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Two alternative procedures for isolating adipofibroblasts from sheep skeletal muscle

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Abstract. Methods to isolate cells used in the study of adipocyte differentiation are lengthy, potentially damaging to the cells collected, and usually result in a mixed group of cells which are difficult to define clearly. Additionally, much work done on the differentiation of fibroblasts to preadipocytes or preadipocytes to adipocytes has relied on the use of populations of cells which are either embryonic in origin or from genetically unique animals. We therefore report two simple and rapid protocols for obtaining relatively pure populations of preadipocytes from perimuscular fat and from intramuscular fat depots of normal sheep skeletal muscle. In the first procedure, a finely minced preparation of perimuscular adipose tissue is placed directly into flasks for ceiling culture. During the second procedure, free-floating adipocytes, resulting from the enzymatic preparation of satellite cells from skeletal muscle, are placed into ceiling culture. Cells from both isolation procedures attach, grow, and are later harvested for use. These cells demonstrate proliferation and differentiation abilities of normal preadipocytes. Cell populations may be expanded for use in cloning pure populations of adipocytes or used directly in studies to identify mechanisms of adipocyte development and intercellular communication with myogenic cells.

Key words: Adipocyte, Ceiling culture, Preadipocyte, Satellite cell

1. Introduction

A goal of the meat industry is to enhance the efficiency and reduce the cost of meat animal production. Of paramount importance to this industry is the development of methods for regulating the proportion of fat in edible muscle of meat-producing animals. While general patterns of adipose cellularity may be manipulated in vivo [20], it is not yet possible to manipulate adipose cellularity within specific muscles using available production practices.

Traditionally, adipocyte cell culture studies have utilized preadipocyte cell lines (ex. 3T3-L1 cells) [10, 11] or primary cultures of preadipocytes isolated directly from large adipose tissue depots [14]. Procedures for isolating adipocytes from adipose tissue of animals include lengthy incubations with caustic enzymes and potentially harmful centrifugation procedures (Figure 1) [3, 7, 15, 17]. In addition, these isolation procedures by their nature yield a heterogeneous and undefined population of stromal-vascular cells and preadipocytes [19]. A major problem in any adipocyte isolation procedure is that a clear biochemical or molecular definition of a cell which may differentiate and accumulate lipid (preadipocyte or adipofibroblast) is not yet available [1, 16]. Our laboratory, for example, has used procedures involving collagenase digestion and Percoll gradient centrifugation to isolate preadipocytes (cells possessing multilocular fat droplets) from fat tissue slices (Figure 2) [21]. These methods were not only time consuming and labor intensive, but the cell isolates were typically exposed to enzyme digestions and rigorous centrifugal separations that may have modified or eliminated some cells from the total, in vivo, cell population.

Intercellular communication mechanisms may be involved in regulating the amount of fat cellularity in muscle [14]. Cell culture systems have been established to study individual adipocytes and myogenic cells for the definition of mechanisms involved in the paracrine regulation of the growth and differentiation of fat by muscle cells. The protocols described herein offer rapid and efficient techniques for the isolation of fat cells from perimuscular adipose tissue and from satellite cell preparations. Because the fat and muscle tissue samples are obtained at the same time, both tissue types may be immediately processed for the isolation of adipocytes and myogenic satellite cells.