Journal of Dairy Research (2004) **71** 398–404. © *Proprietors of Journal of Dairy Research 2004*
DOI: 10.1017/S0022029904000408 *Printed in the United Kingdom*

Octanoate stimulates cytosolic triacylglycerol accumulation and CD36 mRNA expression but inhibits Acetyl coenzyme A carboxylase activity in primary cultured bovine mammary epithelial cells

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Received 3 September 2003 and accepted for publication 23 December 2003

Mammary epithelial cells, which express and secrete leptin into milk, accumulate triacylglycerol (TAG). We examined effects on the accumulation of cytosolic TAG of addition of short- (acetate and butyrate) or medium- (octanoate) chain fatty acids to the medium bathing bovine mammary epithelial cells (bMEC). Octanoate stimulated the accumulation of TAG in a concentration-dependent manner from 1 to 10 mM and increased lipid droplet formation and mRNA expression of CD36 (a fatty acid translocase). Additionally, expression of a peroxisome proliferator activated receptor (PPAR) γ 2 protein that is a lipid-activated transcription factor, was increased by the addition of acetate or octanoate. However, leptin mRNA expression was significantly reduced by addition of acetate or butyrate. Both short- and medium-chain fatty acids inhibited acetyl coenzyme A carboxylase (ACC) activities, which is pivotal in lipid synthesis, but elevated expression of uncoupling protein 2 (UCP2) mRNA, which is important in energy expenditure. These results suggest that octanoate induces cytosolic TAG accumulation and the formation of lipid droplets, and that acetate and butyrate inhibit leptin expression and lipid synthesis in bMEC.

Keywords: SCFA, leptin, CD36, PPAR γ 2, ACC activity, UCP2.

Short-chain fatty acids (SCFA) have several specific biological properties different from those of long-chain fatty acids (LCFA) (Anderson, 1995; Bach et al. 1996). In particular, although SCFA are well known as a major energy source for ruminants, some direct effects of SCFA on peripheral tissues have also been elucidated in exocrine and endocrine cells (Katoh & Obara, 2001).

During lactation, in mammary epithelial cells (MEC), cytoplasmic lipid droplets are formed in the endoplasmic reticulum (ER) to make milk lipid globules. These micro-lipid droplets are budded from the ER, and comprise a core rich in triacylglycerol (TAG) surrounded by membrane composed of proteins and polar lipid (Dylewski et al. 1984; Deeney et al. 1985; Mather & Keenan, 1998). Fatty acids in milk TAG are derived from two sources: first, synthesis *de novo* from volatile fatty acids; and second, the uptake of lipid from plasma by the mammary epithelium (Clegg et al. 2001). The fatty acid composition of milk lipids is highly variable and depends on the lipid composition of the diet. Despite a relatively large content of medium-chain fatty acids in milk, rats fed with a diet high in lipid during pregnancy and lactation markedly increased secretion of oleic and linoleic acids in the milk, accompanied by elevation of lipoprotein lipase (LPL) activity, while rats fed a diet low in lipid increased medium-chain fatty acid (C8–C14) contents accompanied by an elevation of mammary gland lipogenesis but not LPL activity (Prado et al. 1999). Those results indicate that milk lipid synthesis by the mammary gland depends on the intake of fatty acids. However, preferential utilization of fatty acids for lipid synthesis *de novo* in MEC remains to be confirmed.

Leptin is a 16-kDa protein encoded by the *ob* gene and is produced and secreted from adipocytes, playing an important role in regulating food intake, energy expenditure

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and fat mass, probably by acting on the hypothalamus or peripheral tissues through its cognate receptor (Zhang et al. 1994; Bai et al. 1996; Friedman, 1997; Minokoshi et al. 2002). The function of leptin has been demonstrated to be more complex and its role in peripheral tissues has not been fully elucidated. Recently, leptin expression and secretion into milk with lipids has been demonstrated in MEC (Aoki et al. 1999; Smith & Sheffield, 2002). Leptin receptors have also been found in MEC of sheep (Laud et al. 1999). Furthermore, leptin stimulates the proliferation of human breast cancer cells (MCF-7) (Marie-Noelle et al. 2002). These observations suggest that leptin plays an important role in mammary duct growth and morphogenesis.

In addition, PPAR γ 2 is a class of the nuclear hormone receptor family involved in lipid metabolism and adipocyte differentiation (Green, 1995; Schoonjans et al. 1996; Spiegelman & Flier, 1996). PPAR γ 2 is a lipid-activated transcription factor which controls the expression of myriad genes including leptin, CD36, ap2 (an adipocyte-specific fatty acid binding protein), and phosphenolpyruvate carboxykinase (PEPCK) (Tontonoz et al. 1994, 1995; Kallen & Lazar, 1996). Although PPAR γ 2 was at first thought to be expressed exclusively in adipocytes, recent studies have found it in murine and human MEC lines (Elstner et al. 1998; Gimble et al. 1998). On the other hand, CD36 is highly expressed in adipocytes and MEC and is active in the uptake and synthesis of LCFA and the secretion of TAG. Thiazolidinediones, which are PPAR γ ligands that sensitize peripheral tissues to insulin, stimulate the expression of uncoupling protein 2 (UCP2) mRNA in murine white and brown adipose tissues and skeletal muscle (Camirand et al. 1998).

In the present study using bovine MEC (bMEC), we examined whether acetate, butyrate and octanoate stimulate cytosolic TAG accumulation and activities of ACC and the expression of leptin, CD36 and UCP2 mRNA and PPAR γ 2 protein. We discuss relations between these parameters in respect to lipogenesis and energy expenditure in bMEC.

Materials and Methods

Cell culture

Bovine MEC were raised from the mammary gland of a 102-d-pregnant Holstein heifer. Mammary tissue segments were digested by collagenase (500 U/ml) (E.C 3.4.24.3, collagenase II, Sigma–Aldrich, St. Louis, MO, USA) for 30 min at 37 °C, and isolated cells were cultured in Dulbecco's Modified Eagle medium (DMEM) (Sigma–Aldrich) supplemented with 10% fetal calf serum (FCS), 2 mM-glutamine, 100 U penicillin/ml, and 100 μg streptomycin/ml under 5% CO_2 and air at 37 °C. Fluorescence-activated cell sorting (FACS) analysis for cytokeratin expression in the cells at passage 19 revealed that they were composed of 92% MEC. In the present experiment, the cells were used between passages 9 and 19. The cells were seeded at $1{\cdot}0 \times 10^4$ cells/cm^2 in a 6-well plate and 100-mm dish (Corning, NY, USA) and grown in the above same condition until confluency. Then, stimulation with fatty acids was performed for 1–7 d in DMEM containing 10% FCS, 2 mM-glutamine, 100 U penicillin/ml and 100 μg streptomycin/ml with 10 mM-acetate, butyrate or octanoate (Wako, Osaka, Japan) as the sodium salts.

Measurement of TAG contents in the cytosol

Cultured cells on 6-well plates were washed twice with ice-cold phosphate-buffered saline (PBS), scraped off into 0·4 ml of 25 mM-Tris–HCl (pH 7·5) and 1 mM-EDTA, and then homogenized with a micro-homogenizer (Ieda Trading, Tokyo, Japan). Protein concentrations were determined using BCA protein kit (Pierce, Rockford, IL, USA). TAG in the cell lysate was extracted with the same volume of chloroform-methanol (2 : 1, v/v) and quantified enzymically using a Triglyceride G Test Kit (Wako, Osaka, Japan). TAG contents were normalized for protein in each well. All experiments were performed at least in triplicate.

Oil red O staining

After stimulation, cells were washed twice with ice-cold PBS, fixed in 10% formalin for 10 min, rinsed in distilled water and infiltrated into 60% isopropanol for 1 min and stained with oil red O (Wako, Osaka, Japan) for 15 min. Cells were counterstained with haematoxylin for 5 min with rinsing in quarter-saturated $LiCO_3$.

RNA preparation

After stimulation with the fatty acids, cells were washed twice with ice-cold PBS and scraped off into TRIsol reagent. Total RNA was isolated from the cells using the TRIsol extraction method according to the manufacturer's instructions (Gibco BRL, Grand Island, NY, USA). Poly $(A)^+$ mRNA was isolated from total RNA using a mRNA isolation kit (Miltenyi Biotec, Gladbach, Germany). Concentration of isolated mRNA was determined from the optical density at 260 nm and its purity from the wavelength ratio of 260/280 nm.

cDNA cloning and semi-quantitative RT-PCR analysis

cDNA fragments for leptin and glyceraldehyde-3-phosphate dehydrogenase (G3PDH) were obtained by RT-PCR as described previously (Yonekura et al. 2002). cDNA fragments for CD36 and UCP2 were obtained using the following primers: bovine CD36 forward, 5′-AACCACTTTCATCAGACCCG-3′, reverse, 5′-ACGTGTCATCCTCAGTTCCA-3′, and bovine UCP2 forward, 5′-CCTGCTTCCTTTTCCTTCCT-3′, reverse, 5′-TATGGGTGACGTGCTAGTTG-3′. PCR generated 475-bp and 264-bp fragments

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of the bovine CD36 and UCP2 gene, respectively. First strand cDNA was synthesized with random hexamer as a primer and then directly used for the PCR reaction. PCR amplification was done using the cDNA from 20 ng Poly $(A)^+$ mRNA in a final volume of 50 µl. The reaction was performed using a commercial kit (Ready-to-go RT-PCR Beads, Amersham Pharmacia Biotech, Piscataway, NY, USA) according to the manufacturer's instructions. To ensure that amplification of these genes was within the exponential range, different numbers of cycles were run (PC-808 Astec, Tokyo, Japan). Finally, 26, 19, 29, and 28 cycles of amplification were chosen to study leptin, G3PDH, CD36, and UCP2 mRNA expressions, respectively. In all assays, reactions with RT negative amplification were included (results not shown). PCR products were analysed in a 2·0% agarose gel, stained with ethidium bromide and photographed. These PCR products had been cloned and sequenced to be the same as those deposited in the Genbank/EMBL/DDBJ database.

Measurement of acetyl coenzyme A activities

ACC activity was determined by measuring the rate of oxidation of NADPH with a spectrophotometer at 340 nm (Wada & Tanabe, 1983). Protein concentration was determined by BCA protein kit (Pierce). The experiments were repeated at least three times on separate days.

Western blot analysis

After stimulations with the fatty acids, cells were washed twice with ice-cold PBS and scraped off into ice-cold PBS. After centrifugation (400 ***g*** for 10 min and 20 600 ***g*** for 5 min), the pellet was immediately homogenized in a lysis buffer (20 mM-Tris–HCl, pH 7·5, 2 mM-EDTA, 0·5 mM-EGTA, 1% aprotinin, 2 mM-phenylmethylsulphonyl fluoride) on ice and then centrifuged at 20 600 ***g*** for 5 min. The supernatant was collected, and protein content was determined using a BCA protein kit (Pierce). Cell extracts (60 µg) were heated at 100 °C for 5 min in the sample buffer and were separated by SDS-PAGE according to Laemmli (1970), with modification. SDS-polyacrylamide 12·5% gel were electrotransferred onto Sequi-Blot polyvinylidene difluoride (PVDF) membranes (Bio-Rad, Hercules, CA, USA). For immunodetection of PPAR γ 2 proteins, PVDF membranes were blocked with 5% skim milk (Snow Brand Milk Products Co. Ltd., Sapporo, Japan)/PBS overnight at 4 °C, followed by incubation for 1 h at room temperature with primary antisera (Alexis Co., Lausen, Switzerland). The membranes were then washed three times with 0·2% Tween-20 (Sigma–Aldrich)/PBS, followed by incubation for 1 h at room temperature with second antibody (Amersham Pharmacia Biotech). The membranes were washed six times with 0·2% Tween-20/PBS and were visualized by using ECL + pluse Western Blotting Detection system (Amersham Pharmacia Biotech).

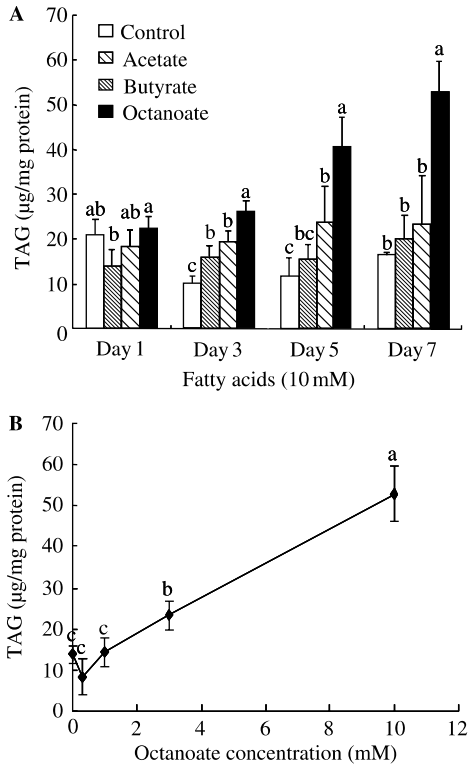

Fig. 1. Effects of acetate, butyrate and octanoate on TAG accumulation in bMEC. (A) bMEC were treated with DMEM containing 10% FCS plus 10 mM-acetate, butyrate, or octanoate for 1, 3, 5 or 7 d, respectively. Values with different letters are statistically different among the columns on the same day ($P < 0·05$). (B) bMEC were treated with various concentrations of octanoate for 7 d. Results are expressed as means ± SEM of triplicate dishes.

Statistical analysis

In all experiments except for Western blot analysis, values are expressed as means ± SEM. Statistical significance was estimated by means of one-way ANOVA followed by Duncan's multiple range test. The test was considered significant at $P < 0·05$.

Results

Cytosolic TAG accumulation

To ascertain whether SCFA induce the accumulation of cytosolic TAG in primary cultured bMEC, we tested it with SCFA at 10 mM for 7 d. Cells cultured with octanoate began to accumulate cytosolic TAG after 3 d or more of culture compared with the control (Fig. 1a). To determine whether the effect of octanoate on bMEC has biological significance, we observed the dependency of TAG accumulation on octanoate concentrations in bMEC cultured during 7 d. The results show that bMEC accumulated cytosolic TAG in a concentration-dependent manner (Fig. 1b).

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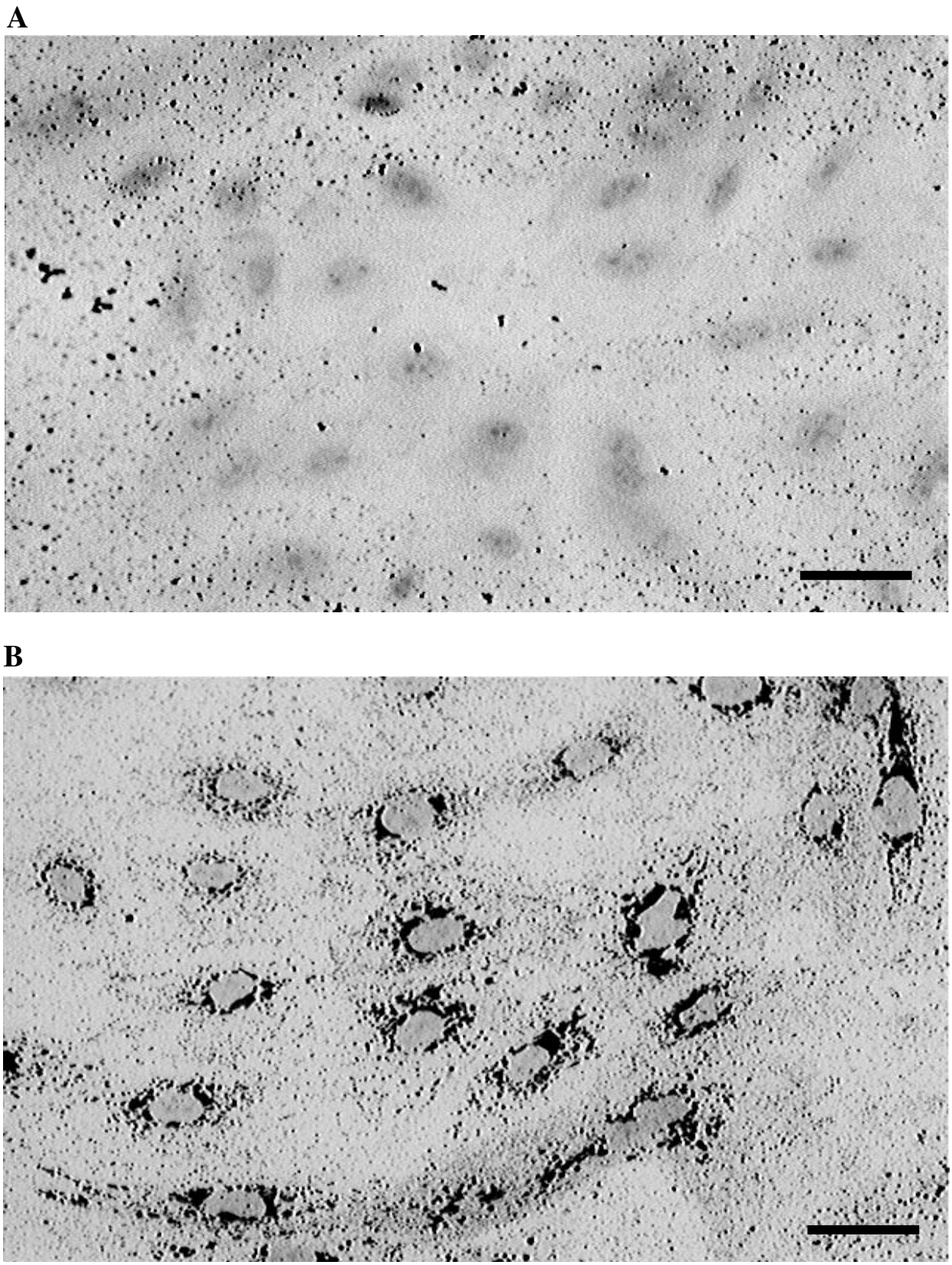

Fig. 2. Effects of octanoate on lipid droplet formation in bMEC. Cells were cultured with octanoate for 7 d and stained with oil red O and haematoxilin. A: no octanoate; B: octanoate (10 mM). Results are representative of three separate experiments. Bar indicates 10 μm.

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Table 1. Effects of SCFA on expression of leptin, CD36 and UCP2 in mammary epithelial cells

Values (in arbitrary units, relative to the expression of glyceraldehyde 3-phosphate dehydrogenase) are means ± SEM for $n=3$

	Leptin	CD36	UCP2
Control	$1{\cdot}179 \pm 0{\cdot}179^{ab}$	$0{\cdot}000 \pm 0{\cdot}000^{c}$	$0{\cdot}637 \pm 0{\cdot}131^{b}$
Acetate	$0{\cdot}877 \pm 0{\cdot}177^{c}$	$0{\cdot}063 \pm 0{\cdot}036^{bc}$	$1{\cdot}047 \pm 0{\cdot}036^{a}$
Butyrate	$0{\cdot}991 \pm 0{\cdot}012^{bc}$	$0{\cdot}119 \pm 0{\cdot}077^{b}$	$1{\cdot}078 \pm 0{\cdot}098^{a}$
Octanoate	$1{\cdot}246 \pm 0{\cdot}187^{a}$	$1{\cdot}576 \pm 0{\cdot}092^{a}$	$1{\cdot}085 \pm 0{\cdot}359^{a}$

Values within a column without a common superscript letter are statistically different ($P < 0{\cdot}05$)

Formation of lipid droplets

To determine whether the formation of lipid droplets is related to the accumulation of cytosolic TAG, we stained lipid droplets in bMEC with the oil red O staining method cultured with each fatty acid for 7 d. Cells cultured only with octanoate formed lipid droplets in the cytosol (Fig. 2). Although lipid droplet formation was also observed in the cells 5 d after culture with octanoate, the size was much smaller than that 7 d after culture (not shown). In our preliminary study, oleate or linoleate (400 μM) dramatically induced the formation of cytosolic lipid droplets.

Effects on expression of leptin, CD36 and UCP2

To investigate the effect of acetate, butyrate and octanoate on the expression of genes involved in lipid metabolism, semi-quantitative RT-PCR analysis for leptin, CD36 and UCP2 mRNA expression was employed in the cells cultured with fatty acids for 7 d. Culture with acetate or butyrate at 10 mM significantly reduced leptin mRNA expression (Fig. 3a and Table 1). This reduction in the cells cultured with acetate and butyrate was 74% and 84% of control, respectively (Table 1). The expression of CD36 mRNA was dramatically elevated in the cells cultured with octanoate (Fig. 3a and Table 1). It is likely that acetate and butyrate also induced the expression of CD36 mRNA, but it was much less than was the case for octanoate. The up-regulation induced by acetate, butyrate and octanoate seemed to depend on the number of carbon atoms in the fatty acids. UCP2 mRNA expression was significantly elevated in the cells cultured with each fatty acid. The expression was increased 1·6-, 1·7- and 1·7-fold in the cells cultured with acetate, butyrate and octanoate, respectively (Fig. 3a and Table 1).

Activities of ACC and expression of PPAR γ 2 protein

Finally, we investigated activity of ACC and the expression of PPAR γ2 protein in the cells treated with fatty acids. All fatty acids significantly suppressed ACC activities (Table 2). The activity of the control was $60{\cdot}7 \pm 0{\cdot}91$ nmol/min per mg protein, which was significantly decreased to $38{\cdot}8 \pm 1{\cdot}21$, $14{\cdot}6 \pm 0{\cdot}46$ and $22{\cdot}9 \pm 0{\cdot}39$ nmol/min per mg protein by treatment with acetate, butyrate and octanoate, respectively. PPAR γ 2 protein expression was increased in the cells cultured with acetate and octanoate (Table 2).

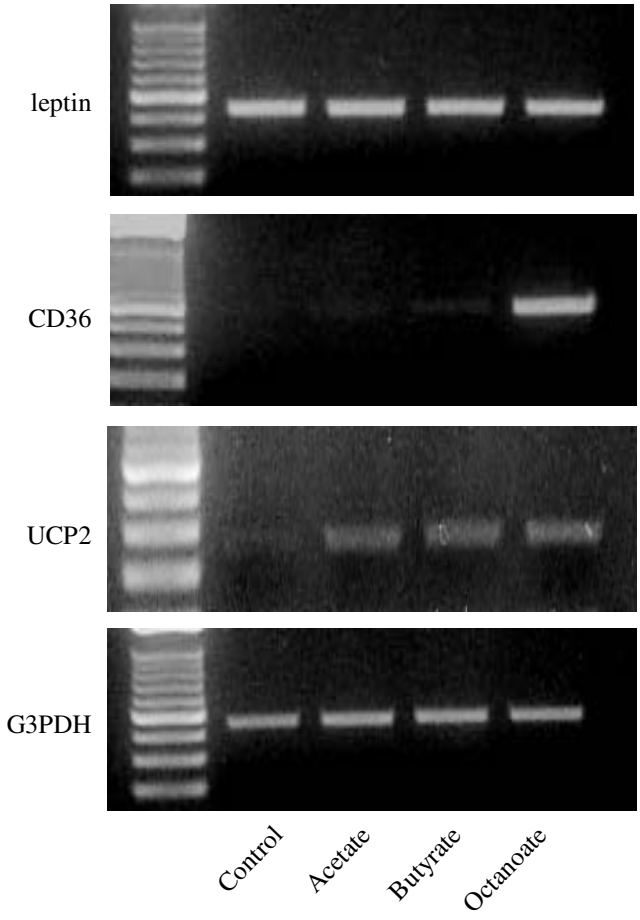

Fig. 3. Expression of leptin, CD36, UCP2 and G3PDH mRNA following acetate, butyrate or octanoate stimulation. Cells were cultured with acetate, butyrate, or octanoate at 10 mM for 7 d, or in the absence of SCFA. Poly $(A)^+$ mRNA (20 ng) was reverse-transcribed followed by PCR amplification with specific sets of primers for bovine leptin, CD36, UCP2, and G3PDH. PCR products were run on a 2·0% agarose gel and stained with ethidium bromide and were analysed densitometrically using NIH image software. Results are representative of three separate experiments.

Discussion

The present study showed that octanoate induced cytosolic TAG accumulation and lipid droplet formation in a concentration-dependent manner in bMEC (Fig. 1a and b). These findings suggest that octanoate has a biological significance in bMEC. In human normal and malignant MEC, treatment with butyrate stimulates accumulation of lipid droplets and differentiation (Davis et al. 2000). However, in bMEC as shown in this study, although butyrate

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Table 2. Effects of SCFA on ACC activity and expression of PPAR γ 2 protein in bovine mammary epithelial cells

Values for ACC activity are means ± SEM for $n = 3$. Values for PPAR γ 2 protein are representative of two separate experiments

	ACC activity (nmol/min per mg protein)	PPAR γ 2 protein (Relative to control)
Control	$60{\cdot}7 \pm 0{\cdot}91^{a}$	1·00
Acetate	$38{\cdot}3 \pm 1{\cdot}21^{b}$	1·93
Butyrate	$14{\cdot}6 \pm 0{\cdot}46^{d}$	0·75
Octanoate	$22{\cdot}9 \pm 0{\cdot}39^{c}$	2·30

Values with different superscript letters are statistically different ($P < 0{\cdot}05$)

stimulated accumulation of TAG, it did not form lipid droplets. The different effects of butyrate may be species specific.

The present results (Figs 2 and 3) indicate that exogenous octanoate stimulated cytosolic TAG accumulation and CD36 mRNA expression in bMEC. However, uptake of plasma octanoate might not depend on CD36. That is because in spontaneously hypertensive rats (SHR), which show phenotypes such as hyperlipidaemia, glucose intolerance and hyperinsulinaemia due to lack of functional CD36, excess uptake of glucose is almost completely ameliorated by feeding a mixture of SCFA (octanoate 65·6%) (Brinkmann et al. 2002), which suggests that cellular uptake of octanoate as well as other SCFA is not caused by CD36. Exogenous octanoate, however, may stimulate uptake of LCFA via up-regulation of CD36, resulting in lipid synthesis *de novo*. This possibility is supported by reduction of ACC activity in the cells treated with octanoate (Table 2), because ACC activity is inhibited by LCFA for lipid synthesis *de novo* (Kim, 1997; Ruderman et al. 1999). In our preliminary experiment, we observed that LCFA stimulated cytosolic TAG accumulation and CD36 mRNA expression more than octanoate did (results not shown). In addition, TAG accumulation and CD36 expression were dependent on the number of carbons in the fatty acid.

Leptin is also well known as a growth factor in several types of cells and tissues. In the murine mammary gland, leptin expression is reduced in a lactation-dependent manner (Aoki et al. 1999). Additionally, leptin stimulates the proliferation of human breast cancer cells (MCF-7) (Marie-Noelle et al. 2002), whilst leptin inhibits that of MAC-T cells (immortalized bMEC) (Silva et al. 2002). Whether leptin plays a proliferative role in MEC is controversial. In human normal and malignant MEC, butyrate induces inhibition of growth and differentiation (Davis et al. 2000; Tsubaki et al. 2001). In the present study, however, leptin mRNA expression was slightly reduced by treatment with acetate and butyrate (Table 1). This reduction may be related to cell proliferation.

It is ACC that controls lipid synthesis *de novo* in cells, and two principal isoforms have been identified, ACC α and ACC β. Among the lipogenic tissues such as liver, adipose tissue and mammary gland, ACC α is the major isoform expressed. Additionally, malonyl-CoA production by ACC strongly inhibits carnitine palmitoyltransferase I (CPTI) which plays a role in transporting LCFA to the matrix for β-oxidation (Ruderman et al. 1999). Although acetate, butyrate and octanoate are not transported into the mitochondrion via the carnitine-palmitoyl shuttle, ACC α mRNA-specific anti-sense causes increased fatty acid oxidation in INS-1 cells (Kim, 1997). This result indicates that ACC α as well as ACC β is pivotal for fatty acid oxidation and lipid synthesis. Our present study revealed that SCFA inhibited ACC and up-regulated UCP2 mRNA expression (Table 2 and Table 1). At first, we expected that acetate would induce cytosolic TAG accumulation in parallel with elevation of ACC activity, because acetyl-CoA derived from it might accelerate the TCA cycle and increase cellular ATP contents, resulting in decreased 5′-AMP-activated protein kinase activity (AMPK), which inhibits the activity of both ACC isoforms, by changing the AMP : ATP ratio. Unexpectedly, acetate neither elevated ACC activity nor induced cytosolic TAG accumulation. These results indicate that the energy level did not simply regulate lipogenesis in bMEC. A recent study demonstrates that leptin inhibits ACC activity via AMPK activation in muscle (Minokoshi et al. 2002). Thus, other cytokines or growth factors may regulate the ACC activity in bMEC. These results suggest that SCFA may inhibit lipogenesis but promote β-oxidation in bMEC.

However, octanoate stimulated cytosolic TAG accumulation in bMEC (Fig. 1a). It is also reported that in 3T3-L1 preadipocytes, the cells exposed to octanoate accumulate TAG although the extent is less than that in the cells exposed to LCFA (Han et al. 2002). In addition, octanoate inhibits the expression of master regulatory genes such as PPAR γ, CCAAT/Enhancer binding protein (C/EBP) α and sterol regulatory element binding protein (SREBP)-1c, but does not inhibit differentiation. In bMEC, however, although exposure to octanoate induces somewhat cytosolic TAG accumulation, it does not reduce PPAR γ 2 protein expression. The difference between the two cell types remains to be clarified.

Finally, thiazolidinediones, which are PPAR γ ligands that sensitize peripheral tissues to insulin, stimulate the expression of UCP2 mRNA in murine white and brown adipose tissues and skeletal muscle (Camirand et al. 1998). However, it is not known whether PPAR γ controls the expression of UCP2 (Medvedev et al. 2001). In the present study, octanoate and acetate, but not butyrate, stimulated PPAR γ protein expression despite increased UCP2 expression (Table 2). Therefore, elevation of PPAR γ expression induced by acetate or octanoate may not be the cause of that of UCP2 expression. However, the possibility that butyrate may raise UCP2 expression via other transcriptional pathways rather than PPAR γ is not excluded.

In summary, our results revealed that octanoate induced the accumulation of cytosolic TAG and the formation of lipid droplets in bMEC. This was accompanied by the elevation of CD36 mRNA expression in bMEC. All of the

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fatty acids used significantly inhibited ACC activities but enhanced UCP2 mRNA expression. In addition, PPAR γ protein expression was enhanced by octanoate and acetate. The greatest action of octanoate amongst short-and medium-chain fatty acids is consistent with the result for ruminant exocrine pancreas (Katoh & Obara, 2001).

We gratefully acknowledge Dr Michael T Rose (University of Wales, Aberystwyth, UK) for his contribution in the preparation of manuscript, and Dr H Aso (Laboratory of Functional Morphology, Tohoku University) and Mr N Uozumi for their technical advices on oil red O staining and ACC activity measurement.

References

Anderson JW 1995 Short-chain fatty acids and metabolism: human studies. In *Physiological Aspects of Short-chain Fatty Acids*, pp. 509–523 (Eds JH Cummings, JL Rombeau & T Sakata). Cambridge: Cambridge University Press

Aoki N, Kawamura M & Matsuda T 1999 Lactation-dependent down regulation of leptin production in mouse mammary gland. *Biochimica et Biophysica Acta* **1427** 298–306

Bach AC, Ingenbleek Y & Frey A 1996 The usefulness of dietary medium-chain triglycerides in body weight control: fact or fancy? *Journal of Lipid Research* **37** 708–726

Bai Y, Zhang S, Kyung-Sup Kim, Jung-Kee Lee & Ki-Han Kim 1996 Obese gene expression alters the ability of 30A5 preadipocytes to respond to lipogenic hormones. *Journal of Biological Chemistry* **271** 13939–13942

Brinkmann JFF, Abumrad NA, Ibbahimi A, Van der Vusse GJ & Glantz JF 2002 New insights into long-chain fatty acid uptake by heart muscle: a crucial role for fatty acid translocase/CD36. *Biochemical Journal* **367** 561–570

Camirand A, Marie V, Rabelo R & Silva JE 1998 Thiazolidines stimulate uncoupling protein-2 expression in cell lines representing white and brown adipose tissue and skeletal muscle. *Endocrinology* **139** 428–431

Clegg RA, Barber MC, Pooley L, Ernens I, Larondelle Y & Travers MT 2001 Milk fat synthesis and secretion: molecular aspects. *Livestock Production Science* **70** 3–14

Davis T, Kennedy C, Chiew Y-E, Clark CL & DeFazio A 2000 Histone deacetylase inhibitors decrease proliferation and modulate cell cycle gene expression in normal mammary epithelial cells. *Clinical Cancer Research* **6** 4334–4342

Deeney JT, Valivullah HM, Dapper CH, Dylwski DP & Keenan TW 1985 Microlipid droplets in milk secreting mammary epithelial cells: evidence that they originate from endoplasmic reticulum and are precursors of milk lipid globules. *European Journal of Cell Biology* **38** 16–26

Dylewski DP, Dapper CH, Valivullah HM, Deeney JT & Keenan TW 1984 Morphological and biochemical characterization of possible intracellular precursors of milk lipid globules. *European Journal of Cell Biology* **35** 99–111

Elstner E, Müller C, Koshizuka K, Williamson EA, Park D, Asou H, Shintaku P, Said JW, Heber D & Koeffler HP 1998 Ligands for peroxisome proliferator-activated receptor γ and retinoic acid receptor inhibit growth and induce apoptosis of human breast cancer cells *in vitro* and in BNX mice. *Proceedings of the National Academy of Science of the USA* **95** 8806–8811

Friedman JM 1997 The alphabet of weight control. *Nature* **385** 119–120

Gimble JM, Pighetti GM, Lerner MR, Wu X, Lightfoot SA, Brackett DJ, Darcy K & Hollingsworth AB 1998 Expression of peroxisome proliferator activated receptor mRNA in normal and tumorigenic rodent mammary glands. *Biochemical and Biophysical Research Communications* **253** 813–817

Green S 1995 PPAR: a mediator of peroxisome proliferator action. *Mutation Research* **333** 101–109

Han J, Farmer SR, Kirkland JL, Corkey BE, Yoon R, Pirtskhalava T, Ido Y & Guo W 2002 Octanoate attenuates adipogenesis in 3T3-L1 preadipocytes. *Journal of Nutrition* **132** 904–910

Kallen CB & Lazar MA 1996 Antidiabetic thiazolidinediones inhibit leptin (*ob*) gene expression in 3T3-L1 adipocytes. *Proceedings of the National Academy of Science of the USA* **93** 5793–5796

Katoh K & Obara Y 2001 Effect of fatty acids on exocrine and endocrine functions of the ruminant. *Trends in Comparative Biochemistry and Physiology* **8** 147–158

Kim K-H 1997 Regulation of mammalian acetyl-coenzyme A carboxylase. *Annual Review of Nutrition* **17** 77–99

Laud K, Gourdou I, Bélair L, Keisler DH & Djiane J 1999 Detection and regulation of leptin receptor mRNA in ovine mammary epithelial cells during pregnancy and lactation. *FEBS Letters* **463** 194–198

Marie-Noelle Dieudonne, Machinal-Quelin F, Serazin-Leroy V, Marie-Christine Leneveu, Pecquery R & Giudicelli Y 2002 Leptin mediates a proliferative response in human MCF7 breast cancer cells. *Biochemical and Biophysical Research Communications* **293** 622–628

Mather IH & Keenan TW 1998 Origin and secretion of milk lipids. *Journal of Mammary Gland Biology and Neoplasia* **3** 259–273

Medvedev AV, Snedden SK, Raimbault S, Ricquier D & Collins S 2001 Transcriptional regulation of the mouse uncoupling protein-2 gene. *Journal of Biological Chemistry* **276** 10817–10823

Minokoshi Y, Young-Bum Kim, Peroni OD, Fryer LGD, Müller C, Carling D & Kahn BB 2002 Leptin stimulates fatty-acid oxidation by activating AMP-activated protein kinase. *Nature* **415** 339–343

Prado M Del, Villalpando S, Gordillo J & Hernández-Montes H 1999 A high dietary lipid intake during pregnancy and lactation enhances mammary gland lipid uptake and lipoprotein lipase activity in rats. *Journal of Nutrition* **129** 1574–1578

Ruderman NB, Saha AK, Vavvas D & Witters LA 1999 Malonyl-CoA, fuel sensing, and insulin resistance. *American Journal of Physiology* **276** E1–E18

Silva LF, VandeHaar MJ, Weber Nielsen MS & Smith GW 2002 Evidence for a local effect of leptin in bovine mammary gland. *Journal of Dairy Science* **85** 3277–3286

Schoonjans K, Staels B & Auwerx J 1996 The peroxisome proliferator activated receptors (PPARs) and their effects on lipid metabolism and adipocyte differentiation. *Biochimica et Biophysica Acta* **1302** 93–109

Smith JL & Sheffield LG 2002 Production and regulation of leptin in bovine mammary epithelial cells. *Domestic Animal Endocrinology* **22** 145–154

Spiegelman BM & Flier JS 1996 Adipogenesis and obesity: rounding out the big picture. *Cell* **87** 377–389

Tontonoz P, Hu E & Spiegelman BM 1994 Stimulation of adipogenesis in fibroblasts by PPAR 2, a lipid-activated transcription factor. *Cell* **79** 1147–1156

Tontonoz P, Hu E, Devine J, Beale EG & Spiegelman BM 1995 PPAR gamma 2 regulates adipose expression of the phosphoenolpyruvate carboxykinase gene. *Molecular and Cellular Biology* **15** 351–357

Tsubaki J, Choi W-K, Ingermann AR, Twigg SM, Kim H-S, Rosenfield RG & Oh Y 2001 Effects of sodium butyrate on expression of members of the IGF-binding protein super family in human mammary epithelial cells. *Journal of Endocrinology* **169** 97–110

Wada K & Tanabe T 1983 Dephosphorylation and activation of chicken liver acetyl-coenzyme-A carboxylase. *European Journal of Biochemistry* **135** 17–23

Yonekura S, Kitade K, Furukawa G, Takahashi K, Katsumata N, Kato K & Obara Y 2002 Effects of aging and weaning on mRNA expression of leptin and CCK receptors in the calf rumen and abomasums. *Domestic Animal Endocrinology* **22** 25–35

Zhang Y, Proenca R, Maffei M, Barone M, Leopold L & Friedman JM 1994 Positional cloning of the mouse obese gene and its human homologue. *Nature* **372** 425–432