401 N. Lindbergh Blvd.
St. Louis, MO 63141
Tel.: 314.993.1700, #546
Toll Free: 800.424.2841, #546
Fax: 800.708.1364

Send via email to: jbode@aaortho.org and cyoung@aaortho.org

AAO Foundation Final Report Form
(a/o 5/30/2022)

Please prepare a report that addresses the following:

Type of Award: **Biomedical Research Award**

Name(s) of Principal Investigator(s): **Iacopo Cioffi**

Institution: **University of Toronto, Faculty of Dentistry**

Title of Project: **Investigating novel mechanisms of muscular temporomandibular disorders**

Period of AAOF Support: **07-01-2020 to 06-30-2022**

Amount of Funding: **30,000 USD**

Summary/Abstract:
Temporomandibular disorders (TMD) are the most common cause of chronic orofacial pain. TMD associated with pain in the masticatory muscles (TMD myalgia, mTMD) is the most common form of TMD accounting for more than 50% of TMD cases. Although there is a high prevalence of mTMD, the underlying mechanisms of this musculoskeletal condition remain unclear with a definitive cause yet to be identified.

Our research lab has shown that abnormalities in the oxygenation of the masseter muscle are present in healthy individuals at risk for mTMD. Similarly, human research studies have shown that masticatory muscle ischaemia is associated with mTMD. Therefore, the development of an ischemic animal model is crucial to determine the role of ischemia in the etiopathogenesis of mTMD.

In this study we developed a novel rodent model of masticatory muscle ischaemia via unilateral ligation of the external carotid artery (ECA) in male Sprague-Dawley rats, followed by an investigation to characterize its downstream effects on mechanosensitivity and cellular features of the masseter and temporalis muscles.

We ligated the right ECA in 18 rats under general anaesthesia. Mechanical detection thresholds (MDTs) at the masseter and temporalis bilaterally were measured immediately before ECA ligation and after euthanasia at 10-, 20-, and 35-days (n=6 rats/timepoint). Tissue samples were harvested for histological analyses and to assess changes in the expression of markers of hypoxia and muscle degeneration (*Hif-1α*,

VegfA and *Fbxo32*) via real time Polymerase Chain Reaction. Data were analyzed using mixed effect and general linear models. Statistical significance was set at $p<0.05$.

Mean surgical time ± SD from anaesthetic induction to completion of wound closure was relative short, amounting to 39±6.4 minutes. Sixteen surgeries were considered successful; two were considered unsuccessful due to post-operative complications. MDTs were higher in the right than left muscles ($p=0.009$) after 20 days. Increased spacing between muscle fibres and fascicles, with widened perimysium and endomysium, and irregular fibre shape were observed in the right temporalis and masseter muscle tissues as early as 10-days and persisted until the 35-day timepoint in both the right muscles compared to the left muscles. *Fbxo32* expression gradually increased in all muscles (all $p<0.05$). *Hif-1α* and *VegfA* did not change significantly with time in all muscles (all $p>0.05$) but were more highly expressed in the right masseter than right temporalis ($p=0.046$ and $p<0.001$, respectively).

We developed a novel surgical method of ECA ligation for the establishment of a model of mTMD. Our investigation revealed that unilateral ECA ligation led to mechanosensory and cellular changes of both the masseter and temporalis muscles. Tissue fibrosis was a common finding in the masseter and temporalis ipsilateral to the ECA ligation. However, the masseter may have a greater adaptive capacity to ischaemia compared to the temporalis, as degenerative changes and fibrosis were more frequently observed in the temporalis than the masseter, consistent with documentation of gene expression changes.

Respond to the following questions:

1. **Were the original, specific aims of the proposal realized?**
   YES
2. **Were the results published?**
   a. **If so, cite reference/s for publication/s including titles, dates, author or co-authors, journal, issue and page numbers**

MSc Thesis. D. Makar. Development of a rat model to characterize the effects of ischaemia on the masseter and temporalis muscles. University of Toronto, 2021 (Supervisor, I. Cioffi). https://tspace.library.utoronto.ca/bitstream/1807/111417/1/Makar_David__202111_MSc_thesis.pdf

The manuscript is currently under review. Preliminary data were presented at the 2021 International Association for Dental Research Conference (virtual, see below).

   b. **Was AAOF support acknowledged?**
      YES
   c. **If not, are there plans to publish? If not, why not?**
      N/A
2. **Have the results of this proposal been presented?**
   YES
   a. **If so, list titles, author or co-authors of these presentation/s, year and** locations

   Makar D, Nazemi A, De Guzman R, Bhardaj N, Gong S-G, Sessle BJ, ***Cioffi I.*** *Short- and long-term effects of experimentally induced masticatory muscle ischemia (2021). International Association for Dental Research Conference. Virtual Conference.* ***Oral presentation.***

b. Was AAOF support acknowledged?
YES

c. If not, are there plans to do so? If not, why not?
N/A

3. To what extent have you used, or how do you intend to use, AAOF funding to further your career?

I have received previous funding from AAOF. AAOF funding has been crucial to develop my research program at the University of Toronto which is currently supported by major national and international research grants.

Accounting for Project: Detailed accounting has been sent to AAOF by University of Toronto Research Office