CEBQ questionnaire among different weight status, but BMI was correlated with the domains 'Enjoyment of food', 'Satiety responsiveness', mothers' BMI and children's age. In the multivariate logistic regression model, the variable that influenced the condition of the children's weight was the mother's weight status (OR=2.75). Oral conditions and functions were not influencing factors for weight status. Overweight/obese children presented significant higher levels of salivary ghrelin than children with normal weight; salivary leptin correlated significantly with salivary ghrelin levels. Both correlated negatively with BMI and ghrelin showed an inverse relationship with 'Enjoyement of food'. Aspects of food related to appetite and the tendency to eat more and maternal nutritional condition, influenced the children's weight status. Oral conditions, occlusion characteristics, and chewing and swallowing had no influence. Salivary leptin and ghrelin can be useful in assessing weight status.

Keywords	Feeding Behavior; Body Mass Index; Ghrelin; Leptin; Saliva; Infant Nutrition
Taxonomy	Behavioral Nutrition, Eating Behavior, Social Influences on Appetite
Manuscript category	Nutrition
Corresponding Author	Maria Beatriz Gaviao
Corresponding Author's Institution	University of Campinas
Order of Authors	Samuel de Carvalho Chaves-Junior, Karina Guedes Sousa, Gabriela Novo Borghi, Maria Beatriz Gaviao
Suggested reviewers	Moira Dean, Esther Papies, Charlotte Hardman

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Abstract 26-02-2020.docx [Abstract]

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Anexo 3 – Parecer Comitê de Ética em Pesquisa

PARECER CONSUBSTANCIADO DO CEP

DADOS DA EMENDA

Título da Pesquisa: Comportamento alimentar, aspectos nutricionais, biomarcadores salivares e condições bucais na primeira infância

Pesquisador: Samuel de Carvalho Chaves Junior

Área Temática:

Versão: 6

CAAE: 50586615.4.0000.5418

Instituição Proponente: Faculdade de Odontologia de Piracicaba - Unicamp

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 2.138.473

Situação do Parecer:

Aprovado

Necessita Apreciação da CONEP:

Não

PIRACICABA, 26 de Junho de 2017

Anexo 4 - *Children's Eating Behavior Questionnaire* (CEBQ)

Questionário do Comportamento Alimentar de Crianças (CEBQ)

Faculdade de Ciências da Nutrição e Alimentação - Universidade do Porto

Este questionário deve ser respondido apenas pela mãe e incide sobre o comportamento alimentar do seu filho(a).
Responda por favor tendo em conta aquilo que o seu filho(a) faz habitualmente com respeito à sua alimentação. As respostas, quaisquer que sejam, são sempre adequadas uma vez que traduzem um modo pessoal de agir.
Assinale nos quadrados respectivos tendo em conta o caso particular do seu filho(a).

A Criança

Nome da criança.....
Sexo:

M	F

Data de Nascimento:……../……/……..
Escolaridade:……

Data de hoje:……/……../……

Peso:…… Estatura:……..... IMC:……

A Mãe

Data de Nascimento:……../……/……..
Escolaridade:……

Profissão:…….....……

Peso:…… Estatura:……..... IMC:……

	Nunca	Quase nunca	As vezes	Muitas vezes	Sempre
1.O meu filho(a) adora comida?					
2.O meu filho(a) come mais quando está preocupado(a)?					
3.O meu filho(a) tem um grande apetite?					
4.O meu filho(a) termina as refeições muito rapidamente?					
5.O meu filho(a) se interessa por comida?					
6.O meu filho está sempre pedindo algo para beber (refrigerantes ou sucos)?					
7.O meu filho(a) recusa novos alimentos?					
8.O meu filho(a) come devagar?					
9.O meu filho(a) come menos quando está irritado(a)?					
10.O meu filho(a) gosta de experimentar novos alimentos?					
11.O meu filho(a) come menos quando está cansado(a)?					
12. O meu filho(a) sempre pede comida?					
13.O meu filho(a) come mais quando está chateado(a)?					
14.Se eu permitisse o meu filho(a) comeria mais?					
15.O meu filho(a) come mais quando está ansioso(a)?					
16. O meu filho(a) gosta de vários tipos de alimento?					
17. O meu filho(a) deixa comida no prato nos finais das refeições?					
18.O meu filho(a) gasta mais de 30 minutos para terminar uma refeição?					
19. Se tivesse oportunidade o meu filho(a) passaria a maior parte do tempo comendo?					
20. O meu filho(a) está sempre esperando chegar a hora das refeições?					
21. O meu filho(a) fica cheio(a) antes de terminar as refeições?					
22. Meu filho(a) adora comer?					
23. O meu filho(a) come mais quando está feliz?					
24. O meu filho(a) dificilmente se contenta com as refeições?					
25.O meu filho(a) come menos quando está perturbado(a)?					
26. O meu filho fica cheio(a) muito facilmente?					
27.O meu filho(a) come mais quando não tem nada para fazer?					
28. Mesmo quando está cheio(a) o meu filho(a) arranja espaço para comer o seu alimento preferido?					
29. Se tiver oportunidade meu filho passaria o dia tomando alguma bebida (refrigerante e sucos)?					
30. O meu filho(a) não consegue comer a refeição se já tiver comido algo antes?					
31. Se tiver oportunidade o meu filho(a) está sempre bebendo algo (refrigerante ou suco)?					
32. O meu filho(a) gosta de experimentar alimentos que nunca provou antes?					
33. O meu filho(a) decide que não gosta de um alimento mesmo que nunca tenha provado antes?					
34. Se tiver oportunidade meu filho(a) estaria sempre com comida na boca?					
35. O meu filho(a) come cada vez mais devagar ao longo da refeição?					