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Exploring Adipose Tissue Functions

Jennifer Jager[1], **Jérôme Gilleron**[1]

[1] Team Cellular and Molecular Pathophysiology of Obesity and Diabetes, C3M, Université Côte d'Azur, Inserm

Corresponding Author

Jérôme Gilleron
gilleron@unice.fr

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Editorial

Characterized by an increase in adipose tissue (AT) mass and pro-inflammatory remodeling of AT immune cells, obesity has arisen as a major worldwide public health issue since it dramatically increases the risk of developing cardiovascular disease, type 2 diabetes, and liver diseases. Hence, exploring AT structure and function has become an important clinical challenge. In this methods collection, we present several state-of-the-art methods developed to investigate AT structure and function under physiological and pathological conditions.

AT is heterogeneous tissue composed of several cell populations including mature adipocytes, adipose progenitor cells (APCs), fibro-inflammatory progenitors (FIPs), endothelial cells, and immune cells. Therefore, in order to characterize this tissue, single cell phenotyping can be performed. In their articles, Cho et al. and Peics et al. provide protocols to isolate and characterize mouse white AT-resident APCs by fluorescence activated cell sorting (FACS)[1,2]. This step is pivotal not only in counting the number of resident APCs, which is an important factor in determining the capacity of AT expansion, but also in further exploring the function of these cells at the single-cell level. Peics et al. also provide a method to isolate mouse white AT-resident FIPs[2], non-adipogenic collagen-producing cells that may contribute to the development of a pro-inflammatory phenotype known to play a part in AT dysfunction. Similarly, Estrada-Gutierrez et al. describe a protocol to simultaneously isolate viable mature adipocytes and stromal vascular fraction cells from human visceral AT biopsies[3]. Altogether, these protocols are the gold-standard to generate a single cell suspension from human and mouse AT, which is the first critical step to further count and functionally phenotype the different AT cell sub-populations.

The relationship between the structure and function is highly relevant in AT. A hallmark of AT dysfunction during obesity is related to the increase in adipocyte size and a profound remodeling of the AT. This remodeling impacts not only the adipocyte and the immune cell populations, but also the lymphatic and the blood vessels network. To appreciate these alterations in the whole AT, Gilleron et al. develop a very simple, inexpensive, and fast AT clearing protocol[4]. This straightforward protocol three-dimensionally visualizes the morphology of whole mouse AT and large human AT biopsies. This includes the neuronal and vascular networks, the adipocytes, and the innate and adaptative immune cell distribution, which are all important parameters to study in obesity and its associated pathologies. To

characterize the impact of obesity on AT lymphatic and blood vessels, Czepielewski et al. provide an *in vivo* method to simultaneously stain the lymphatic arborization and blood vessels by injecting fluorochrome-conjugated lectins[5]. This protocol provides a way to analyze the *in vivo* morphology of both networks, including their density, volume, and branching. Interestingly, combining this labelling strategy with an AT clearing procedure[4] allows high resolution mapping of the whole mouse AT in three-dimensions (3D)[5]. Altogether, these approaches can characterize the structure of AT under physiological and pathophysiological conditions, gaining insight into the correlation between AT structure and function.

Due to its high heterogeneity and tremendous remodeling capacity, analyzing the function of adipocytes within AT *in vivo* is not simple, and so several culture systems have been developed. The main advantage of all these cell culture systems is the high level of control over the cell population and micro-environment. Jager et al. develop a simple protocol to manipulate RNA expression in 3T3-L1 differentiated mature adipocytes[6]. Although this culture system is far from the ideal physiological conditions, 3T3-L1 adipocytes remain functional with respect to insulin signaling, glucose uptake, lipogenesis, and lipolysis. Therefore, this protocol provides a powerful tool to manipulate protein and non-coding RNA expression and study their role on adipocyte functions. To get closer to ideal physiological conditions, Poret et al. describe a method to generate functional mature adipocytes by using adipose-derived stem cells (ADSCs) obtained from rhesus macaque AT[7]. This protocol explains how to isolate primary ADSCs and how to induce their proliferation and differentiation. The authors further suggest that this procedure may be adapted to other species. Although this culture system is closer to ideal physiological conditions compared to the 3T3-L1 cell line, the mature adipocytes derived from primary ADSCs are grown in 2D, which is different from *in vivo*. To cope with this issue, Batista Jr. et al. provide an efficient protocol for a three-dimensional printing tissue culture system[8]. In this work, the authors generate adipospheroids from mouse stromal vascular fraction cells, and they differentiate adipocyte precursors directly within this 3D culture system. This approach is irrevocably closer to ideal physiological conditions than the 2D culture method, but the lack of vessels that might bring nutrients to the adipocytes in the center of the spheroids should be taken into consideration. Modulating protein and non-coding RNA expression, as described[6], within these culture systems may lead to further insight on the functional role of these targets in more physiologically relevant adipocytes. However, such a complex system remains to be established. The major interest of these *ex vivo*/*in vitro* culture systems is the high level of control over the microenvironment, which also includes the factors secreted by the cells. In this respect, the protocol by Akbar et al. isolates and characterizes the extracellular vesicles (EVs) secreted by human adipocytes[9]. Recently, EVs were found to be important metabolic regulators; this method is mandatory to analyze the metabolic impact of these adipocyte-derived EVs under different metabolic situations.

In the present methods collection, we give an overview of state-of-the-art protocols covering several aspects of AT analysis, including *ex vivo* and *in vitro* culture systems, 3D whole tissue exploration, and single cell analysis. Although no culture system is perfect, it is important to choose the culture system that is adapted to the biological question. Therefore, this methods collection provides a large toolbox of procedures to isolate, differentiate, and manipulate adipocytes *in vitro*. Combining all the different methods described here will lead to insight into the morphology and the function of AT.

Disclosures

The authors have nothing to disclose

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