A METHODOLOGY FOR ASSESSING ECTOPIC LIPID DEPOSITION IN SKELETAL MUSCLE TISSUES USING OIL RED-O STAINING

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Abstract. Ectopic lipid deposition in skeletal muscle tissues has been implicated in various metabolic disorders, including obesity and type 2 diabetes. A reliable method for its assessment is important for understanding the underlying mechanisms and developing therapeutic interventions. This article presents a comprehensive methodology for determining ectopic lipid deposition in skeletal muscle tissues utilizing the oil red-O staining. The protocol includes section preparation, oil red-O staining procedures, microscopic imaging, and imageJ quantitative analysis. As a result, the data show clear positive oil red-O staining of intracellular and extracellular lipid contents in skeletal muscle cross section. The densities of lipid accumulation within skeletal muscle indicated that the highest content of lipids is in extracellular positions. Therefore, this method provides researchers with a valuable tool for investigating the role of ectopic lipids in skeletal muscle pathology and evaluating the efficacy of interventions aimed at metabolic dysregulation.

Keywords: Lipid deposition, skeletal muscle, oil red-O staining, imageJ.

1. INTRODUCTION

Disperse lipid deposition, characterized by the accumulation of lipids in non-adipose tissues such as skeletal muscle, has emerged as a significant factor contributing to metabolic dysregulation and associated disorders such as obesity and type 2 diabetes (Van Herpen and Schrauwen-Hinderling, 2008). Skeletal muscle, a major site for glucose uptake and utilization, plays an important role in maintaining metabolic homeostasis, especially lipid and glucose metabolisms (Merz and Thurmond, 2020). However, ectopic lipid accumulation within skeletal muscle burdens its normal function, such as dysregulating lipid metabolism, impairing insulin sensitivity, and increasing local inflammatory responses. All together, these at least partly leads to metabolic disorders (Guebre-Egziabher et al., 2013). Unfortunately, whether intracellular or extracellular lipids in skeletal muscle tissues have a higher effect on metabolic homeostasis remains to be elucidated.

The accurate assessment of ectopic lipid deposition in skeletal muscle tissues contributes to the understanding of insight mechanisms of metabolic disorders and for the development of therapeutic strategies. Several methods have been employed to quantify ectopic lipid content in skeletal muscle, including biochemical assays and immunohistochemical stainings (Schrauwen-Hinderling et al., 2006). Among them, the oil

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red-O staining has been proven as a widely utilized and reliable approach for visualizing and quantifying lipid depositions within tissues. The oil red-O staining, a histological method, specifically stains neutral lipids such as triglycerides and cholesterol esters, enabling the visualization of lipid content within tissues under light microscopy. This staining method provides researchers with a valuable tool for assessing the extent and distribution of ectopic lipid deposition in tissues. However, the successful implementation of the oil red-O staining depends on various factors such as tissue processing, staining protocols, and image analysis methods (Du et al., 2023).

In the current study, we present a reliable methodology for determining ectopic lipid deposition in skeletal muscle tissue using oil red-O staining. Steps of the procedure were shown, including cross section preparation, staining, microscopy imaging, and quantitative analysis. By providing a bascal protocol for determining ectopic lipid accumulation in skeletal muscle tissues, this methodology holds promise for evaluating the efficacy of therapeutic interventions targeting ectopic lipid deposition and restoring metabolic homeostasis in skeletal muscle.

2. MATERIALS AND METHODS

2.1. Animals

The eight-week-old C57BL/6 male mice were purchased from the Orient (South Korea) and individually housed in plastic cages in a specific pathogen-free animal facility with a 12-h light, 12-h dark cycle. The mice were fed a high-fat diet (HFD) (60% of calories from fat; Research Diets Inc., New Brunswick, NJ) for 9 weeks, and given free access to food and water. The animals were killed by CO₂ asphyxiation and skeletal muscles were dissected.

2.2. Cross Section Preparation and Oil Red-O Staining

Gastrocnemius muscles were fixed overnight at room temperature in 10% formaldehyde and embedded in paraffin. The embedded gastrocnemius skeletal muscle was cut at the middle of muscle bundle in 8 micron-thick cross sections of by using a cryostat (Kalstain, Paris, France) and mounted on glass slides. For staining with oil red-O, the cross sections were thawed at room temperature and washed gently with PBS. The sections were then treated with 60% aqueous isopropanol for 1 min and next stained with 0.18% oil red-O in 60% aqueous isopropanol for 2 hr at room temperature. Finally, the stained sections were washed in 60% aqueous isopropanol and monitored using Axio-Star Plus microscope (Carl Zeiss, Gottingen, Germany). Staining densities were measured with an ImagJ analyzer (Biocompare, San Francisco, USA).

2.3. Statistical Analysis

The results are presented as means ± standard error of the mean. Variables were compared using Student’s t test or analysis of variation with Duncan’s multiple-range test. Differences were considered significant at p < 0.05.
3. RESULTS AND DISCUSSION

3.1. Recognition of positive oil red O staining

Referring from the methodology that has been discovered before (Kim et al., 2003), here, the skeletal muscle cross sections mounted on the glass slides were thawed and washed in PBS. The sections were treated with 60% isopropanol for 1 min, and after that were stained with 0.18% oil red-O in 60% isopropanol for 2 hours at room temperature. After washing in 60% fresh isopropanol, the sections were visualized under microscopic. The result in Figure 1 shows the positive oil red-O staining positions. Oil red-O is a histological stain commonly used to visualize lipid droplets in tissues. When tissues are stained with oil red-O, it selectively binds to triglycerides and lipids with long hydrocarbon chains (Koopman et al., 2001). This staining procedure allows researchers and histologists to identify and quantify lipid accumulation within cells and tissues.

![Figure 1. Expression of positive oil red O staining in the section. Histochemical staining for lipid-specific oil red-O in paraffin embedded section of gastrocnemius muscle of HFD-fed mice. Magnification × 200.](image)

3.2. Localization of lipid distribution in skeletal muscle tissue

Lipid deposition in skeletal muscle tissue plays a pivotal role in muscle metabolism and function (Morales et al., 2017). Here, we observed strength positive oil red-O staining lipid both inside and outside of skeletal muscle cells in Figure 2B and Figure 2C, respectively. A weak oil red-O staining was seen in Figure 2A. Ectopic lipid dipositions other tissues, such as adipose tissue and liver tissue, were recognized to be intracellular and extracellular lipid positions (Narayanan et al., 2016). Consistent with this, in the current study, the data showed intracellular lipid (Figure 2B) and extracellular lipid (Figure 2C) in skeletal muscle tissue. Intracellular lipid deposition refers to the lipids found within the muscle cells. It consists of subsarcolemmal and intermyofibrillar lipids. On another hand, extracellular lipid deposition may be clusters of adipose tissue dispersed within skeletal muscle. Studying the deposition of lipids in skeletal muscle tissue is
important for understanding exercise physiology and metabolic disorders such as obesity and diabetes.

Figure 2. Deposition of lipids in skeletal muscle. Histochemical staining for lipid-specific oil red-O in paraffin embedded section of gastrocnemius muscle of HFD-fed mice: A and B positions for intracellular lipids; C positions for extracellular lipids. Magnification × 200.

3.2. Determine lipid levels in histological section by ImageJ

Figure 3. ImageJ analysis for lipid densities in skeletal muscle section. Lipid positive oil red-O staining at A, B, C positions in Figure 2. (A) data analysis of densities of oil red-O staining. (B) comparison of density levels. Data represent the results of three positions of A, B, and C in Figure 2. Values are means (X) ± standard error (SE). *p < 0.05; ***p < 0.001 compared between each two groups.

The deposition of lipids in skeletal muscle tissue can vary depending on factors such as metabolic homeostasis, age, gender (Kiens and Bente, 2006). Normally, increased lipid content in skeletal muscle tissue is related with several metabolic disorders such as obesity and type 2 diabetes (Van Herpen and Schrauwen-Hinderling, 2008). Therefore, the present study uses the ImageJ method to determine relative lipid content in skeletal muscle
The ImageJ method was used to measure density of oil red-O positive stained lipid in every 3 positions in A, B, C of Figure 2. The results in Figure 3A and 3B show that the highest level of lipid content is in extracellular position C of Figure 2. The lowest level of lipid content is in intracellular position A of Figure 2. Those comparisons are significant differences (Figure 3A, B). The ImageJ method has been used to measure density of tissue section staining in many studies (Koopman et al., 2001; Kim et al., 2003). Therefore, the differences in staining density among positions A (intracellular), B (intracellular), and C (extracellular) in Figure 2 demonstrate the usability of the ImageJ method.

4. CONCLUSIONS

In conclusion, the methodology presented in the current study offers a reliable approach for assessing ectopic lipid deposition in skeletal muscle tissues using oil red-O staining. By providing a detailed protocol including tissue preparation, staining steps, microscopy imaging, and quantitative analysis, this method helps researchers with the applicable tools to study the role of lipids in skeletal muscle pathology and metabolic disorders. Oil red-O staining, by visualizing and quantifying lipid contents within skeletal muscle sections, indicates a preference for ectopic lipid accumulation in extracellular positions within skeletal muscle tissues. In future research perspectives, the methodology mentioned in this research can be further applied to understand the mechanisms underlying ectopic lipid deposition in the metabolic tissues. The distinct effects of intracellular and extracellular lipids in skeletal muscle tissue on systemic metabolic homeostasis should be uncovered. Thus, this methodology gives a contribution for the development of targeted therapeutic strategies for combating metabolic disorders and improving human health.

REFERENCES


PHƯƠNG PHÁP ĐÁNH GIÁ TÍCH TỤ LIPID TRONG MÔ CƠ XƯƠNG SỬ DỤNG NHUỘM OIL RED-O

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Abstract: Sự tích tụ lipid lạc chổ trong các mô cơ xương có liên quan đến các rối loạn chuyển hóa như béo phì và đái tháo đường type 2. Các phương pháp đánh giá đáng tin cậy là rát quan trọng để hiểu được cơ chế cơ bản và phát triển các biện pháp can thiệp. Bài viết này trình bày một phương pháp để xác định sự tích tụ lipid lạc chổ trong mô cơ xương bằng cách sử dụng phương pháp nhuộm oil red-O. Phương pháp này bao gồm việc chuẩn bị và quét hình ảnh, phân tích định lượng bằng imageJ. Kết quả cho thấy oil red-O đúng tính rộ rệt với hàm lượng lipid nội bào và ngoài bào trong mật cát ngang của cơ xương. Mạt độ tích lipid trong cơ xương cho thấy hàm lượng lipid cao nhất có chiều hướng tích tụ ở các vị trí ngoài bào. Do đó, phương pháp này cung cấp cho các nhà nghiên cứu công cụ có giá trị để nghiên cứu vai trò của lipid lạc chổ trong bất thường cơ xương và đánh giá hiệu quả của các biện pháp can thiệp giúp giảm thiểu rối loạn chuyển hóa.

Keywords: Tích tụ lipid, mô cơ xương, nhuộm oil red-O, imageJ.

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