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The aim of this study was to determine the role of protein kinase , in regulating glucose uptake in lactating bovine mammary epithelial cells , The , were cultured and treated with different concentrations of phorbol- myristate- acetate , / , - , , , , - , and ng/m , the classical activator of , , for h compared with the cells with no , / , treatment , and - ng of , / /m significantly stimulated the glucose uptake of the , , whereas the glucose uptake by the cells treated with the lowest and the highest amounts of , / and ng/m , respectively did not show a significant difference consistently, the mRNA expression of glucose transporter *GLUT* - and showed a similar pattern of increase under the treatments of , / urthermore, when the cells were pretreated with - , , , , - , and , *M*, an inhibitor of , , for min before exposed to , / , ng/m , the , / induced glucose uptake and *GLUT1* and *GLUT8* expression were decreased by - , , in a dose dependent manner These results demonstrate that , is involved in the regulation of glucose uptake by , , and this function may work, at least partly, through upregulating the expression of *GLUT1* and *GLUT8*

Key words: bovine, glucose transporter, mammary epithelial cell, protein kinase

Glucose is an important substrate and energy source in milk synthesis and large amounts of glucose are required to sustain lactation ant et al, Glucose is taken up from the bloodstream by mammary secretory epithelial cells **MEC** through facilitative glucose transporters **GLUT**, mainly T- and possibly T hao, -

rotein kinase **PKC** is an important cellsignaling molecule that modulates a variety of cellular processes, such as secretion, gene expression, proliferation, differentiation, and muscle contraction ishizuka, - n addition, it is well known that , is a key regulator ing glucose uptake in a variety of cells atson and essin, - os h et al, uiken et al, owever, whether , regulates glucose uptake in , is not known pression of , proteins in reases in human mammary epithelial tissue from puberty to pregnancy , associated el h et al, - / tiation of , enhances proliferation and survival rossoni et al, and plays an important role in prolactin induced milk protein synthesis in mouse , , arter et al, - urthermore, hanges in , expression or activity have been observed during mammary arin genesis rtreger et al, - Therefore, may also be an important regulator in regulating glucose uptake in, The objective of the current study was to determine the role of , in regulation of glucose transport and glucose transporter gene expression in lactating bovine mammary epithelial cells **BMEC**

The lactating , were isolated, characterized and cultured as previously described hao et al, - iu et al, - The cells were starved with serum free medium for - h and then treated with - different concentrations , - , , , , - , and ng/m of phorbol - myristate - acetate **PMA** (sigma, st ouis, , a classical activator of , , for h and with different concentrations , , , , - , and , *M* of - , , er kloster ienes, armstadt, ermany, an inhibitor of , , for min before being treated with , / ng/m for another h The difference in glucose content in the culture media before and after the treatments, determined by an enzymatic oloring glucose oxidase/peroxidase assay method Tiffany et al, - and normalized by total cell protein ontent, was considered as the net glucose uptake / orsi et al,

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T ol reagent n itrogen orp arlsbad / was used to isolate total / from the ells The first strand of / was trans ri ed using a re erse tran s ription kit Takara Tokyo apan The m/ a undan e of *GLUT1* and *GLUT8* was analy ed y uantitati e re erse trans ription using reen as des ri ed efore hao et al - The rela ti e m/ haages of ea hene were al ulated using the T where T y le threshold method i ak and hmitgen - and normali ed to a tin m/ in the same sample ata were analy ed y one way / using software ersion- orp/ rmonk / and un an s multiple rage tests were used for multiple omparisons a h e periment was performed with to repli ates and repeated times using pooled isolated from olstein la tating ows ata from - representati e e periment is reported here ignifi ant differen es were onsidered at *P* n this study we used a a ti ator / and a inhi itor - y to study the role of in regulation ofglu ose uptake and *GLUT* e pression in hor ol- myristate- a etate is a diester of phor ol whi h has een widely used in iomedi al resear h to a ti ate lassi al os h et al oel et al e ause of its stru tural similarity to one of the natural a ti ators of lassi isoforms dia ylgly erol ompared with the ontrolgroup g/m treatment of with and- g of / m signifi antly in reased the ellularglu ose uptake *P* ure- whereas theglu ose uptake in the ells treated with either the lowest on entration g/m or the highest on entration g/m of / did not show a signifi ant differen e *P* > ure- n addition when were treated with a pan inhi itor - y erts et al uyen arese and a an - at the on entrations of - and *M* efore / treatment g/m / a ti atedglu ose uptake was signifi antly inhi ited y - y at a on entration of *M* or higher *P* ure These results demonstrate a regulatory role of in glu ose transport in onsistent with pre ious oser ations in adipo ytes and intestinal ells i s et al - - a e et al - - heng and arr - urthermore we found that in regu lates m/ e pression of *GLUT1* and *GLUT8* the mainglu ose transporters e pressed in the mammary gland hao et al - n parti ular and - g of / /m signifi antly in reased the m/ e pression of *GLUT1* and *GLUT8* in *P* ure / and ompared with the ontrol g of / /m whereas and g/m had no effe t *P* > These oser ations are in line with the report

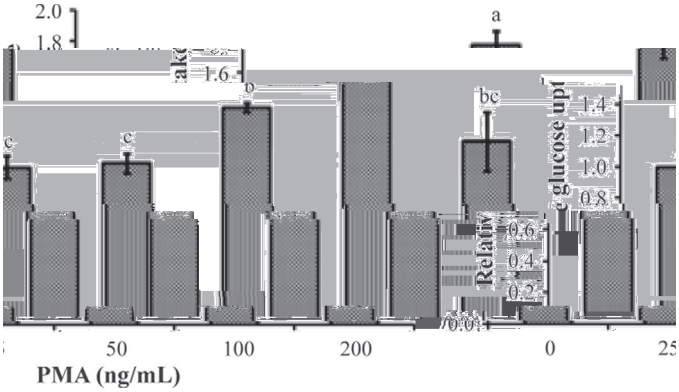

Figure 1. lu ose uptake in o ine mammary epithelial ells treated with different on entrations of phor ol- myristate- a etate / The glu ose uptake in the ontrol ells without / treatment g/m is assigned a alue of- alues with different letters a are signifi antly different *P* n rror ars indi ate the

that *GLUT1* m/ le els were in reased y a ti ation of in o ine rain apillary endothelial ells ar rell et al and sugest that the a ti ated glu ose uptake in may e mediated y enhan ed e pression of *GLUT1* and *GLUT8* n addition *GLUT8* m/ was higher at - g/m of / ompared with g/m *P* ure whereas no dif feren e was seen in *GLUT1* e pression etween these groups *P* > ure / imilar to theglu ose uptake the / indu ed *GLUT1* m/ e pression in was signifi antly inhi ited y - y at a on entration of *M* or higher *P* ure / whereas the / indu ed *GLUT8* m/ e pres sion was signifi antly inhi ited y - y at a

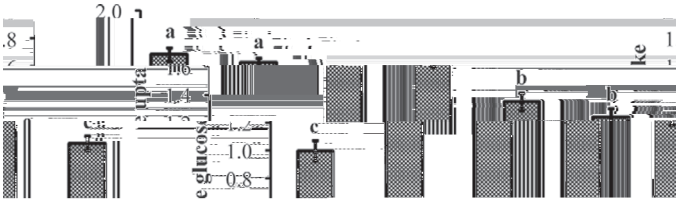

Figure 2. ffe t of - y an inhi itor of protein kinase on phor ol- myristate- a etate / indu edglu ose uptake in o ine mammary epithelial ells ells were in u ated in media with different on entrations of - y - and *M* for min and then treated with or without / g/m The glu ose uptake in theglu ose group without / and - y treat ments is assigned a alue of- alues with different letters a are signifi antly different *P* n rror ars indi ate the

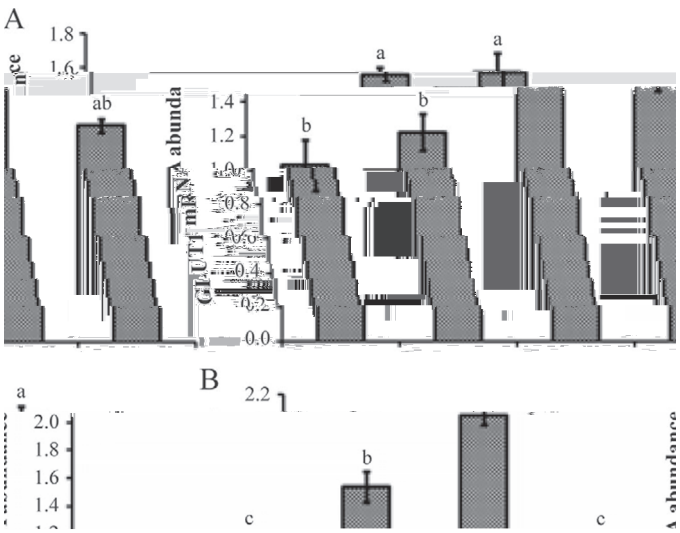

Figure 3. The m! / a undan e of s lu ose transporter- *GLUT1* / and *GLUT8* s enes in o ine mammary epithelial ells treat ed with different on entrations of phor ol- myristate- a etate / The m! / le els of *GLUT1* and *GLUT8* in the ells without / treatment are assi ned alues of- alues with different letters a are si nifi antly different *P* n rror ars indi ate the

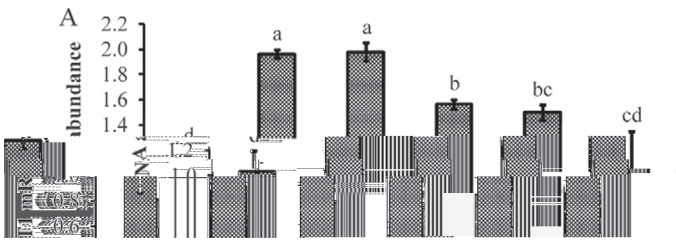

Figure 4. ffe t of - y an inhi itor of protein kinase on phor ol- myristate- a etate / indu ed s lu ose trans porter- *GLUT1* and *GLUT8* m! / e pression in o ine mammary epithelial ells ells were in u ated in media with different on entra tions of - y and *M* for min and then treated with or without / m /m The *GLUT1* and *GLUT8* m! / le els in thes roup without / and - y treatments were assi ned alues of- alues with different letters a d are si nifi antly different *P* n rror ars indi ate the

on entration of *M* or hi her *P* i ure t appeared that the m! / e pression of *GLUT8* in may e more sensiti e to a ti ation than the e pression of *GLUT1*

re ious studies ha e shown that a ti ation indu es a ti ator protein **AP-2** mediated tran s riptional a ti ation awa et al - yman et al - oth phor ol ester response elements and / indin sites ha e een identified in the rat *GLUT1* s ene in li er ells ehroo and smail e i - The *GLUT1* s ene is hi hly onser ed a ross mammalian spe ies hao and eatin and the rat phor ol ester response element and human / element indin sites ha e een found in the o ine *GLUT1* s ene Therefore it is likely that may a t on these sites dire tly or indire tly to a ti ate T- e pression in The o ine *GLUT8* s ene has not een well hara teri ed thus it is not known whether these elements are present in o ine *GLUT8* s ene

e annot rule out the possi ility that may stimulate s lu ose uptake y the me hanisms other than enhan in *GLUT* m! / e pression os h et al reported that / stimulates s lu ose uptake in T - adipo ytes y in reasin the T- protein ontent in the total ell homo enate oreo er the translo ations of T- i s et al - os h et al and T in rat adipo ytes tandaert et al otani et al atson and essin - and T in rat intestinal ells hei and arr - were o ser ed in re ulated s lu ose transport Therefore it is possi le that may also re ulate protein translation translo ation or transport kineti s of T in to enhan e s lu ose uptake n summary this study pro ided e iden e that may play a role in the re ulation of s lu ose uptake and e pression of *GLUT1* and *GLUT8* in

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