Journal of Morphological Sciences
J Morphol Sci
Brazilian Society of Anatomy

Original Article
J Morphol Sci 2022;39:37-41

Ostarine Avoids Masseter Atrophy Caused by Changes in the Diet Consistency

João Vitor Tadashi Cosin Shindo[1], Júlia Pedrozo Bellini[1], Cláudia Cristina Biguetti[2] Maira Couto[1], Karina Torres Pomini[1], Mariza Akemi Matsumoto[3], Iris Santos German[1], Jesus Carlos Andreo[1], Rogério Leone Buchaim[1], André Luís Shinohara[1]

[1]Department of Anatomy, Bauru School of Dentistry, University of São Paulo, FOB-USP, São Paulo, SP, Brazil
[2]Department of Bioengineering of the University of Texas at Dallas(UTD), TX,United States of America
[3]Department of Basic Sciences, School of Dentistry, São Paulo State University (UNESP), Araçatuba, SP Brazil

Disclose and conflicts of interest: none to be declared by all authors

ABSTRACT

Introduction: modern eating habits, which softer and processed foods predominate in, requiring less effort to chew and have been correlated with an increase in the incidence of malocclusion and problems in the temporomandibular joint (TMJ) also, reducing the cross-sectional area (CSA). This vital muscle fiber variable indicates muscle function and strength. Selective androgen receptor modulators (SARMs) were developed to treat muscular and skeletal system problems to replace traditional androgens. This study aimed to verify if the use of ostarine associated with a soft-diet could exert the same anabolic and anti-catabolic effects on the masseter muscle described in the literature.

Material and Methods: fifteen six-months age C57BL/WT mice were used and divided into three groups and fed on either a solid (Control group - CG) or a soft-diet (Soft Diet Control Group - SDCG) with and without ostarine (Ostarine Group - OG).

Results: as a result, the soft-diet (SDCG) showed a lower median than the hard-diet (CG), but without statistical differences. However, the OG associated with a soft-diet presented a higher CSA than the SDCG with a statistical difference.

Conclusions: although there was no statistical difference between CG and SDCG group, slight atrophy occurred, and the use of ostarine (OG) reversed this process, becoming equal to the CG with a hard-diet.

Keywords: Masseter Muscle; Androgens; Atrophy; Diet Consistency; Nutrition.

Introduction

The complex interaction between the genotype and the environment can alter the craniofacial morphology and generate adaptations in the jaw structure and in the physiological overload of chewing[1,2]. The alteration of external stimuli, such as modifying the diet consistency, alters the mass of masticatory muscles[3,4]. Modern eating habits, which softer and processed foods predominate in, requiring less effort to chew, have been correlated with an increase in the incidence of malocclusion and problems in the temporomandibular joint (TMJ)[5,6].

A soft diet reduces the demand on masticatory muscles and reduces the overload applied to the jaw during the mastication process, causing bone remodeling similar to that found in individuals with partial or total edentulism[7,8]. Changing the food consistency reduces the cross-sectional area, a vital muscle fiber variable that indicates muscle function and strength[9,10]. Urushiyama *et al.*, demonstrated in their work that during one week under a soft consistency diet, the smaller diameter of the masseter muscle fibers isreduced by 19% compared to animals with more solid consistency[11].

The relation between masticatory muscles and nutritional status has been correlated[12]. A perfectly functioning oral cavity, associated with good nutritional intake, especially protein, is crucial to treat and prevent sarcopenia and malnutrition[13,12]. The low protein intake in elderly individuals may result from problems of the oral cavity, such as low masticatory function, even decreasing the appetite[14]. Malnutrition reduces the daily activities of the elderly and increases the risks of infection and mortality[15].

Selective androgen receptor modulators (SARMs) were developed to treat problems in the muscular and skeletal system in order to replace traditional androgens, which generate known adverse effects on the liver, prostate, heart, and skin[16].

SARMs are described in the literature as substances capable of increasing muscle mass and bone density in several experimental and clinical trials[17] decreased quality of life, and increased mortality. Although several therapeutic agents are currently in development for the treatment of muscle wasting or cachexia in cancer, the majority of these agents do not directly inhibit muscle loss. Selective androgen receptor modulators (SARMs)[18,19]. Among all, ostarine has the most well-documented studies and effects, proving be safe for human use [20]. However, so far, no experimental trial

DOI: 10.51929/jms.39.37.2022
ISSN 2177-0298

has been performed using this class of substances, especially ostarine, to observe its effects on the muscles of mastication.

Because of the embryological differences between the masticatory muscles and the limbs[21], we aimed to verify if the use of ostarine associated with a soft-diet, could exert the same anabolic and anti-catabolic effects on the masseter muscle described in the literature. Thus, if a positive outcome is observed, this substance may be used to prevent or even reverse the loss of muscle mass in the stomatognathic system and assist in malocclusion and orthognathic treatments.

Materials and Methods

Animals

Fifteen six-months age C57BL/6 WT mice were used, according to previous studies[11], which were purchased from the animal laboratory of the Ribeirão Preto campus of the University of São Paulo. The animals were housed in conventional cages containing five animals each at the Animal Laboratory of Bauru School of Dentistry – University of São Paulo, with feeders and drinkers "ad libitium" (irradiated feed – Nuvilab rodents and filtered water) at temperature-controlled rooms (22-25°C). All experiment procedures in the animals were conducted with the approval of the Institutional Review Board in Animal Studies of the Bauru School of Dentistry, University of São Paulo (Protocol: CEPA – 001/2018).

Experimental design:

The division of the groups was done according to the consistency of the diet in the presence or not of the ostarine: Control Group (CG) – solid consistency; Soft Diet Control Group (SDCG) – soft consistency diet (Figure 2) which was prepared at from the standard ration (Nuvilab) under a 2:5 water mixture used by[22] and Ostarine Group (OG) – soft consistency diet + administration of ostarine (SARM) at a dosage of 10 mg/kg/day (Enhanced Chemicals, MK2866), according to previous studies[23].

Figure 2. Soft diet prepared from Nuvilab feed under a 2:5 water mixture[22].

Ostarine was administered once a day and orally for four weeks as it has excellent oral bioavailability[24]. The drug was offered using a micropipette (LabMate Pro) to avoid possible esophagus aspiration or perforation and reduce more significant stress to the mice.

At the end of the experiment, the animals were killed according to the protocols approved by the Guidelines for the Care and Use of Laboratory Animals. We dissected the superficial masseter muscle, which was immediately frozen in liquid nitrogen and stored at -80°C for later histological analysis.

Histological Processing of Frozen Muscles

After the mice euthanasia, the superficial masseter muscle was removed and embedded into the OCT (Optimal Cutting Temperature) (Tissue-Tek; Sakura Finetek, Torrance, CA, USA) and frozen in liquid nitrogen and stored at -80°C. All the samples were brought to -22 °C, and serial transverse sections (8цm) were made with a cryostat (Leica 1850).

Hence, histological slides were stained with

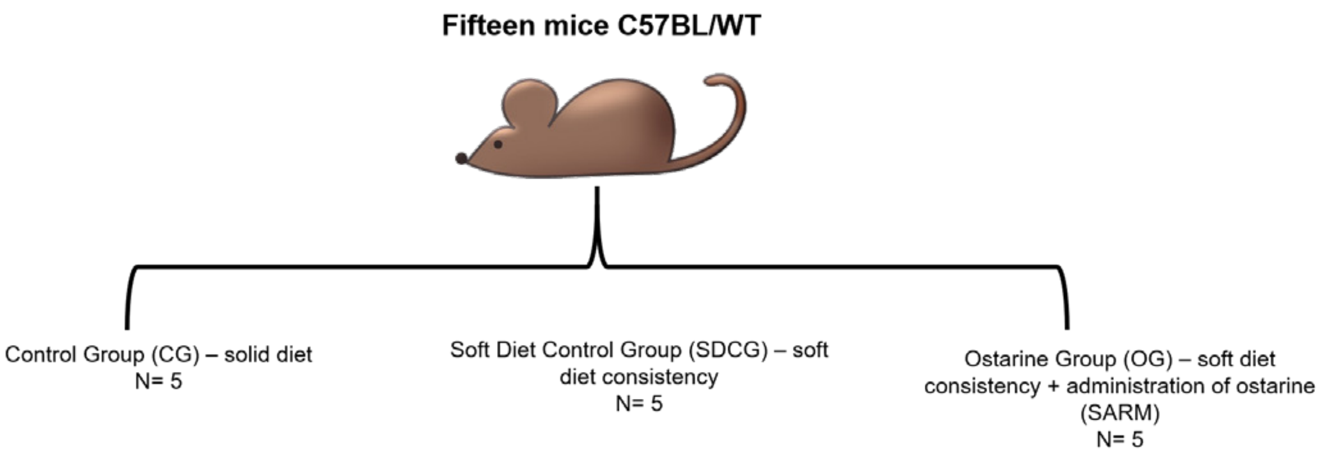

Figure 1. Illustration of the experimental group's design. The authors prepared them.

hematoxylin and eosin (H&E) to measure the muscle fiber's cross-sectional area (CSA). The microscope (model Olympus BX50) with 40x magnification was used to capture the images at the anatomy department of Bauru School of Dentistry, University of São Paulo (FOB-USP). We used the SigmaProScan 5.0 software, bypassing the perimeter of each fiber individually from each muscle, from each animal from the group to evaluate the muscle fiber's CSA. We measured 220 muscle fiber's from each sample from each animal per group.

Statistical Analysis

Given this, to evaluate the quantitative variables and verify the difference between the groups, the Kruskal-Wallis test was used, followed by the Tukey-test for multiple comparisons between them. Values of p <0,05 were considered significant.

Results

The perimeter of the fibers of the masseter muscle was traced to obtain the cross-sectional area (CSA), and compared to the control group with a hard-diet (CG), the group with a soft-diet (SDCG) showed a lower median, but without statistical differences. However, the ostarine group (OG) associated with a soft-diet presented a higher CSA than the soft-diet group control (SDCG) with a statistical difference. Indeed, although there was no statistical difference between the CG and SDCG group, slight atrophy occurred, and the use of ostarine (OG) reversed this process, becoming equal to the control group (CG) with a hard-diet (Figure 3).

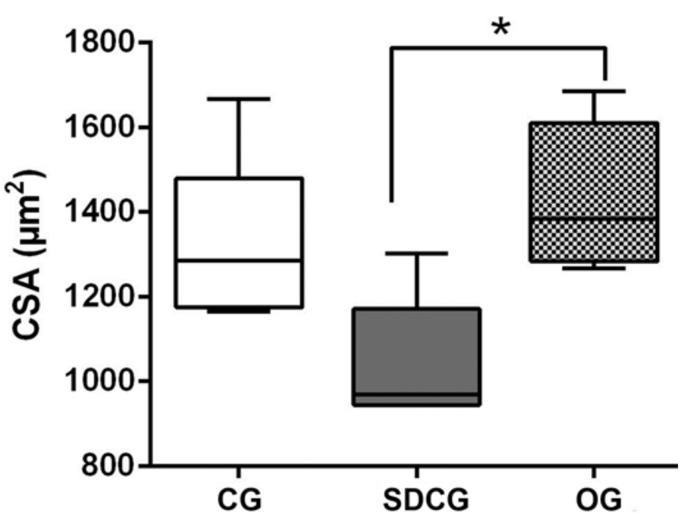

Figure 3. Mean cross-sectional area (μm²) of the superficial masseter. Values are means + S.D. Significant differences **P* <0,05.

Discussion

The cross-sectional area (CSA) of the muscle fiber is an important variable that indicates muscle function and strength[9]. It is reported that the changes in the consistency of the food reduce this variable[10]. Thus, in our study, we sought to evaluate the effect of changing the consistency of the diet and its impact on the stomatognathic apparatus with and without the use of ostarine, a selective androgen receptor modulator (SARMs).

In this context, reports show that the masseter muscle is one of the main muscles activated during chewing and the bite[25]. Thus, the consistency of a soft-diet, typical in the modern days, would reduce the overload on the masticatory muscles, having a direct relationship with craniofacial changes and higher rates of malocclusion[26]. In our study, despite not being statistically significant, the CSA of the SDCG was lower than the CG (Figure 3), a result similar to that from another study[27]. However, the group with a soft-diet associated with ostarine (OG), prevented the mild atrophy caused by the changes in the consistency of the diet (Figure 3), exertingits anti-catabolic action as described in other studies[28,29].

As a result of the reduction in the overload generated by the contraction of the masseter muscle on the bite force, generates a remodeling in this muscle, which is the reduction in the CSA of its muscle fibers, which will impact the functionality of this muscle[25]. The activation of the ubiquitin-proteasome system (UPS) is responsible for 70-90% degradation of misfolded or damaged proteins, is the mechanism by which this occurs through the unloading on the masseter muscle by changing the diet consistency[30,31,32].

Hence, no other work had used ostarine or any other selective androgen receptor modulator (SARMs) in masticatory muscles until now. Our study showed that ostarine exerts anti-catabolic actions in the same way that it does on the muscles of the limbs [(20)].

On the other hand, our study presented a limitation, which we believe to have been the reason, that the atrophy caused by the modification of the consistency of the diet, was not as reported by other studies[11,2,33]. Although they have already reported that many results involving this model of atrophy are not necessarily consistent [(34)], we believe that the non-observance of significant atrophy may be related to the ratio administered to the animals. The ratio used in this work was described by[35] and, compared to the feed used in some studies, ours offers 13% more calories in 100g[11,2,36]. We believe that this has been a limiting factor in our work since the regulatory pathways of hypertrophy and atrophy, such as Akt/FoxO/UPS, are also regulated by caloric intake[37]. Therefore, future studies will be carried out using a normocaloric diet to verify and, if confirmed, remove this bias.

Conclusions:

Although our study has had presented a limitation, we believe that ostarine exerts anti-catabolic actions in the same way that it does on the muscles of the limbs. However, future studies must be carried out using a normocaloric diet to verify and, if confirmed, remove the bias that may affect the caloric intake of the animals.

References

1. Klingenberg CP, Leamy LJ. Quantitative genetics of geometric shape in the mouse mandible. Evolution. 2001;55(11):2342–52.
2. Kawai N, Sano R, Korfage JAMM, Nakamura S, Kinouchi N, Kawakami E, *et al*. Adaptation of rat jaw muscle fibers in postnatal development with a different food consistency: An immunohistochemical and electromyographic study. Journal of Anatomy. 2010;216(6):717–23.
3. Ringqvist M. Fiber types in human masticatory muscles. Relation to function. European Journal of Oral Sciences. 1974;82(4):333–55.
4. Maeda N, Kawasaki T, Osawa K, Yamamoto Y, Sumida H, Masuda T, *et al*. Effects of Long-Term Intake of a Fine-Grained Diet on the Mouse Masseter Muscle. Acta Anat (Basel). 1987;128(4):326–33.
5. Waugh LM. Influence of diet on the jaw and face of the American Eskimo. Journal of the American Dental Association [Internet]. 1937;24(10):1640–7. Available from: http://dx.doi.org/10.14219/jada.archive.1937.0295
6. Tiilikainen P, Raustia A, Pirttiniemi P. Effect of diet hardness on mandibular condylar cartilage metabolism. J Orofac Pain. 2011;25(1):68–74.
7. Helkimo EVA, Carlsson GEG, Helkimo M. Bite force and state of dentition. Acta Odontologica Scandinavica. 1977;35(6):297–303.
8. Slagter AP, Bosman F, Van der Glas HW, Van der Bilt A. HUMAN JAW-ELEVATOR MUSCLE ACTIVITY AND FOOD COMMINUTION IN THE DENTATE AND EDENTULOUS STATE. Archives of Oral Biology. 1993;38(3):195–205.
9. Palma L De, Marinelli M, Pavan M, Orazi A. Ubiquitin ligases MuRF1 and MAFbx in human skeletal muscle atrophy. Joint Bone Spine. 2008;75:53–7.
10. Kim HK, Suzuki T, Saito K, Yoshida H, Kobayashi H, Kato H, *et al*. Effects of Exercise and Amino Acid Supplementation on Body Composition and Physical Function in Community-Dwelling. Journal of the Americal Geriatrics Society. 2012;60:16–23.
11. Urushiyama T, Akutsu S, Miyazaki J-I, Fukui T, Diekwisch TGH, Yamane A. Change from a hard to soft diet alters the expression of insulin-like growth factors, their receptors, and binding proteins in association with atrophy in adult mouse masseter muscle. Cell and Tissue Research [Internet]. 2004;315(1):97–105. Available from: http://link.springer.com/10.1007/s00441-003-0787-0
12. Hasegawa Y, Yoshida M, Sato A, Fujimoto Y, Minematsu T, Sugama J, *et al*. Temporal muscle thickness as a new indicator of nutritional status in older individuals. Geriatrics and Gerontology International. 2019;1–6.
13. Fukutomi E, Kimura Y, Wada T, Okumiya K, Matsubayashi K. Long-term care prevention project in Japan. The Lancet [Internet]. 2013;381(9861):116. Available from: http://dx.doi.org/10.1016/S0140-6736(13)60049-5
14. Matsuo K, Masuda Y, Kito N, Ogawa K, Izumi A. Effects of textured foods on masticatory muscle activity in older adults with oral hypofunction Running. Journal of Oral Rehabilitation. 2019;47(2):180–6.
15. Correia MITD, Waitzberg DANL. The impact of malnutrition on morbidity , mortality , length of hospital stay and costs evaluated through a multivariate model analysis. Clinical Nutrition. 2003;22(3):235–9.
16. Narayanan R, Coss CC, Dalton JT. Development of selective androgen receptor modulators (SARMs). Molecular and Cellular Endocrinology [Internet]. 2018;465:134–42. Available from: http://dx.doi.org/10.1016/j.mce.2017.06.013
17. Crawford J, Prado CMM, Johnston MA, Gralla RJ, Taylor RP, Hancock ML, *et al*. Study Design and Rationale for the Phase 3 Clinical Development Program of Enobosarm, a Selective Androgen Receptor Modulator, for the Prevention and Treatment of Muscle Wasting in Cancer Patients (POWER Trials). Current Oncology Reports [Internet]. 2016;18(6):37. Available from: http://link.springer.com/10.1007/s11912-016-0522-0
18. Gao, Wenqing. Dalton JT. Expanding the therapeutic use of androgens via selective androgen receptor modulators (SARMs). Drug Discov Today. 2007;86(3):573–9.
19. Hoffmann DB, Komrakova M, Pflug S, von Oertzen M, Saul D, Weiser L, *et al*. Evaluation of ostarine as a selective androgen receptor modulator in a rat model of postmenopausal osteoporosis. Journal of Bone and Mineral Metabolism [Internet]. 2018;(0123456789):1–13. Available from: https://doi.org/10.1007/s00774-018-0929-9
20. Srinath R, Dobs A. Enobosarm (GTx-024, S-22): a potential treatment for cachexia Reshmi. Future Oncol. 2014;10:187–94.
21. Stål P, Eriksson PO, Schiaffino S, Butler-Browne GS, Thornell LE. Differences in myosin composition between human oro-facial, masticatory and limb muscles: enzyme-, immunohisto-and biochemical studies. Journal of Muscle Research and Cell Motility. 1994;15(5):517–34.
22. Bresin A, Kiliaridis S, Strid KG. Effect of masticatory function on the internal bone structure in the mandible of the growing rat. European Journal of Oral Sciences. 1999;107(1):35–44.
23. Gao W, Gao, Wenqing, J. Reiser, Peter; Coss, Chirstopher C.; Dalton JT. Selective Androgen Receptor Modulator Treatment Improves Muscle Strength and Body Composition and Prevents Bone Loss in Orchidectomized Rats. Endocrinology. 2005;146(11):4887–97.
24. Kim J, Wang R, Veverka KA, Dalton JT. Absorption, distribution, metabolism and excretion of the novel SARM GTx-024 [(S)-N-(4-cyano-3-(trifluoromethyl)phenyl)-3-(4-cyanophenoxy)-2-hydroxy-2-methylpropanamide] in rats. Xenobiotica. 2013;43(11):993–1009.
25. Thomas NR, Peyton SC. AN ELECTROMYOGRAPHIC STUDY OF MASTICATION IN THE FREELY-MOVING RAT. Archives of Oral Biology. 1983;28(10):939–45.
26. Yamada K, Kimmel DB. The effect of dietary consistency on bone mass and turnover in the growing rat mandible. Archives of Oral Biology. 1991;36(2):129–38.
27. Sfondrini G, Reggiani C, Gandini P, Bovenzi R, Pellegrino MA. Adaptations of masticatory muscles to a hyperpropulsive appliance in the rat. American journal of orthodontics and dentofacial orthopedics : official publication of the American Association of Orthodontists, its constituent societies, and the American Board of Orthodontics. 1996;110(6):612–7.
28. Coss CC, Jones A, Dalton JT. Selective androgen receptor modulators as improved androgen therapy for advanced breast cancer. Steroids [Internet]. 2014;90:94–100. Available from: http://dx.doi.org/10.1016/j.steroids.2014.06.010
29. Ponnusamy S, Sullivan R, Thiyagarajan T, Tillmann H, Getzenberg R, Narayanan R. Tissue Selective Androgen Receptor Modulators (SARMs) Increase Pelvic Floor Muscle Mass in Ovariectomized Mice. J Cell Biochem. 2017;118(3):640–6.
30. Jung T, Catalgol B, Grune T. The proteasomal system. Molecular Aspects of Medicine [Internet]. 2009;30(4):191–296. Available from: http://dx.doi.org/10.1016/j.mam.2009.04.001
31. Kiliaridis S, Engstrom C, Thilander B. The relationship between masticatory function and craniofacial morphology: I. A cephalometric longitudinal analysis in the growing rat fed a soft diet. European Journal of Orthodontics [Internet]. 1985;273–83. Available from: http://www.ncbi.nlm.nih.gov/pubmed/7674427
32. Bodine SC, Baehr LM. Skeletal muscle atrophy and the E3 ubiquitin ligases MuRF1 and MAFbx/atrogin-1. American Journal of Physiology - Endocrinology and Metabolism. 2014;307(6):E469–84.
33. Kiliaridis S, Engström C, Thilander B. Histochemical analysis of masticatory muscle in the growing rat after prolonged alteration in the consistency of the diet. Archives of Oral Biology. 1988;33(3):187–93.
34. Kitagawa Y, Mitera K, Ogasawara T, Nojyo Y, Miyauchi K, Sano K. Alterations in enzyme histochemical characteristics of the masseter muscle caused by long-term soft diet in growing rabbits. Oral Diseases. 2004;10(5):271–6.
35. German IJS, Pomini KT, Bighetti ACC, Andreo JC, Reis CHB, Shinohara AL, *et al*. Evaluation of the use of an inorganic bone matrix in the repair of bone defects in rats submitted to experimental alcoholism. Materials. 2020;13(3).
36. Kono K, Tanikawa C, Yanagita T, Kamioka H, Yamashiro T. A Novel Method to Detect 3D Mandibular Changes Related to Soft-Diet Feeding. Frontiers in Physiology. 2017;8(August):1–12.
37. Sandri M, Barberi L, Bijlsma AY, Blaauw B, Dyar KA, Milan G, *et al*. Signalling pathways regulating muscle mass in ageing skeletal muscle. the role of the IGF1-Akt-mTOR-FoxO pathway. Biogerontology. 2013;14(3):303–23.

Mini Curriculum and Author's Contribution

1. João Vitor Tadashi Cosin Shindo – MsC student. Contribution: conceived and designed the experiments also, wrote the paper. ORCID: 0000-0002-0230-5962

2. Júlia Pedrozo Bellini – MsC student. Contribution: Performed the experiments and collected data. ORCID: 0000-0002-2527-7482

3. Cláudia Cristina Biguetti – PhD. Contribution: Perfomed the experiments, collected data, helped with her skilled assistance in histological staining. ORCID: 0000-0003-4503-3108

4. Maira Couto - BSc. Contribution: Maira helped during the experiments also with her skilled assistance in histological staining.

5. Karina Torres Pomini - PhD student: Contribution: She performed the experiments and collected data. ORCID: 0000-0003-2858-4717

6. Mariza Akemi Matsumoto - PhD. Contribution: All the resources used in this work were provided by teacher Mariza Akemi Matsumoto. ORCID: 0000-0001-5389-0105

7. Iris Santos German - PhD student: She performed the experiments, collected data, and helped writing the paper. ORCID: 0000-0002-7624-7689

8. Jesus Carlos Andreo - PhD. Jesus Carlos Andreo is an anatomy teacher of the Bauru School of Dentistry (FOB-USP) and has great experience in the study of skeletal muscle, focusing on the atrophy process. Contribution: Supervised the project. ORCID: 0000-0001-7227-1908

9. Rogério Leone Buchaim – PhD. Rogério Leone Buchaim is an anatomy teacher of Bauru School of Dentistry (FOB-USP) and has experience in the study of bone and biomaterials. However, he also has works in skeletal muscle field associated with nerve injury. Contribution: Supervised the project. ORCID: 0000-0002-5881-2218

10. André Luís Shinohara - PhD. André Luís Shinohara is an anatomy teacher of the Bauru School of Dentistry (FOB-USP) and has experience in the study of skeletal muscle. His new aims are to work to sarcopenia. Contribution: Supervised the project. ORCID: 0000-0003-4444-3949

Received: November 6, 2021
Accepted: December 3, 2021

Corresponding author
João Vitor Tadashi Cosin Shindo
E-mail: jvshindo@gmail.com