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Role of Satellite Cells in Long Term Fiber Type Shifts

Honglu Liu
University of Kentucky, liu.honglu@gmail.com

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Dr. Charlotte A. Peterson
Dr. Christopher Fry
Ms. Jyoti Mula

Abstract

Muscle fiber type shifts in respect to satellite cells, muscle stem cells, are not well understood currently. The Peterson Lab has generated a mouse model (PAX7-DTA) that ablates satellite cells to determine if these muscle stem cells contribute to mouse muscle fiber type changes over an eight week period. In the study, control and satellite-cell-ablated mouse groups were split into control and overload groups (via synergist ablation surgery) and placed under similar environmental conditions. Eight weeks post-experiment, muscles were dissected to obtain the plantaris muscles of animals from all groups. Muscle cross-sections obtained were immunohistochemically stained, imaged, quantified by fiber type, and statistically analyzed. Results of the study found that in a two-way ANOVA analysis, there was no significant difference ($P > 0.05$) between groups. This result suggests that satellite cells play little apparent role in muscle fiber type shifts in mouse muscle in response to overload.

Introduction

Muscle, when overloaded, exhibits hypertrophy (growth) and fiber type shifting. Historically, satellite cells, muscle stem cells, have been considered required to promote muscle growth (1). The role of satellite cells in fiber type switching has not been determined.

Human muscles contain three fiber types, Type I, Type IIa, and Type IIx. These muscle fiber types differ in size, function, and myosin heavy chain (MyHC) isoform expression. Type I fibers are slow-oxidative fibers. These fibers are involved in, for instance, posture, where long, sustained activity, such as sitting up for extended periods of time, is needed (3). Slow-oxidative refers to the rate of contraction of the fiber (slow) dependent on ATP hydrolysis by type I myosin heavy chain and the requirement of oxygen for function. Type I fibers do not fatigue easily and are small compared to type IIa and type IIx muscle fibers. Type IIa fibers are fast-glycolytic/oxidative fibers. These are fast-twitch fibers that utilize oxygen and are recruited in activities such as long distance running or long course swimming (3). These fibers are more powerful and thus larger than type I fibers. Type IIa myosin heavy chain also possesses faster ATPase activity compared to Type I MyHC. Type IIx fibers are fast-glycolytic fibers. Unlike humans, rodents also contain a fourth myosin heavy chain gene, IIb, that encodes an isoform with the fastest ATPase hydrolysis rate. Each myosin heavy chain isoform can be separated via gel electrophoresis (1). Type IIb and IIx fibers are larger than both other types of fibers and are fast twitch fibers that require rapid energy sources. Both of these fiber types rely on glycolysis to break down glucose, a simple sugar utilized for basic anaerobic energy production (3). Type IIb and IIx fibers are anaerobic and are responsible for rapid force generation and cannot endure long periods of use due to high fatiguing.

When muscle is overloaded, fiber types shift to adjust and adapt to demands as needed. For instance, if a human subject trained by lifting weights, that subject would likely see a shift in their fiber type to a higher ratio of type IIx muscle fibers. By contrast, if a subject trained aerobically for a marathon, it is expected that a shift from type IIx to type IIa fibers occurs in

response to increased demand for low fatiguing and high endurance activities. It is likewise possible to have mixed fiber types as a result of training. Here, muscles are in a transition state between fiber types. Currently, the exact role of satellite cells in fiber type shifts is poorly understood. A 2006 study by Martins et al. utilizing gamma-radiation for satellite cell ablation in rat models over a three week period revealed via fiber typing through immunohistochemistry, staining utilizing myosin heavy chain isoform-specific antibodies, that satellite cells perform a facilitative role in fast-to-slow fiber type shifts (2). Whether or not satellite cells play a specific role in fiber type shifts over an eight week period is currently unknown. The Peterson Lab has generated a mouse model (PAX7-DTA) that ablates satellite cells to determine if satellite cells contribute to changes in muscle fiber type in response to overload. It is hypothesized that there is some relation between satellite cell function and fiber type shifts.

Methods

1. PAX7-DTA Mouse Model

The Peterson Lab PAX7-DTA mouse model was utilized for this project. The mice were randomly labeled and divided into four groups (Vehicle Sham, Vehicle Overload, Tamoxifen Sham, and Tamoxifen Overload). The Vehicle groups denote the control groups and the Tamoxifen groups denote the satellite-cell-ablated groups. Mice in the Tamoxifen groups were hand-injected intraperatoneally with tamoxifen for satellite cell ablation; the Vehicle groups received injection of sunflower oil in which the Tamoxifen was dissolved. The Sham groups were then split from the Overload groups; the latter groups had their gastrocnemius and soleus muscles surgically removed, such that the synergist plantaris muscle experienced mechanical overload for eight weeks.

2. Fiber Typing via Immunohistochemsitry

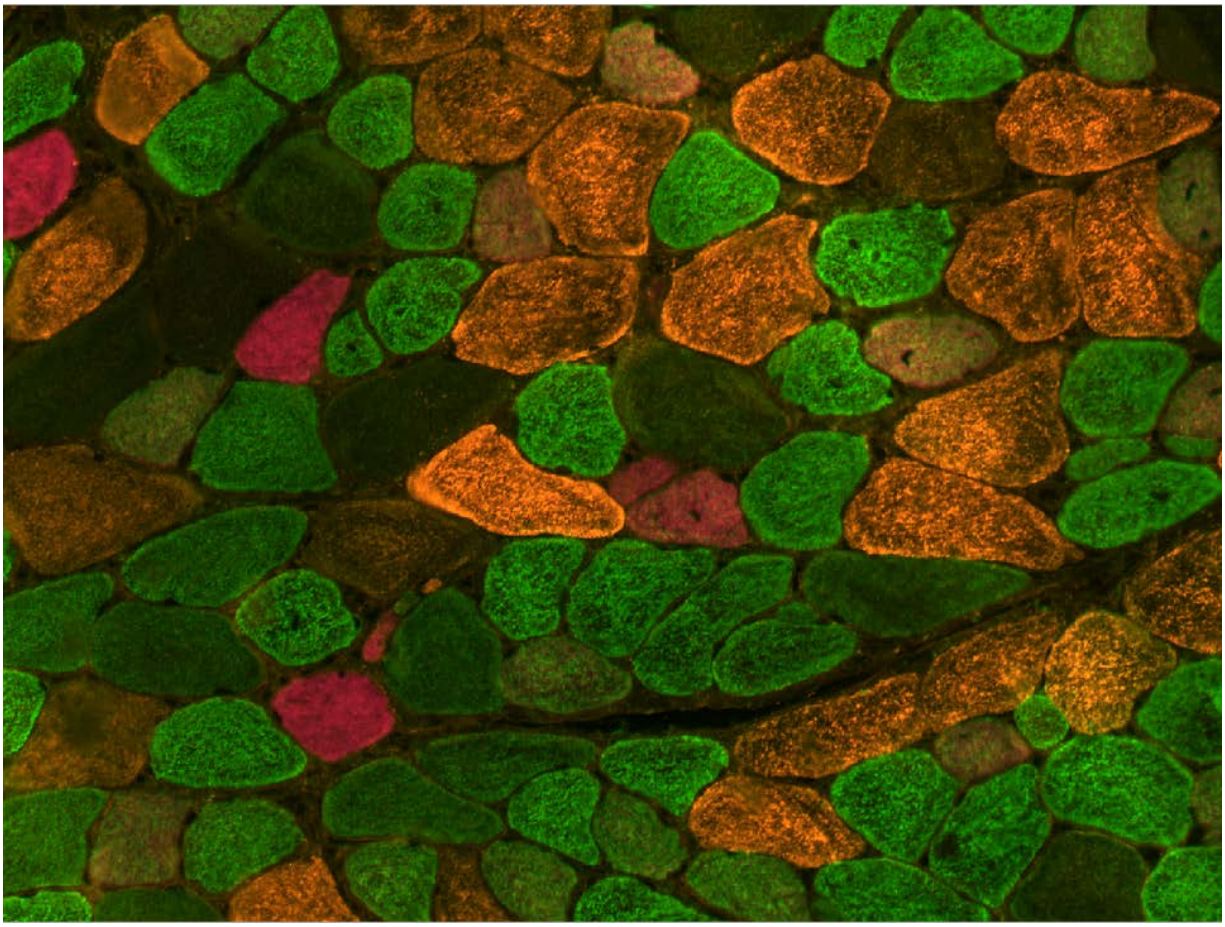
Following the eight weeks, the mice were sacrificed and plantaris muscles were removed and frozen. The muscles were then sliced into 7 μm cross-sections via a Micron HM 525 cryostat. The muscles were examined for damages via microscopy and were re-sliced until undamaged cross-sections were obtained. Staining was performed via immunohistochemical methodology, where differing myosin heavy chain isoform-specific antibodies were utilized to mark each fiber type. Secondary antibodies with fluorescence tags were used for visualization. Type IIx fibers were not stained due to unavailability of a type IIx myosin heavy chain isoform-specific antibody that works on mouse tissue.

3. Imaging

Once stained, slides were placed under a Zeiss Axio Imager M1 Upright microscope and firmly attached utilizing clips on the microscope platform. AxioVision software was utilized to capture images for each muscle cross-section at 20x magnification utilizing fluorescent imaging. Several images were produced per muscle cross-section to account for various areas of the cross-section, too large to be produced as one single image. Fluorescent imaging technique was utilized in

conjunction with immunohistochemistry to allow for clear exhibition of antibody reactions with respective muscle fibers, appearing in varying colors (**Figure 1**). Images were then organized by animals.

Figure 1. Image of fiber typed muscle retrieved from AxioVision fluorescent imaging



4. Fiber Type Quantification

Criterion for stained fiber type colors were established in an investigation team meeting. Type IIx fibers were assumed to be negative fibers as no clearly-reacting antibody was available. The criterion for fiber typing appearance established by the investigation team was as follows:

Table 1. Fiber Typing Standardization Criterion

Fiber Type	Stain Color
Type I	Pink
Type IIa	Green
Type IIb	Yellow or Red
Type I/IIa	Pink and Green
Type IIa/IIb	Green and Yellow or Green and Red
Type IIx	Negative

Unclear fibers, elongated cross sectional fibers, and damaged cross sectional fibers were discounted. Darkened fibers with staining were counted as their original color. Images obtained from imaging were randomly selected and hand counted utilizing the Count tool in AxioVision software, allowing for marker placement on the centers of fibers. The Count tool was re-selected for each fiber type and changed in color markings to accommodate differing data and to reduce confusion (**Figure 2**). When counting different fiber types, different color filters within AxioVision were also applied to verify if stains were positive or negative (**Figure 3**). The overall counts were cross-referenced between different counters to increase reliability of the experiment. The data were then compiled into Microsoft Excel for analysis.

Figure 2. Count tool in AxioVision with different colored "X"-markers to denote various fiber types

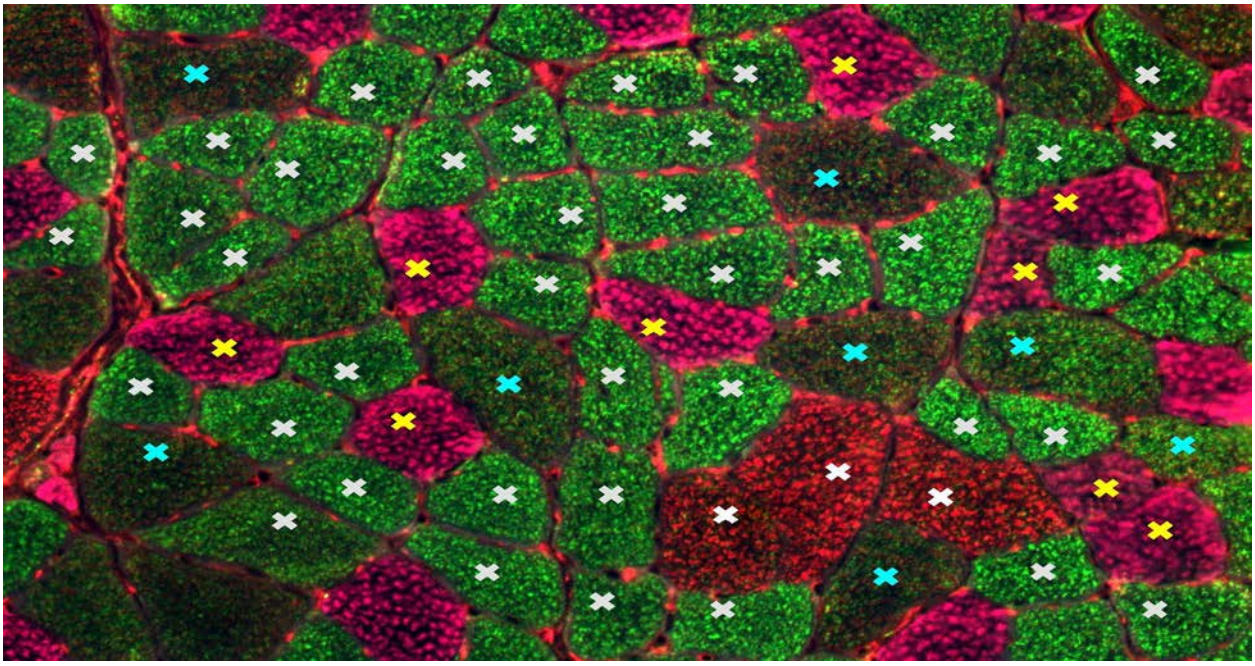
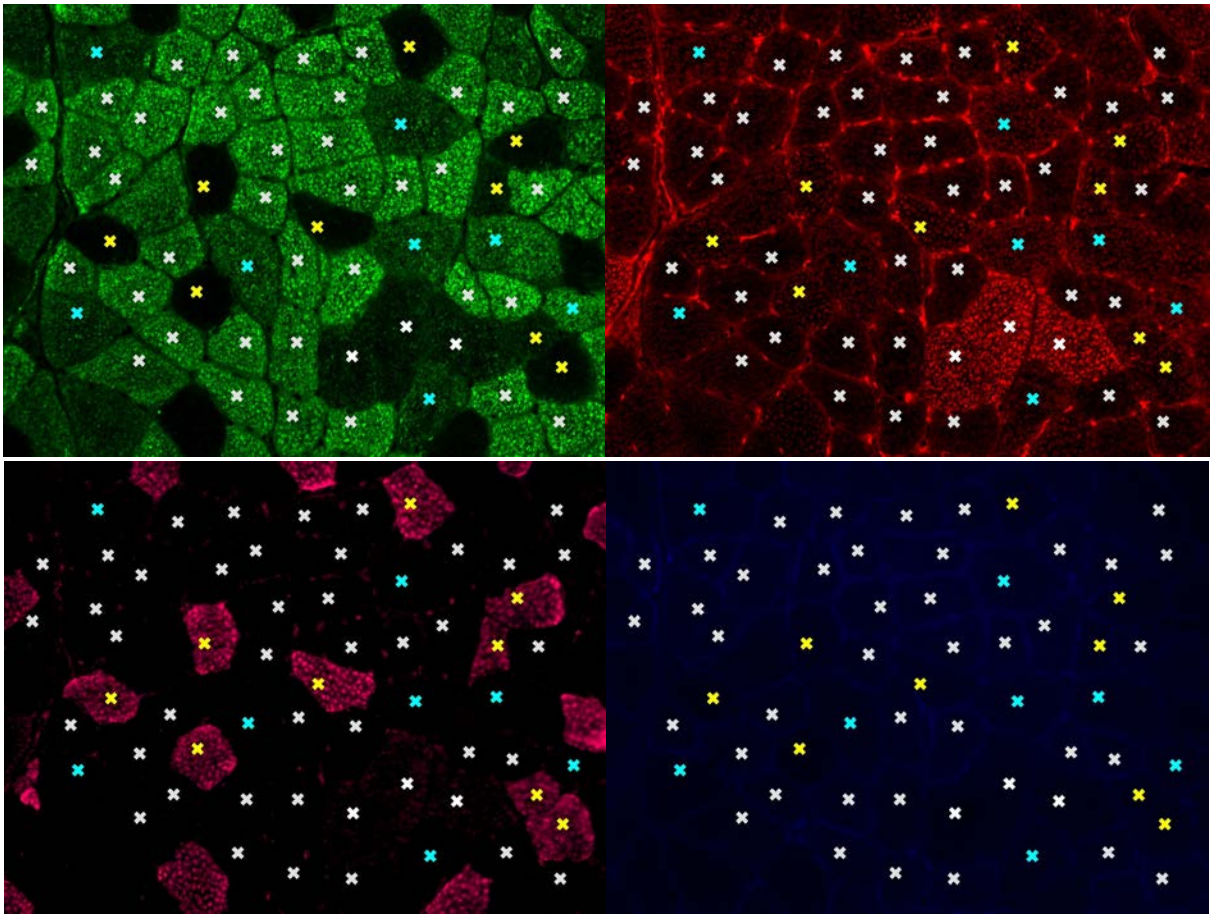


Figure 3. Application of varying color filters to verify positive versus negative stains



5. Statistical Analysis

For all animal groups, data were collected, fiber types were averaged, and means were normalized. A two-way ANOVA test was conducted to determine the overall differences between Sham (Control) and Overload (Synergist Ablated) groups, Vehicle (Control) and Tamoxifen (Satellite Cell Ablated) Groups, and their overall differences and / or interaction with respect to their fiber type relative averages, where,

1) H_0 = No difference in relative fiber percentage between Sham and Overload groups;
   H_1 = Difference in relative fiber percentage between Sham and Overload groups;

2) H_0 = No difference in relative fiber percentage between Vehicle and Tamoxifen groups;
   H_1 = Difference in relative fiber percentage between Vehicle and Tamoxifen groups;

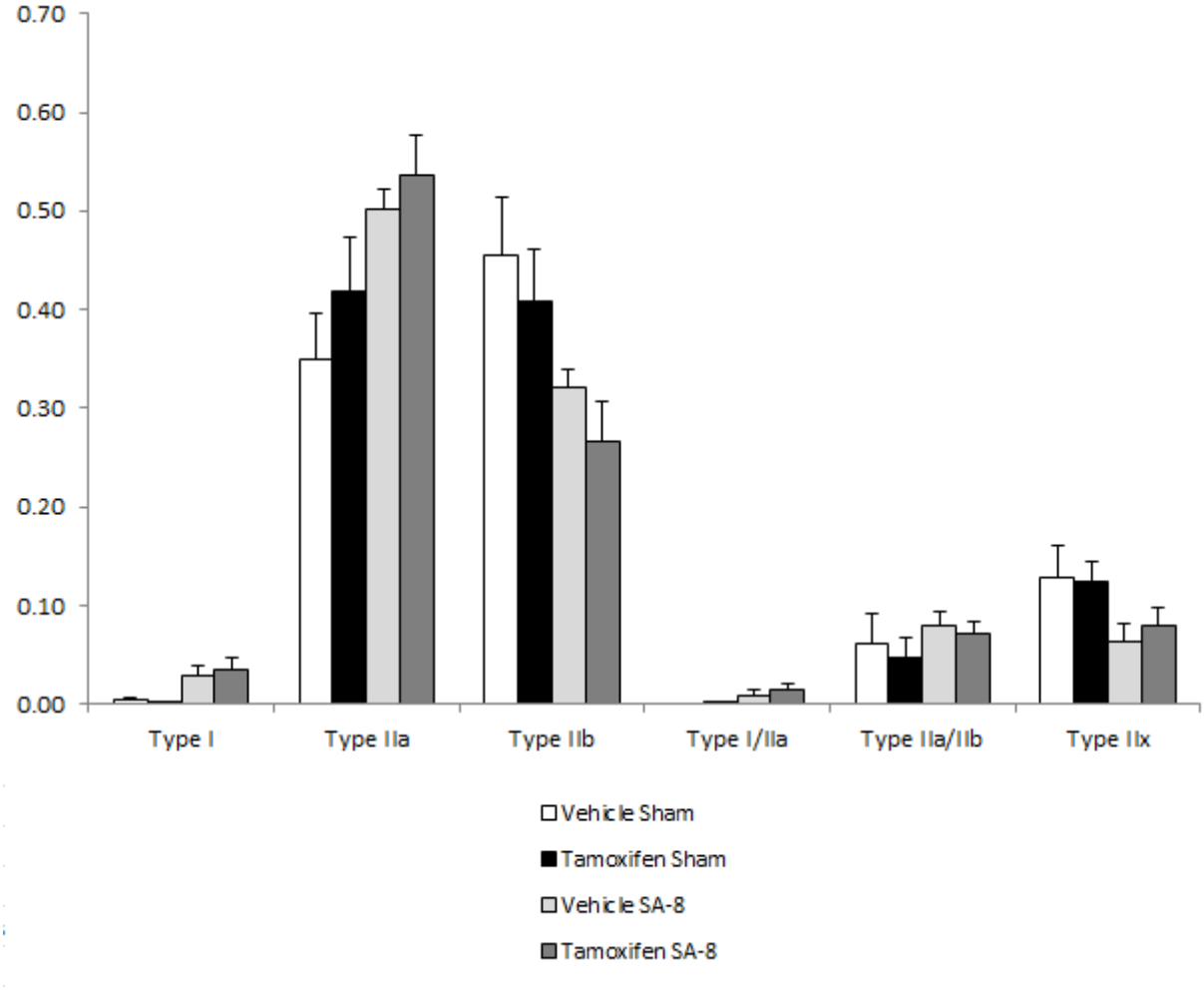
3) H_0 = No difference between these two groups above;
   H_1 = Differences between these groups.

Certain animals were dropped from the analysis due to poor imaging results.

Results

The following figure denotes comparisons drawn amongst the fiber type data. The overall results for the fiber typing can be visually interpreted utilizing the following graph of relative average fiber types for all animals groups.

Graph 1. Relative Average fiber types for all animal groups



In an overall statistical analysis of the data set, no significant differences were observed following analysis by the two-way ANOVA ($P > 0.05$).

Discussion

The graph values (**Graph 1**) highlight fiber type comparisons, where all groups visually exhibit similar normalized percentages for specific fiber types. In an overall statistical analysis, the two-way ANOVA test yielded statistical insignificance in all three null hypotheses ($P > 0.05$). This p-value thus denotes failure to reject three null hypotheses. Therefore, this statistical result determines that there exists: 1) No overall difference in relative fiber percentage between Sham

and Overload groups; 2) No overall difference in relative fiber percentage between Vehicle and Tamoxifen groups; and 3) No overall interaction, and thus, no differences between the two groups of Sham and Overload and Vehicle and Tamoxifen groups. Also, under all conditions, no extreme changes within the data were found, suggesting the reliability and validity of the methods.

Conclusion

1. General Conclusion

Satellite cells appear to play little role in fiber type shift under overload conditions over an eight week period in mouse muscle, as comparative data from the Vehicle Sham and Vehicle Overload groups paired with the Tamoxifen Sham and Tamoxifen Overload groups denote. This finding suggests that the effect observed by Martins et al. may be due to the non-specific effects of irradiation on cell types other than, or in addition to, satellite cells in fiber type shifts.

2. Implications

The implications of this experiment are in how understanding functions of muscle and its components can aid in disease state alleviation and in contribution to the advancement of scientific knowledge. Although there exists limited understanding of the current role of satellite cells, identifying their function can potentially aid in treating, for instance, atrophic muscular diseases. Bettering understanding of satellite cell function in muscle can also stimulate further exploration in this field to better understand other aspects of varying muscle components and their functions.

3. Limitations

One limitation in this study is the lack of a reactive Type IIx-specific antibody. Although blank fibers can be inferred to be pure type IIx, it is not possible to identify mixed fiber types co-expressing type IIx (i.e., type IIa/IIx).

As well, the number of animals (N = 6) per group perhaps limited power. More animals may have been useful in determining more valid statistical measures in fiber type shifts. That said, the resources and time placed into this project by manual quantification was relatively large. Had there been a faster method to obtain data, perhaps more data could have been collected to contribute to a fuller picture.

4. Future Research

The full function of satellite cells is still not fully defined. Based on the results of this project, further research with an effective Type IIx antibody in this domain with more animals or perhaps even human muscles can promote further understanding of satellite cell function with respect to overall aspect of muscle physiology.

As well, historically, fiber typing analysis via quantification has been performed by manual quantification. Recently, the Bioinformatics Department and the Center for Muscle

Biology at the University of Kentucky have co-developed an automated quantification program capable of detecting different muscle fiber types stained immunohistochemically. Although still a work in progress, this project, in addition to determining satellite cell function, can serve as a testbed for the automated quantification program in comparison with manual (human) quantification.

References

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