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One modified method for culture of adipocyte precursors

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***Abstract*—The paper details a modified method for isolation and culture of adipocyte precursors from pig adipose tissue. The optimized conditions for adipose more cell culture is the collagenase concentration of 1mg/ml, digestion duration of two hour and FBS concentration of 15%. The method which overcomes the shortcomings of adipocyte suspension culture, can be used to study adipocyte cell biology.**

Keywords- adipocyte precursors; culture

Excessive weight gain arises from the interactions among environmental factors (dietary intake and physical activity), genetic predisposition and the individual behaviours [1, 2]. Thus, genetic predisposition is important determinants for the increasing prevalence of the metabolic syndrome and associated complications, namely obesity [3-5]. The events occurring during the stages of adipocyte metabolism are relatively well characterized, but the molecular mechanisms underlying the stages of adipogenesis remain unknown.

One explanation for the increase in the number of adipocytes is that it uptake of exogenously derived fatty acids (FA) and *de novo* of biosynthesis of FA was increased and then hydrolyzing acylglycerols was decreased. Among the different mechanisms that could lead to fat deposition and obesity, the epigenetic regulation of gene expression has emerged in the last years as a potentially important contributor [1]

A first step towards a better elucidation of the problems mentioned above would be the separation of fat cell precursors from adipose tissue for subsequent characterization. The present work describes such isolation procedures resulting in a homogeneous fraction of cells that develops into mature adipocytes upon subsequent culture.

Epididymal fat pads of healthy pig (aged 18–20 days, Landrace) were removed under sterile conditions and transferred to ice-cold Dulbecco phosphate-buffered saline (DPBS) medium containing penicillin, streptomycin and fungizone (Sangon Biological Engineering Technology &Services Co., Ltd, Shanghai, China). Preadipocytes were isolated as described by Jürgen Janke [6] and modified. In briefly, after excision of blood vessels, adipose tissue was minced into small pieces, washed in DPBS twice, and centrifuged for 10 min at 300g. Then the tissue was decanted into isolation medium (1.0 g tissue/ml DPBS supplemented with 25 mmol/l HEPES, 200 μg/ml kanamycin, 100 units/ml penicillin, 100 μg/ml streptomycin, 3% BSA, and 1.0 mg/ml collagenase) and digested for 2 h at 37°C with constant shaking. Suspended cells were then filtered through a double-layered cotton mesh and spun for 5 min at 700g.

Adipocytes were decanted, washed, and spun (250g, 5 min) four times in the isolation medium without collagenase. Then, 25 ml adipocytes were resuspended in 50 ml adipocyte medium for four hours (DMEM/Ham's F-12 50/50, supplemented with 14 mmol/l $NaHCO_3$, 16.5 μmol/l biotin, 8.5 μmol/l panthotenate, 15 mmol/l glucose, 2 mmol/l l-glutamin, 10.0 mmol/l HEPES, 200 μg/ml kanamycin, 100 units/ml penicillin, 100 μg/ml streptomycin, and 15% FBS). Then, adipocytes were spun (200g, 5 min), decanted, resuspended in the adipocyte medium, washed three times, and cultured for 24 h in the adipocyte medium depending on their attachment efficiency [7]. Repeated counting revealed equal cell numbers in the wells of multiwell plates [6].

Preadipocytes were incubated at 37°C in a humidified atmosphere with 5% CO2. Cells were stimulated to proliferate over 10 days. Under these conditions, 80–90% of the cells could proliferate. Media were changed every 96 h [8]. All cell culture reagents were from Sangon Biological Engineering Technology &Services Co., Ltd, Shanghai, China.

Multiple epidemiologic studies have shown that obesity markedly increases the risk of cardiovascular morbidity and mortality and is a significant risk factor for coronary artery disease and congestive heart failure [9-11]. A recent study demonstrated that obesity promotes atherosclerosis even in adolescent and young adult men [12].

The adipose tissue is now recognized as an endocrine organ that secretes multiple cytokines and growth factors [13,14]. It has also been found to cause progression of atherosclerosis, presumably by promoting endothelial proliferation and neovascularization in the atherosclerotic plaque [15]. The combination of obesity and cardiovascular risk factors is commonly referred to as Syndrome X or metabolic syndrome. However, in addition to influencing other risk factors, obesity also acts as a cardiovascular risk factor, independent of changes in blood pressure (BP), insulin resistance, or cholesterol levels [1]. While the

hemodynamic changes associated with obesity are thought to contribute to the ventricular dysfunction observed in obese patients [16], less is known about the independent mechanisms by which obesity promotes atherosclerosis.

Adipose tissue fat stores in humans are mainly dependent upon fatty acid (FA) supply, FA esterification to triglycerides (TG), and TG breakdown, or lipolysis.

Obesity, defined as increased adipose tissue mass, is a major risk factor for metabolic disorders such as diabetes mellitus, hypertension, and atherogenic diseases. These diseases are influenced by gender, ethnic distinction, genetic factors and daily lifestyle factors such as exercise, habitual smoking and food consumption. Therefore, we are driven by necessity to explore the cause of obesity in consideration of hereditary predisposition and circumstantial conditions.

It has been reported that obesity is a major risk factor for metabolic disorders such as diabetes mellitus, hypertension, and atherogenic diseases [1]. Obesity has become a major global health problem, but its association with hereditary factors, lifestyle factors and biochemical profiles is not understood. The aim of this study was to isolate and culture of adipocyte precursors. Isolated adipocytes floated to the top of the medium in culture plates/flasks. During the first days of culture, they adhered loosely to the ceiling surface. Starting from day 2, the cytoplasm began to spread on the ceiling surface. While the main cell body remained spherical, the cytoplasmic rim became flattened and tentacle-like. Large adipocytes exhibited attachment, but their cytoplasm did not spread out widely. Figure 1 shows adipocytes that were attached to the ceiling surface after 16 h and 48 h of incubation. Attachment efficiency to the ceiling surface of coverslips varied between 50 and 80%. Cell viability was checked by exclusion of trypan blue and was >85% at the end of 7–14 days of incubation. Adipocytes maintained their unilocular characteristic as shown in Fig. 2. Multiple small lipid droplets were observed at the periphery of some cells, indicating lipogenic activity.

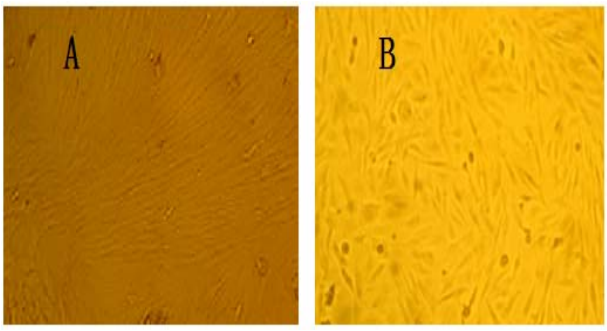

Figure 1. Cells from adipose tissue in modified ceiling culture.

(A)16 h -old adipocytes. (B) 48 h -old adipocytes.

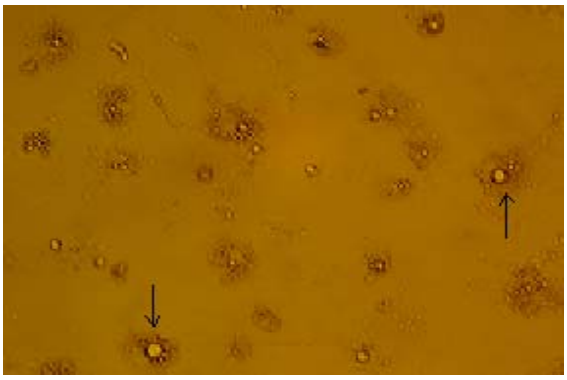

Figure 2. 7-day-old adipocytes in modified ceiling culture (Arrows indicate the multiple small lipid droplets).

This short communication describes the isolation and culture of adipocyte precursors in practice. In summary, the optimized conditions for adipose cells culture are collagenase concentration of 1mg/ml, digestion duration of 2 hour and FBS concentration of 15%, and another global manipulation is as described by Jürgen Janke [6]. To assess the validity of the modified ceiling culture method, we have demonstrated proliferation of the modified ceiling-cultured adipocytes under controlled conditions. The finding shows that the modified ceiling-cultured adipocytes are functional and can be used to study adipocyte cell biology.

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