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Effects of Boron on Fat Synthesis in Porcine Mammary Epithelial Cells

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Abstract

This study aimed to investigate the effect of boron on porcine mammary epithelial cells (PMECs) survival, cell cycle, and milk fat synthesis. PMECs from boron-treated groups were exposed to 0–80 mmol/L boric acid concentrations. Cell counting kit-8 and flow cytometry assays were performed to assess cell survival and the cell cycle, respectively. Triacylglycerol (TAG) levels in PMECs and culture medium were determined by a triacylglycerol kit while PMECs lipid droplet aggregation was investigated via oil red staining. Milk fat synthesis–associated mRNA levels were determined by quantitative real-time polymerase chain reaction (qPCR) while its protein expressions were determined by Western blot. Low (0.2, 0.3, 0.4 mmol/L) and high (> 10 mmol/L) boron concentrations significantly promoted and inhibited cell viabilities, respectively. Boron (0.3 mmol/L) markedly elevated the abundance of G2/M phase cells. Ten mmol/L boron significantly increased the abundances of G0/G1 and S phase cells, but markedly suppressed G2/M phase cell abundance. At 0.3 mmol/L, boron significantly enhanced ERK phosphorylation while at 0.4, 0.8, 1, and 10 mmol/L, it markedly decreased lipid droplet diameters. Boron (10 mmol/L) significantly suppressed ACACA and SREBP1 protein expressions. The FASN protein levels were markedly suppressed by 0.4, 0.8, 1, and 10 mmol/L boron. Both 1 and 10 mmol/L markedly decreased FASN and SREBP1 mRNA expressions. Ten mmol/L boron significantly decreased PPARα mRNA levels. Low concentrations of boron promoted cell viability, while high concentrations inhibited PMECS viabilities and reduced lipid droplet diameters, which shows the implications of boron in pregnancy and lactation.

Keywords PMECs · Boron · Cell cycle · Milk fat biosynthesis

Introduction

During lactation, the mammary glands are the most active lipid synthesis and secretion organs [1]. The mammary glands of mammals secrete milk, which triacylglycerols account for the majority of milk fat [2–4]. The fat in breast milk is an important source of energy and essential fatty acids for the growth and development of newborns. The late gestation period of the sow is associated with rapid mammary gland development and epithelial cell proliferation, which are important cells in milk lipid and milk protein synthesis. In the sow, the number of mammary epithelial cells may double at parturition, usually in tandem with increased milk production [5]. During lactation, milk components are majorly synthesized from small molecule substrates in mammary epithelial cells (MECs) [6]. Amino acids, fatty acids, glucose, and other hormones as well as bioactive compounds can regulate lactation by stimulating MECs through different sensors and signaling pathways [7]. Valine induces nuclear accumulation of sterol regulatory element–binding protein 1 (SREBP1), which promotes the expressions of fatty acid synthesis–associated proteins, thereby elevating intracellular triacylglycerol levels [8]. Physiologically, SREBP1 preferentially promotes the expressions of fatty acid biosynthesis–associated genes, such as acetyl coenzyme A carboxylase alpha (ACACA) and fatty acid synthase (FASN). Therefore, SREBP1 expressions in the mammary glands or MEC are correlated with milk fat levels and milk fatty acid composition [9, 10]. Acyl-CoA

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synthetase long-chain family member 3 (ACSL3), as a member of the long-chain acetyl coenzyme A synthase family, has the main biological function of regulating fatty acid metabolism in vivo and catalyzing the conversion of fatty acids into long-chain acetyl coenzyme A, which is important for lipid synthesis, protein modification and β-oxidation. Fatty acid–binding protein 3 (FABP3) is involved in various life activities such as lipid metabolism, energy metabolism, and immune regulation through mediating fatty acid transport. The roles of 1-acylglycerol-3-phosphate O-acyltransferase 1 (AGPAT1), glycerol-3-phosphate acyltransferase 4 (AGPAT6), and glycerol-3-phosphate acyltransferase, mitochondrial (GPAM) are TAG synthesis and lipid droplet formation. The role of peroxisome proliferator–activated receptor alpha (PPARα), insulin-induced gene 1 (INSIG1) is the transcriptional regulation of lipid. Nutritional strategies that are aimed at promoting milk fat production and mammary epithelial cell proliferation in lactating animals can ensure offspring growth and health. Boron, a mineral element that is located in the second cycle of the periodic table of elements (IIIA), has excellent physical as well as chemical properties and is widely used in agriculture, chemical industries, cosmetics, food, medical, and health fields [11]. Adequate amounts of boron in humans and animals have regulatory effects on lipid metabolism, embryonic development, and energy substrate utilization [12]. Boron can result in a significant decrease in serum total cholesterol, low-density lipoprotein (LDL), LDL cholesterol, and triacylglycerol levels [13]. Low boron concentrations have been reported to promote cell proliferation and inhibit apoptosis. Therefore, we treated PMECs with different boron concentrations to elucidate on its molecular mechanisms in milk fat synthesis and provide a reference point for dosing of boric acid during lactation.

Materials and Methods

Cell Cultures

The porcine mammary epithelial cells line was a gift from Guan Wutai's group at South China Agricultural University. The porcine mammary epithelial cells line was established as described previously [14]. Cells were cultured in modified DMEM/F12 medium containing 10% fetal bovine serum (FBS) (Gibco, USA), 5 μg/mL insulinotransferrin selenium (ITS) (ScienCell, USA), 10 ng/mL epidermal growth factor (EGF) (MCE, USA), 10 ng/mL IGF-1 (MCE, USA), 5 μg/mL hydrocortisone (MCE, USA), and 1% PSN double antibodies (Gibco, USA). The cells were cultured in an incubator at 37 °C with 5% CO_2. The culture medium was changed after every 1 day. At a cell fusion rate of 90%, cells were passaged with 0.25% trypsin–EDTA.

Cell Viability Assay

The effects of boric acid on porcine mammary epithelial cells were evaluated using the CCK-8 kit (MCE, USA), as instructed by the manufacturer. Briefly, cell suspensions were inoculated in 96-well cell culture plates (100 μL/well) at a density of 5×10^3 cells/well and incubated at 37 °C in a humidified 5% CO_2 atmosphere for 24 h. Then, cells were exposed to different boric acid concentrations (0, 0.1, 0.2, 0.3, 0.4, 0.8, 1, 10, 20, 40, 80 mM) for 24 h. Subsequently, they were supplemented with 10 μL of CCK-8 solution and incubated at 37 °C for 2 h. Finally, absorbance was measured at 450 nm using a Full Wavelength Enzyme Labeler (Thermo Fisher Scientific, USA).

Cell Cycle Analysis

The PMECs were inoculated in 6-well cell culture plates at density of 5×10^5 cells per well and grown for 24 h. After incubation for 24 h, according to the results of CCK-8 kit, the medium was changed to a medium containing boric acid (0, 0.3, and 10 mM). Then, cells were collected by trypsin digestion and transferred into 70% ethanol for 2 h. Cell cycle analysis was performed using a MoFlo XDP flow cytometer (Beckman-Coulter, USA) and a commercial EZCell™ Cell Cycle Analysis Kit (Biovision, USA), as instructed by the manufacturers.

Intracellular Lipid Droplet Staining Assay

Intracellular lipid accumulation was assessed by Oil Red O (BBI, Canada) staining. Suspensions of PMECs were inoculated in 24-well cell culture plates at a density of 5×10^4 cells per well and incubated at 37 °C in a humidified 5% CO_2 environment for 24 h. Then, according to the results of CCK-8 kit, they were exposed to media containing different concentrations of boric acid (0, 0.2, 0.3, 0.4, 0.8, 1, and 10 mM) for 24 h, rinsed twice using PBS, fixed in 4% paraformaldehyde for 30 min at room temperature, and rinsed thrice (10 min each) using PBS. Cell culture plates were supplemented with 200 μL of oil red preparation solution for 3 h, followed by several washes using PBS. Observation and imaging of cells were performed using an inverted microscope. Lipid droplet diameters were measured using the Image-Pro Plus 6.0 software (media, cybernetics, USA).

Quantification of Intracellular TAG Levels

Intracellular TAG levels were determined using an enzymatic colorimetric assay kit (Solarbio, Beijing, China). Briefly, PMECs suspensions were inoculated in 6-well plates

at a density of 5×10^5 cells per well and incubated for 24 h. Cells were exposed to medium containing different boric acid concentrations (0, 0.2, 0.3, 0.4, 0.8, 1, and 10 mM) for 24 h. Then, the medium was carefully collected, after which cells were rinsed thrice using PBS, collected by centrifugation in a centrifuge tube with 1 ml of n-heptane: isopropanol reagent and lysed by ultrasound for 1 min (intensity 20%, sonication for 2 s, pause for 1 s). After centrifugation at 8000 × g for 10 min at 4 °C, determination of intracellular and medium TAG levels according to kit instructions, absorbance was measured at 420 nm using a Full Wavelength Enzyme Labeler (Thermo Fisher Scientific, USA). Each experiment was performed in triplicates and repeated at least three times.

Extraction of RNA and Real-time Quantitative PCR

In this assay, PMECs were inoculated in 6-well cell culture plates at a density of 5×10^5 cells per well and incubated for 24 h. The medium was changed to a medium containing different boric acid concentrations (0, 0.2, 0.3, 0.4, 0.8, 1, and 10 mM) and incubated for 24 h. Subsequently, total RNA was extracted from cells using the commercial EZ-press RNA Purification Kit (EZBioscience, USA), as instructed by the manufacturer. Reverse transcription was performed using the commercial Color Reverse Transcription Kit (EZBioscience, USA). Expressions of adipogenic pathway–related genes and the internal reference gene (β-actin) were determined by quantitative real-time polymerase chain reaction (qPCR) and a commercial 2 × SYBR Green qPCR Master Mix kit (EZBioscience, USA), as instructed by the manufacturer. Primers (Table 1) were designed using the Primer Premier 6 software and working efficiency of each designed primer is above 92%.

Western Blot Analysis

The PMECs were inoculated in 6-well cell culture plates at a density of 5×10^5 cells per well and incubated for 24 h. Then, they were supplemented with 120 μL of RIPA lysis buffer (Beyotime, Nanjing, China) containing 1% PMSF (MCE, USA), homogenized and treated with 30 μL of 5 × SDS-PAGE protein loading buffer. Proteins were separated by 5–12% polyacrylamide gel electrophoresis and transferred to a nitrocellulose membrane that was blocked with 5% BSA. The membrane was probed overnight with the following primary antibodies: anti-FASN (1:1000, ab99539), anti-ACACA (1:1000, ab72046), anti-DGAT1 (1:1000, ab118180), anti-SREBP1 (1:1000, ab3259) and anti-β-actin (1:1000, ab8226) from Abcam, UK as well as with anti-ERK (1:3000, 9102S) and anti-P-ERK (1:3000, 9101S) from Cell Signaling Technology, USA. After washing, membranes were incubated with a secondary antibody, anti-Rabbt IgG (1:5000; Abbine, USA). Chemiluminescence signals were detected using a HRP reagent (Millipore, Billerica, MA), while the fluorescent bands were quantified using the ImageJ processing software.

Data Analysis

Data are expressed as mean ± standard deviation (SD). Data analyses were performed using the SPSS 25.0 software. Comparisons of means among groups was performed using one-way analysis of variance (ANOVA), followed by the Dunnett's post hoc test for between group comparisons. In this study, $p \leq 0.05$ and $p \leq 0.01$ were set as the thresholds for significant and extremely significant differences, respectively.

Table 1 Characteristics of primers used for real-time quantitative PCR analysis

Gene name	Forward (5′-3′)	Reverse (5′-3′)
ACACA	TGGAGGTGTATGTGCGAAGG	GGTGGAGTGAATGCGTTGTC
ACSL3	TTGGCTGTCCTATGAAGAAGTC	AATGATGTTGGTCACCTCTGTT
FASN	CGTGGTGGTGGACAGGAAT	CCAGGATAGGCTTGAGATGCT
AGPAT1	GCTGCTACTGCTGCTCTTC	TGGTTGGAGACGACGACATA
AGPAT6	CCATCTTCGGCGTCTCCTTC	GTTCTTCTCCTTGGCTCCTCTC
INSIG1	GTTATTGCCACCATCTTCTCCT	TGTGCGGTTCTCCAAGGT
PPARα	TCACGGAGTTCGCCAAGT	GTTCATCACAGAGGACAGCAT
SREBP1	GCTACCGCTCCTCCATCAAT	TCTGGCTTCATCTGGCTGTC
FABP3	GACCAAGCCTACCACAATCAT	TTGCCTCCATCCAGTGTCA
GPAM	CTGTGCTACCTGCTCTCCAA	CGCTGTCTTCATCTTCCTCATC
β-actin	GATCTGGCACCACACCTTCTACAAC	TCATCTTCTCACGGTTGGCTTTGG

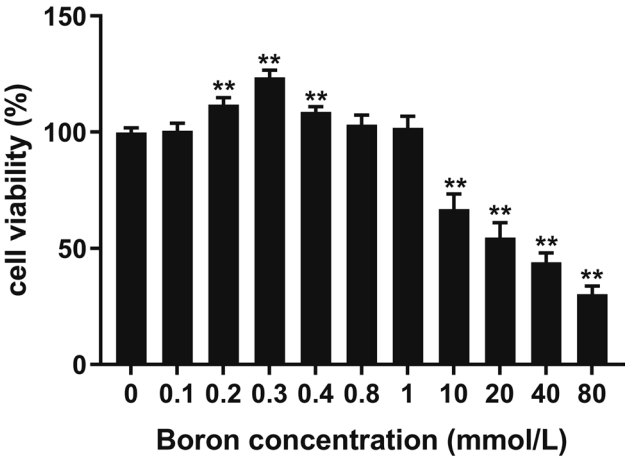

Fig. 1 Effects of boron on the viability of porcine mammary epithelial cells. The data are expressed as the mean ± SD ($n = 6$). A single asterisk (*) indicates significant difference compared to 0 mmol/L boron ($p < 0.05$). Double asterisks (**) indicate significant difference compared to 0 mmol/L boron ($p < 0.01$)

Results

Effects of Different Boron Concentrations on PMEC Viabilities

The effects of different boron concentrations on PMECs viabilities were evaluated by the CCK-8 assay (Fig. 1). Compared to 0 mmol/L, low boron concentrations (0.2, 0.3, and 0.4 mmol/L) significantly enhanced PMEC viabilities ($p < 0.01$), while high doses (10, 20, 30, 40, and 80 mmol/L) significantly inhibited PMEC viabilities ($p < 0.01$).

Effects of Different Boron Concentrations on PMEC Cycle

After 24 h of 10 mmol/L boron treatment, abundances of PMECs were significantly high in the G0/G1 ($p < 0.01$) and S ($p < 0.05$) phases, but were markedly low in the G2/M phase ($p < 0.01$). After 24 h of 0.3 mmol/L boron

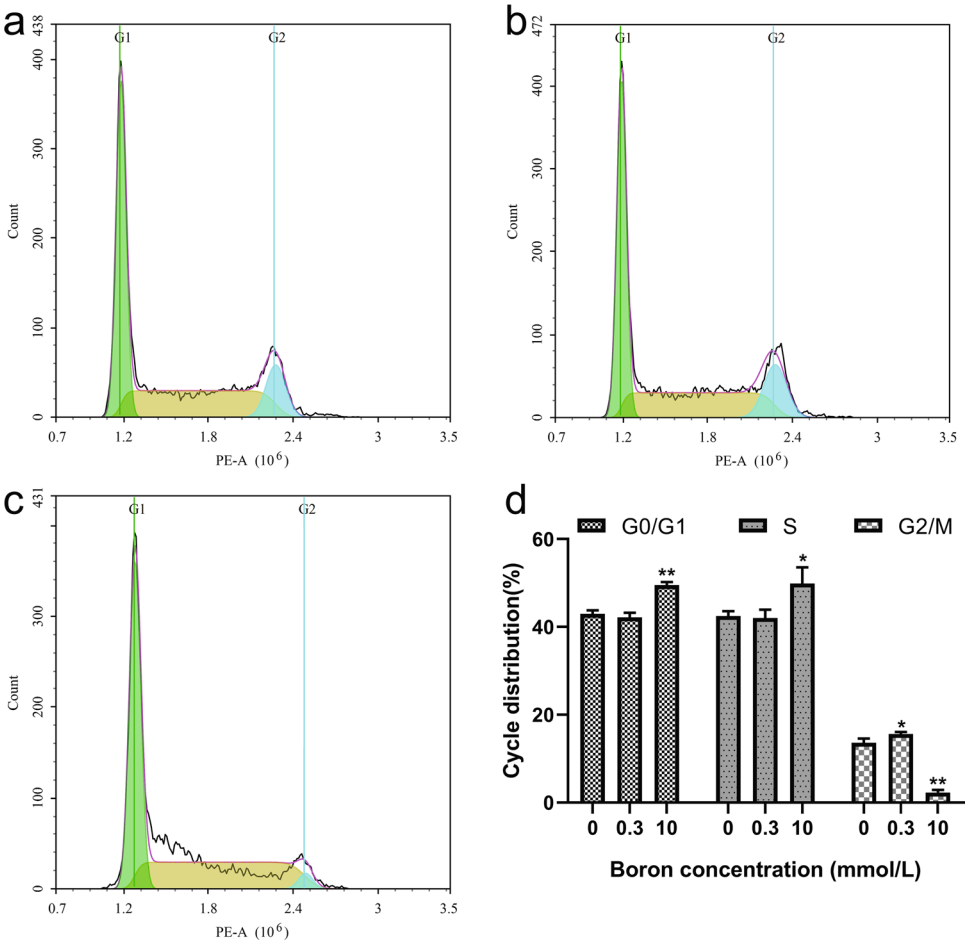

Fig. 2 Effect of boron on the cell cycle of porcine mammary epithelial cells assayed using flow cytometry. **a** Control group; **b** 0.3 mmol/L group; **c** 10 mmol/L group; **d** statistical graph of the effect of boron on the cell cycle of porcine mammary epithelial cells. The data are expressed as the mean ± SD ($n = 4$). A single asterisk (*) indicates Significant difference compared to 0 mmol/L boron ($p < 0.05$). Double asterisks (**) indicate significant difference compared to 0 mmol/L boron ($p < 0.01$)

treatment, the abundance of PMECs in the G2/M phase was significantly high ($p < 0.05$) (Fig. 2).

Effects of Different Boron Concentrations on Intra- and Extracellular Triglyceride Levels

After 24 h of boron treatment, compared with 0 mM, TAG levels in the medium were significantly reduced ($p < 0.01$) while intracellular TAG levels did not exhibit significant differences ($p > 0.05$) (Fig. 3).

Effects of Different Concentrations of Boron on Maximum Lipid Droplet Diameters of PMECs

Oil red O staining confirmed that cytosolic lipid droplet formation in PMECs was suppressed by 24 h of boron treatment, and their maximum diameters decreased with increasing boron concentrations. Compared to 0 mM, the diameters were significantly reduced by 0.4, 0.8, 1, and 10 mM boron concentrations ($p < 0.01$) (Fig. 4).

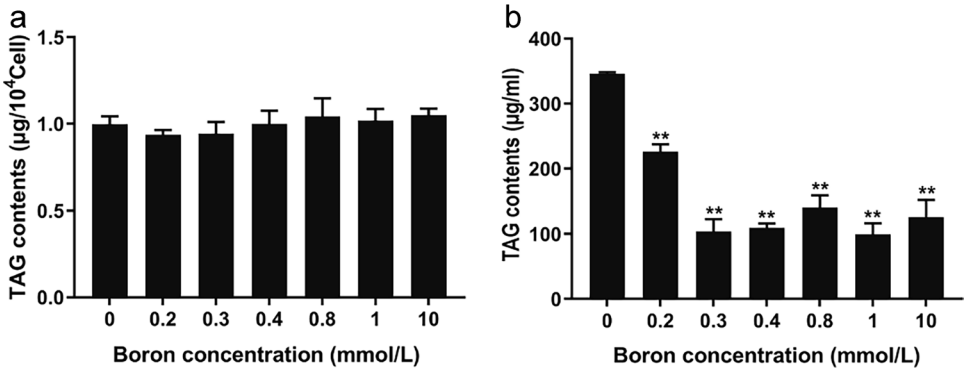

Fig. 3 Effect of boron on triacylglycerol secretion by porcine mammary epithelial cells. **a** Intracellular; **b** culture fluid. The data are expressed as the mean ± SD ($n = 6$). A single asterisk (*) indicates significant difference compared to 0 mmol/L boron ($p < 0.05$). Double asterisks (**) indicate significant difference compared to 0 mmol/L boron ($p < 0.01$)

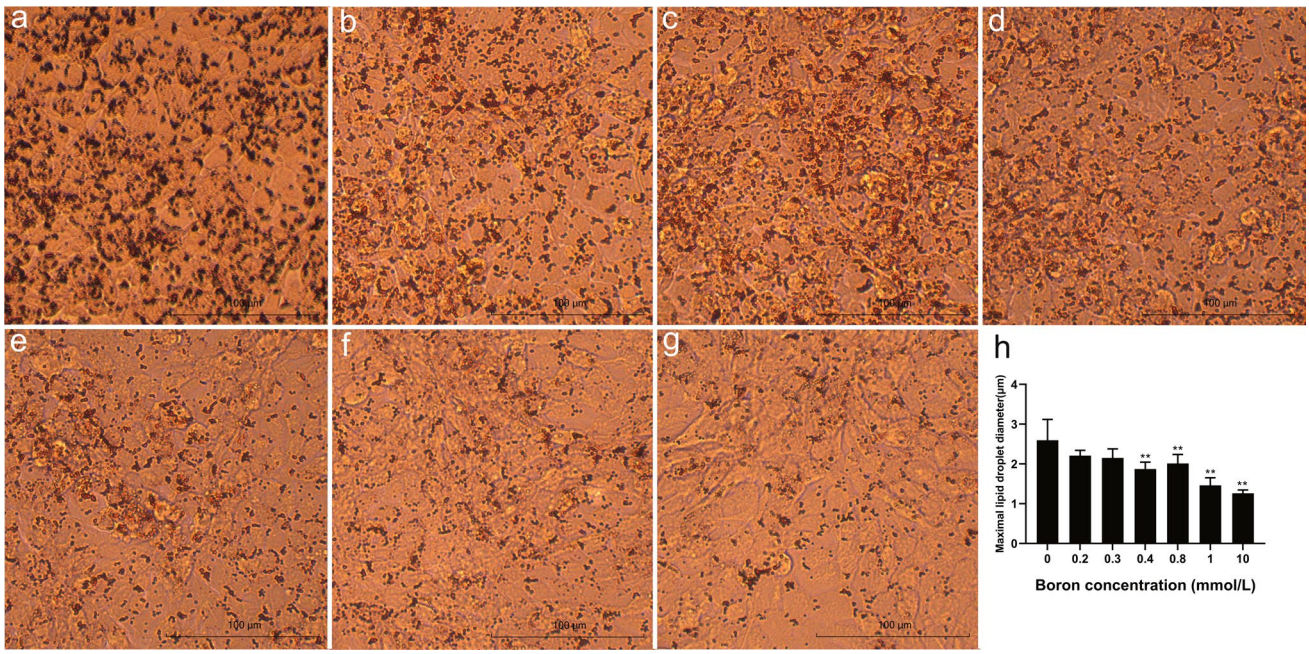

Fig. 4 Effects of boron on lipid droplet formation in porcine mammary epithelial cells. **a** 0 mmol/L boron; **b** 0.2 mmol/L boron; **c** 0.3 mmol/L boron; **d** 0.4 mmol/L boron; **e** 0.8 mmol/L boron; **f** 1 mmol/L boron; **g** 10 mmol/L boron; **h** statistical results of the largest lipid droplet diameter graph bar: 100 μm. The data are expressed as the mean ± SD ($n = 6$). A single asterisk (*) indicates significant difference compared to 0 mmol/L boron ($p < 0.05$). Double asterisks (**) indicate significant difference compared to 0 mmol/L boron ($p < 0.01$). bar: 100 μm

Expressions of Lactation-related Proteins in PMECs Treated with Different Boron Concentrations

After 24 h of boron treatment, compared with the 0 mM boron treatment group, protein expressions of fatty acid ab initio synthesis of ACACA were significantly suppressed in the 10 mM boron treatment group ($p < 0.01$), SREBP1 protein expression was significantly inhibited in the 10 mM boron treatment group ($p < 0.05$), while FASN expressions decreased with increasing boron concentrations. That is, they were significantly suppressed in 0.4 and 0.8 mM boron treatment groups ($p < 0.05$) but were significantly suppressed in 1 and 10 mM boron treatment groups ($p < 0.01$). Moreover, ERK phosphorylations were enhanced in the 0.3 mM boron treatment group, compared to the 0 mM boron treatment group. All boron treatment groups of DGAT1 were not significantly different compared to the 0 mM boron treatment group ($p > 0.05$) (Fig. 5).

Expressions of Lactation-associated Genes in PMECs Treated with Different Boron Concentrations

Treatment with boron for 24 h suppressed the expressions of genes involved in de novo synthesis and transcriptional regulation of fatty acids in PMECs. At 0.2–0.8 mM, boron treatment did not affect the mRNA expressions of FASN ($p > 0.05$); however, 1 and 10 mM boron treatment significantly downregulated the mRNA expressions of FASN ($p < 0.05$). Compared to 0 mM boron treatment, PPARα mRNA expressions were significantly downregulated ($p < 0.05$) by 10 mM boron treatment while SREBP1 mRNA expressions were significantly downregulated ($p < 0.05$) by both 1 and 10 mM boron treatments. Compared to 0 mM boron treatment, all boron treatment groups, boron treatment did not affect the mRNA expressions of ACACA, ACSL3, AGPAT1, AGPAT6, INSIG1, FABP3, GPAM ($p > 0.05$) (Fig. 6).

Discussion

Low concentrations of boron (0.2–0.4 mmol/L) significantly enhanced the viabilities of PMECs, which were markedly suppressed by higher boron concentrations (10 ≥ mmol/L) in current study. The finding imply that the optimal concentration range of boron for PMECs survival is 0.2–0.4 mmol/L. The flow cytometry assay found that the 0.3 mmol/L boron dose significantly increased the number of PMECs in the G2/M phase; however, higher doses of boron (concentrations above 10 mmol/L) exhibited significant toxic effects, inhibiting cell viabilities and increasing the number of cells remaining in G0/G1 and S phases. Xiaoting Zhang [15]

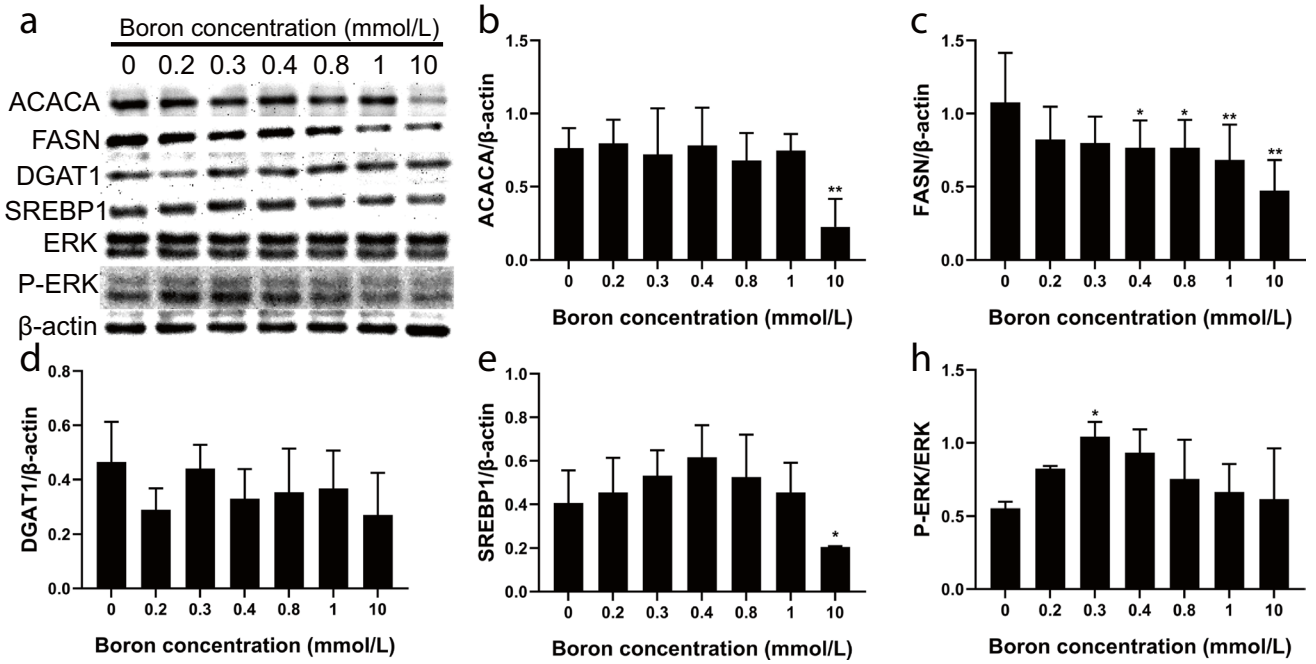

Fig. 5 Effects of boron on the expression of proteins involved in lipid synthesis in porcine mammary epithelial cells. **a** Representative protein bands of ACACA, FASN, DGAT1, SREBP1, ERK, and P-ERK. Bar graph of protein expression level relative to β-actin, including **b** ACACA; **c** FASN; **d** DGAT1; **e** SREBP1; **f** ERK/P-ERK. The data are expressed as the mean ± SD ($n = 3$). A single asterisk (*) indicates significant difference compared to 0 mmol/L boron ($p < 0.05$). Double asterisks (**) indicate significant difference compared to 0 mmol/L boron ($p < 0.01$)

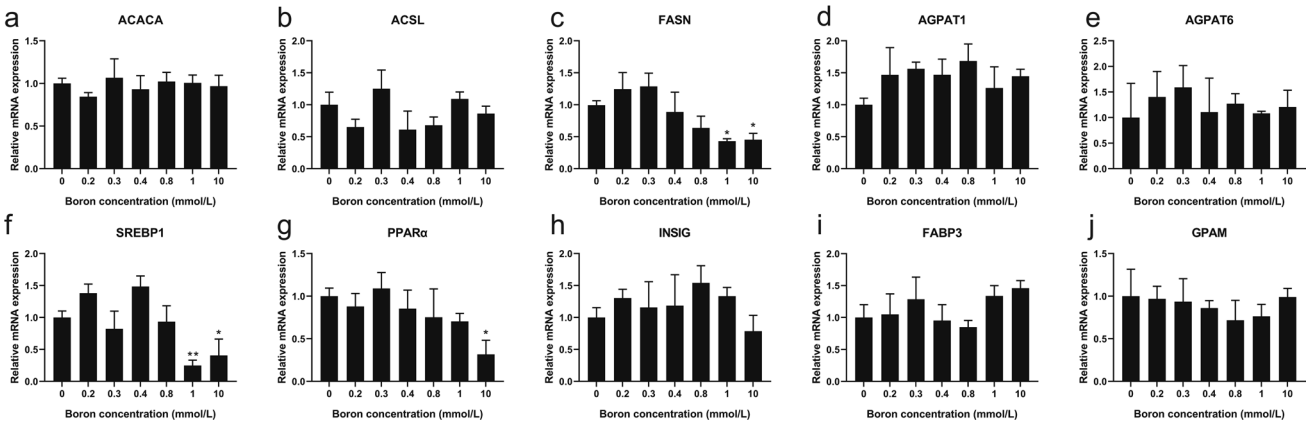

Fig. 6 Effects of boron on the expression of gene involved in lipid synthesis in porcine mammary epithelial cells. **a** ACACA; **b** ACSL3; **c** FASN; **d** AGPAT1; **e** AGPAT6; **f** SREBP1; **g** PPARα; **h** INSIG1; **i** FABP3; **j** GPAM; the data are expressed as the mean ± SD ($n = 3$). A single asterisk (*) indicates significant difference compared to 0 mmol/L boron ($p < 0.05$). Double asterisks (**) indicate significant difference compared to 0 mmol/L boron ($p < 0.01$)

reported that low concentrations (0.01–0.1 mmol/L) of boron promoted the proliferation and inhibited the apoptosis of ostrich spleen lymphocytes by enhancing cell membrane functions as well as intracellular catalase activities, whereas high concentrations (25–100 mmol/L) of boron exerted the opposite effects on cells. Comparable with previous studies [16, 17], boron concentrations below 0.5 mmol/L significantly improved the viabilities of testicular Sertoli cells in vitro and inhibited their apoptosis. However, high doses of boron (concentrations above 5.0 mmol/L) exhibited significant toxic effects, inhibiting cell viabilities, accelerating Sertoli cell apoptosis, and arresting the cell cycle in the G0/G1 phase. Moreover, we established that low doses of boron can achieve better results in promoting immune responses in rat duodenum, and the physiological amount of boron is a regulator of energy substrate metabolism [18]. In present experiment, 0.3 mmol/L boron significantly promoted ERK phosphorylations. The ERK signaling pathway is majorly involved in cell differentiation as well as proliferation and is highly associated with pro-proliferative and anti-apoptotic roles [19]. Therefore, promotion of cell viabilities by low boron concentrations may be related to activation of the ERK signaling pathway. High boron concentrations inhibited cell viabilities, which may be attributed to their toxic effects [20].

The nutrient composition and level of sow milk is regulated by nutrients. Milk fat is the main component that determines the value of sow milk [21]. We established that low boron concentrations promoted the viabilities of PMECs while TAG levels in the medium were markedly decreased after 24 h of boron treatment. Moreover, a decreasing trend of lipid droplet diameter was revealed by oil red staining. Enzymes in the biosynthesis pathway of fatty acids, triacylglycerols, and cholesterol are regulated by sterol regulatory element–binding proteins (SREBP), which are among the most important families of transcription factors involved in lipid homeostasis [22]. SREBP1 is the most physiologically relevant, and its activity indicates the lipid synthesis capacities [23]. Amino acids, fatty acids, glucose, as well as some hormones and bioactive substances, sense extracellular stimuli through different sensors and signaling pathways (mainly mTOR with SREBP1) to regulate milk synthesis [24]. Therefore, the reasons for the decrease in TAG levels and the decreasing trend of lipid droplet diameter in the present study may be attributed to various factors. First, DGAT1 protein expression and SREBP1 protein expression at low boron concentrations were unchanged. But SREBP1 protein expressions were significantly decreased at high boron concentrations (10 mmol/L). Second, expressions of ACACA and FASN, which are key proteins in fatty acid ab initio synthesis were decreased. Although the protein expression of ACACA and FASN did not change at low boron concentrations. Expressions of ACACA were significantly decreased at high boron concentration treatment, while FASN expressions gradually decreased with increasing boron concentrations. Third, no significant difference in mRNA expression of all genes in low boron treatment; meanwhile, the relative mRNA expressions of FASN and SREBP1 were significantly suppressed at high boron treatment (1 and 10 mmol/L). Moreover, mRNA expressions of PPARα, a free fatty acid receptor that plays a key role in fatty acid β-oxidation and energy metabolism, were significantly decreased in high boron concentrations. The result implies that boron inhibits TAG synthesis by suppressing the expressions of PPARα and target adipogenic genes. Fatty acids and eicosanoids are natural ligands of PPAR, and the binding of these ligands to PPARα can regulate gene expressions as well as lipogenesis rates [25].

According to our results, the reduction of triacylglycerol levels and lipid droplets in cell cultures may also be attributed to boron inhibiting fatty acid ab initio synthesis-related genes (ACACA and FASN), possibly via SREBP1 regulation. Physiologically, SREBP1 is a key regulator of genes encoding proteins involved in ab initio synthesis of fatty acids (ACACA and FASN) in mammary epithelial cells [26]. Boron inhibits adipogenesis by the Wnt/β-linked protein pathway in progenitor cells [27]. Boron was reported to significantly inhibit adipogenic differentiation of hADSCs, with progressive decreases in lipid deposition as its concentrations increased, and it suppresses the main regulators of the adipogenic transcriptional program at mRNA and protein levels [28]. These findings are consistent with the present research, whereby it is possible that boron inhibited the transcription as well as translation of ACACA and FASN by targeting SREBP1 or inhibited the activities of key enzymes, including oxidoreductase, transferase, hydrolase, and isomerase. Moreover, boron competitively inhibits pyridine as well as flavin nucleotides as coenzymes of oxidoreductase and affects energy substrate utilization by competing with these enzymes for NAD^+ and flavin [29]. In current study, low boron concentrations increased the cell viabilities, and the reason of the regulation might be boron, as a mineral element, promotes cell proliferation at low concentrations. High boron concentrations are cytotoxic and inhibit the key pathway of milk lipid synthesis, SREBP1, and fatty acid synthesis from scratch (ACACA and FASN). Due to the effects of high boron concentrations, TAG levels in the cell culture medium were reduced while lipid diameters also showed gradual decreases.

Conclusions

Low boron concentrations promoted PMECs viabilities, while high concentrations inhibited PMECs viabilities and reduced lipid droplet diameters. These findings provide a basis for supplementation of boron during pregnancy and lactation as well as in feed additives.

Author Contribution All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by Yanan Yang and Ya Yang. The first draft of the manuscript was written by Yanan Yang, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data Availability The raw data supporting this study will be available by authors to any qualified researcher upon request.

Declarations

Competing Interests The authors declare no competing interests.

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