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Effects of fatty acids on cytosolic TAG accumulation in primary cultured bovine mammary epithelial cells

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ABSTRACT

We examined the effects of short-, medium- or long-chain fatty acids on the accumulation of cytosolic triacylglycerol (TAG) in bovine mammary epithelial cells (bMEC). Octanoate and long-chain fatty acids stimulated TAG accumulation in a concentration-dependent manner from 1 to 10 mM and from 50 to 400 μM, respectively, as well as mRNA expression of CD36 and UCP2. Octanoate, oleate and linoleate increased lipid droplet formation. Leptin mRNA expression was significantly reduced by addition of acetate and butyrate but was elevated by addition of oleate and linoleate. Long-chain fatty acids stimulated αs1-casein mRNA expression.

KEY WORDS: mammary epithelial cell, fatty acids, cytosolic TAG accumulation, lipid droplet

INTRODUCTION

Mammary epithelial cells are well known to accumulate TAG in the cytosol. During lactation, lipid droplets, which comprise a TAG-rich core, are formed and enveloped by a surface coat composed of proteins and polar lipid in endoplasmic reticulum (ER) (Dylewski et al., 1984; Deeney et al., 1985; Mather and Keenan,1998). The sources of TAG are derived either from plasma lipoproteins or by *de novo* synthesis from small molecule precursors. Although mammary epithelial cells are lipogenic, preferential utilization of fatty acids for TAG synthesis and the gene expression involed in lipid metabolism are still unclear.

In this study, using bovine mammary epithelial cells (bMEC) we examined whether cytosolic TAG accumulation is stimulated by the addition of various free fatty acids to the cell culture medium. Short- (acetate and butyrate), medium- (octanoate) or long- (palmitate, stearate, oleate and linoleate) chain fatty acids were

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used. Furthermore, we related these treatments to the expression of leptin, CD36 (a fatty acid translocase), UCP2 (uncoupling protein 2) and αs1-casein mRNA.

MATERIAL AND METHODS

Materials

Fatty acids sodium salts, Dulbecco's modified Eagle's medium (DMEM), foetal calf serum (FCS), and fraction V fatty acid-free bovine serum albumin (BSA) were purchased from Sigma-Aldrich, St. Louis, MO, USA. Triglyceride G Test Kit was from Wako, Osaka, Japan.

Cell culture

Bovine MEC isolated from a 102-day-pregnant Holstein heifer were cultured in DMEM containing 10% FCS as previously described (Katoh et al., 2001). At confluency, stimulation with short-, medium- or long-chain fatty acids was performed for 1day or 7 days, by the addition of 10 mM acetate, butyrate or octanoate, or 400 μM palmitate, stearate, oleate, or linoleate complexed with fatty acid-free bovine serum albumin, respectively.

Measurement of TAG contents and oil red O staining

TAG in the cell lysate was extracted with the same volume of chloroform-methanol (2:1, v/v) and quantified enzymatically using a Triglyceride G Test Kit. The TAG contents were normalized for protein in each well. Cells were stained with oil red O and were counterstained with haematoxylin.

Northern blot analysis and semi-quantitative RT-PCR

Expression of mRNA was quantified using Northern blot analysis or semi-quantitative RT-PCR.

Statistical analysis

In all experiments values are expressed as means ±S.E. of the mean. Statistical significance was estimated by means of one-way ANOVA followed by the Duncan's multiple range test. The test was considered significant at $P<0.05$.

RESULTS

Cytosolic TAG accumulation

Acetate and butyrate did not stimulate TAG accumulation during 7 days of culture (Figure 1). The cells cultured with octanoate began to accumulate cytosolic TAG after 3 days or more of culture compared to the control. The addition of palmitate, stearate, oleate, or linoleate significantly increased cytosolic TAG contents compared to the control. These fatty acids induced TAG accumulation in a concentration-dependent manner.

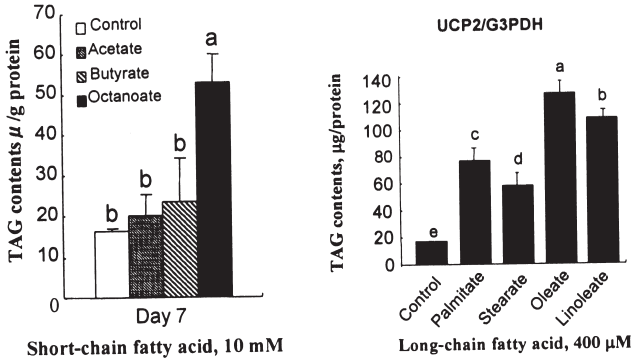

Figure 1. Effects of fatty acids on TAG accumulation in bMEC

Formation of lipid droplets

The cells cultured with octanoate formed lipid droplets in the cytosol. In the cells treated with long-chain fatty acids, lipid droplets were observed only when cultured with unsaturated fatty acids, but not with saturated fatty acids. Octanoate induced the formation of lipid droplets with lower TAG contents than those stimulated with the saturated fatty acids.

Effects on mRNA expression of leptin, CD36 and UCP2

Culture with acetate or butyrate significantly reduced leptin mRNA expression ($P<0.05$). However, cultured with oleate and linoleate significantly incresed leptin mRNA expression ($P<0.05$). The expression of CD36 mRNA was dramatically elevated in the cells cultured with octanoate and long-chain fatty acids. UCP2 mRNA expression was significantly elevated in the cells cultured with all fatty acids (Figure 2).

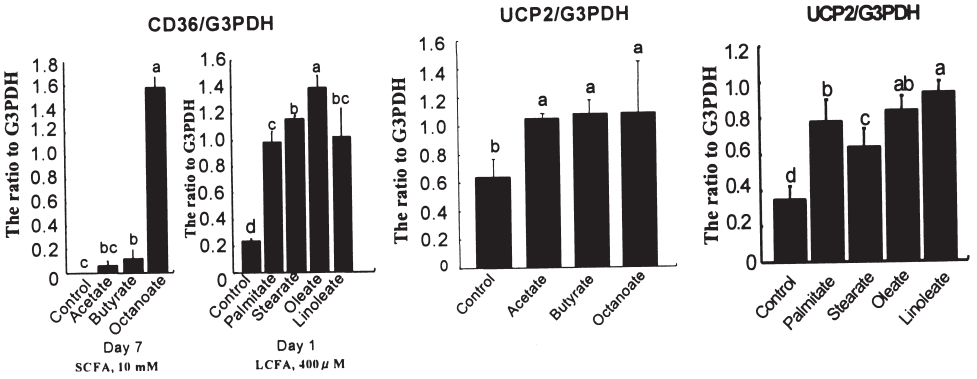

Figure 2. The expression of CD36 and UCP2 mRNA following fatty acids

DISCUSSION

Exogenous octanoate and long-chain fatty acids induced cytosolic TAG accumulation in a concentration-dependent manner. Furthermore, mRNA expression of CD36 and UCP2 was markedly elevated by these fatty acids. These findings indicate that octanoate and long-chain fatty acids have a biological significance for TAG accumulation *via* elevated CD36 synthesis in bMEC.

However, cellular metabolism appeared to be different between saturated (palmitate and stearate) and unsaturated (oleate and linoleate) fatty acids because saturated fatty acids did not induce lipid droplet formation, despite increased TAG accumulation, whereas unsaturated fatty acids did induce lipid droplet formation. Furthermore, we found that octanoate induced the formation of lipid droplets, despite lower cellular TAG contents, than those stimulated with saturated fatty acids.

Leptin mRNA expression was significantly elevated in the cells cultured with unsaturated fatty acids, although that expression was slightly reduced by treatment with acetate or butyrate. This finding suggests that leptin mRNA expression associated with the formation of lipid droplets. The expression of αs1-casein mRNA was markedly enhanced along with the accumulation of cytosolic TAG. This finding suggests that the accumulation of cytosolic TAG may be an indicator of the differentiation of mammary epithelial cells.

CONCLUSIONS

Octanoate and long-chain fatty acids induced the accumulation of cytosolic TAG and markedly enhanced the mRNA expression of CD36 and UCP2 in bMEC, although effects on lipid droplet formation and leptin mRNA expression were different among fatty acids. long-chain fatty acids also induced the expression of αs1-casein mRNA.

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