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A combination of octanoate and oleate promotes *in vitro* differentiation of porcine intramuscular adipocytes

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Abstract

To understand the relationship between intramuscular adipogenesis in the pig and the supply fatty acids, we established a clonal porcine intramuscular preadipocyte (PIP) line from the marbling muscle tissue of female Duroc pig. Confluent PIP cells exhibited a fibroblastic appearance. Their adipogenic ability was investigated using confluent PIP cells after exchanging growth medium for adipogenic medium containing 50 ng/mL insulin, 0.25 μM dexamethasone, 2 mM octanoate, and 200 μM oleate. Appropriate concentrations of octanoate and oleate for the induction of adipogenesis were determined from the ability of cells to accumulate lipid and the toxicity of fatty acids. When cells were cultured in differentiation medium for 8 days, large numbers of lipid droplets were observed in differentiated PIP cells, and their cytosolic TG content increased in a time-dependent manner. While oleate only induced the expression of PPARγ mRNA, but not that of C/EBPα, octanoate significantly induced the expression of both PPARγ and C/EBPα mRNA. Octanoate and oleate accelerated the inducing effect of insulin and dexamethasone on the expression of aP2 mRNA. These results indicate that a combination of octanoate and oleate synergistically induced PIP adipogenesis, and that the stimulation of octanoate was essential to the trigger for the adipogenesis in PIP cells.

Keywords: Adipocyte; Adipogenesis; Octanoate; Oleate; Porcine; Intramuscular preadipocyte; PPARgamma; C/EBPalpha; aP2

1. Introduction

Adipose tissue is the main organ accumulating excess consumed energy as neutral lipid, and this energy is mobilized when energy consumption does not cover the body's need. This organ plays a pivotal role in systemic metabolism; however, prolonged abnormal energy balance causes metabolic syndromes such as obesity and type 2 diabetes (Holst and Grimaldi, 2002; MacDougald and Mandrup, 2002). Adipose tissues are composed of adipocytes that are differentiated from precursor cells such as mesenchymal stem cells. Muscle satellite cells also have a potential of differentiation into adipocytes (Asakura et al., 2001; Abdallah et al., 2004; Shefer et al., 2004; Yamanouchi et al., 2006). Most *in vitro* studies on adipocyte differentiation and metabolism have been performed using the murine preadipocyte cell line, 3T3-L1, which has enabled researchers to establish a great deal of knowledge on adipogenesis (Ntambi and Kim, 2000; Feve, 2005). However, adipogenesis in this cell line has not always reflected that of other species (Aso et al., 1995; Kim et al., 2000; Matsubara et al., 2005). In one study in pigs, Nakajima et al. (2003) established a clonal preadipocyte line (PSPA) from the subcutaneous tissue of a fetal pig. The authors noted that PSPA differentiate into adipocytes following stimulation with octanoate, and further that growth arrest of PSPA cells following octanoate stimulation is required for PSPA adipogenesis.

Fatty acids are not only an essential nutrient source for mammals, but also modulate adipocyte differentiation. Octanoate, a medium-chain fatty acid (MCFA), accelerates adipocyte differentiation (Aso et al., 1995; Nakajima et al., 2003; Takenouchi

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et al., 2004). In contrast, other studies have proposed that octanoate attenuates adipogenesis in 3T3-L1 preadipocytes (Han et al., 2002) and inhibits triglyceride synthesis in 3T3-L1 and human adipocytes (Guo et al., 2003). These contradictory reports suggest that octanoate has different effects among different species. Long-chain fatty acids (LCFAs) also have several biological properties. LCFAs promote preadipocyte differentiation (Gao et al., 2000; Ding and Mersmann, 2001; Matsubara et al., 2005) and activate PPARγ, a master regulator of adipogenesis (Keller et al., 1993; MacDougald and Lane, 1995). LCFAs also stimulate adipogenic differentiation in human vascular smooth muscle cells (Davies et al., 2005). This is particularly the case for oleate, a monosaturated fatty acid that induces adipogenesis more fully than other LCFAs (Ding and Mersmann, 2001; McNeel and Mersmann, 2003). It was recently reported that oleate stimulates adipogenesis of in 3T3-L1 cells and OP9 mouse stroma cells, and that oleate is an important reagent for adipogenesis (Wolins et al., 2006).

Adipocyte differentiation begins during development, and adipose tissue is formed in various parts of the body before birth, while intramuscular adipose tissue is the final adipose tissue to be formed. It was previously reported that lipid metabolisms and differentiation levels of intramuscular adipocyte differed from that of other adipose tissues (Gardan et al., 2006, 2007). A clonal line of bovine intramuscular preadipocyte (BIP) has been established for the *in vitro* study of intramuscular adipogenesis in cattle (Aso et al., 1995). Adipogenesis of BIP cells occurs when cultured with insulin, dexamethasone, acetate and octanoate. A method has been established for the adipogenesis of porcine stromal–vascular (SV) cells from neonatal pig semitendinosus muscles as well as co-cultures of myotubes and preadipocytes (Hausman and Poulos, 2004, 2005). However, there have hitherto been no reports on the establishment of a porcine intramuscular preadipocyte line.

In order to understand porcine intramuscular adipogenesis, we have established a clonal porcine intramuscular preadipocyte (PIP) line from the *Musculus longissimus thoractis* of a Duroc pig, a breed that is able to produce meat with a high degree of marbling (Wood et al., 1999; Suzuki et al., 2003). We used this cell line for the investigation of adipogenic differentiation in these PIP cells using a combination of octanoate and oleate.

2. Materials and methods

2.1. Animal

In this study, a female Duroc pig (*Sus scrofa*, 5 months old) was kept at the Graduate School of Agricultural Science, Tohoku Univ. and tissues were taken for the preparation of the clonal cell line. The strain of Duroc pig used was selected over 7 generations for high levels of intramuscular fat accumulation (Suzuki et al., 2005). The experiments were conducted in accordance with the Guideline for Animal Experimentation at Tohoku University.

2.2. Establishment of clonal PIP line and cell culture

Isolated preadipocytes were obtained using a modified method of Forest et al. (1987). Marbling muscle tissue was obtained from the *Musculus longissimus thoractis* by biopsy. Immediately, the tissue was washed with serum-free low-glucose Dulbecco's modified Eagle's medium (DMEM, 1 g/L glucose) (Gibco-BRL, Grand Island, NY, USA) three times, minced and digested by gently shaking in DMEM containing 2 mg/mL collagenase (Wako Pure Chemicals, Osaka, Japan) and 2% bovine serum albumin (Sigma Aldrich Chemicals, St. Louis, MO, USA) at 37°C for 30 min. The digested tissue was then filtered through a stainless steel mesh (pore size 105 μm), centrifuged for 7 min at 200 g, and the pellet was collected. The pellet was washed once with serum-free low-glucose DMEM, two times with first culture medium, low-glucose DMEM supplemented 10% fetal bovine serum, 100 U/mL penicillin and 100 μg/mL streptomycin. The cells were resuspended in culture medium and seeded into an 80-cm^2 flask (Nalge Nunc International, Rochester, NY, USA). The medium was exchanged every other day. One month later, the cells reached confluence. They were detached using 0.02% trypsin and 0.01% EDTA in phosphate-buffered saline (PBS, pH 7.4) and seeded into another flask at a density of 10^4 cells/cm^2. The cells were passaged when they were confluent. After 10 passages, the cells were cloned by a process of limiting dilution. Five weeks later, the different clones were grown separately and a clonal PIP cell was selected on the basis of its ability to proliferate. At the 16th

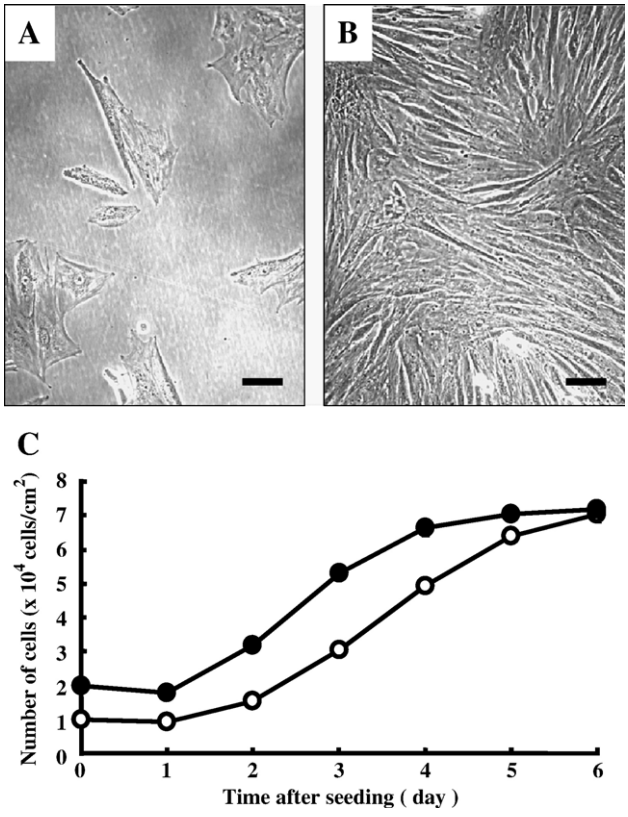

Fig. 1. Morphology and growth of PIP cells. Photographs are PIP cells at 1 day (A) and 5 days (B) after seeding at a density of 10^4 cells/cm^2. Confluent PIP cells exhibit the appearance of fibroblast. Cells were seeded into 6 well plates at a density of 1.0 and 2.0×10^4 cells/cm^2 and counted on the days indicated using the trypan blue dye exclusion method with a hemocytometer (C). Cells were confluent after 4–6 days. The data are expressed as means ± SEM ($n=3$). Scale bar: 50 μm.