ƒ ‹ F | Œfia‹ ˙'e›ß• |–M^D‹ ª¤s›p e ƒ¤"G‡ł§i

⁄j¥ „«⁄"¯ ƒ¥H⁄ ¸¥ ⁄Ufl " † ›M¥Hokadaic acid ¤°¿Efl ‰Ł⁄ , ⁄§‹ ª¤s

›p e^ §¡G §O«‹ ›p e

›p e‰ ¸s„¡GNSC91-2314-B-038-046-

ıƒ · ¶¡¡G91 ƒ~08 ⁄°01 ⁄ ƒ 93 ƒ~07 ⁄°31 ⁄Ø

ıƒ ‡ ƒ ¡G» O ¥´ ˙⁄j ˙, › ˙‹

›p e¥ D ø⁄¡HG¶ z†[

›p e »P⁄H›ß¡G‡ fl « ‹ ª¤s¥˝

‡ł§i^ «‹¡G" °†‡ł§i

‡ B † zŁ¡fG¡¥»›p e¥ i ⁄ ‰ ¶‹ d,

⁄⁄ ¥` Œ 93 ƒ~12 ⁄°20 ⁄Ø

ƒ ‹ F | Œfia‹ ˙'e›ß• |, §U–M^D‹ ª¤s›p eƒ¤"G‡ł§i

⁄j¥ „«⁄"¯ƒ¥H⁄ ¸¥ ⁄Ufl " † ›M¥H okadaic acid

¤°¿Efl ‰Ł⁄ , ⁄§‹ ª¤s

›p„"‰s, „¡G NSC 91-2314-B-038-046

ıƒ ·`¶¡¡G 91 ƒ~08 ⁄°01 ⁄Øƒ 93 ƒ~07 ⁄°31 ⁄Ø

¥D«ø⁄H¡G¶ z†[˘——´

›p„" »P⁄H›ß¡G‡fk‹ ‹ ª¤s¥˝

ıƒ "c⁄ ¸‡ ƒ ƒW" ¡G¥x¥_´ ˙⁄j ˙, › ˙‹

⁄@¡B⁄⁄⁄ "K›n

fi L¥h""‹ ª¤s– o "¡A ' ⁄ "ƒ¥H¥ ⁄U⁄§fl " † ´ · ¡ A g¥ (ƒp", »X catecholamines) " " ¤ ° ¿ E ¡ A ¥ i ¥ H « P ¶ i fl " † › M ¶ i ƒflcAMP , ¡C¤ §@¥ ¸ T fi ¶˙¡A‹ ¡˘ cyclic AMP-dependent protein kinase (PKA) ¡ ¶iƒ «P¤˘† M‰Ł⁄"" hormone-sensitive lipase (HSL) ` ©˜⁄˘ƒ ‹ ¡˘¡A¤^¥ † ›M‰Ł †ƒfl " " w " ›«P¤˘fl ‰ Ł ⁄ , ƒ¡G¥~¡ AH phosphatase ""§ ¤ flokadaic acid ¤ ° ¿ E fl " † ›M"¡A"‡ ¥›M ‰Ł• | o ¥ "«©˜⁄˘""† ¶H¡Aƒ P fi ⁄ ¶ |fl ‰ Ł ⁄,ƒ ¡©ƒ„^ "«" " §¥¤¸^⁄ £ ¶…W ¥[HSL ""‹ ¡'°¡Aƒ ‹ O R¶HSL ´ †ƒfl " " w " ›¡Cƒ]ƒ„fl " " w " ›‡ ¥ ‰Łƒbfl ‰Ł ⁄ , ⁄Ł›–§ Œ " t‹«"›"¤ ƒ ¡C" æ ƒ¤ o ‡⁄"ƒfl " † ⁄›M"HSL ‹ ¡'°, ß ¥⁄fU " † ›M ""HSL ‹ ¡'° "¡ F ¥ B ‡" ›M⁄"fl " " w " ›‡ ¥ ‰Ł¥ ⁄£" ‹ ƒP¡C' ¥H¥ » „ Œ ¥¸˜⁄j¥ „«⁄"¯ƒ¥H⁄ ¸¥ ⁄Ufl " † ‹›M §fi¡˘Aƒb isopreterenol ¥H⁄ ¸ okadaic acid ¤ ¿E« «Æ¡A ;œ ¤^⁄æ, ßfl ‰ Ł «Æ" " fl"»˜˜ æ¶pC ϕ{ H ¿«@ ⁄ W ¡A· ´ fl " »˜""§ t ¶‰,TŒ..W ¥[¡Cƒ §Q¥ ¸ SDS-PAGE „ ¢¡a⁄Ł"k⁄ "Rfl " " w " ›‡ ¥ ‰ Ł"¯ ⁄˘¡A⁄] o†ƒb isopreterenol ¥H⁄ ¸ okadaic acid ¤ ¿E«Æ¡ A fl"" w " ›‡ ¥ ‰ Ł"" ^»§P ¶q» PL^ "«¤° ¿ E"„ •†⁄æ, ß⁄]ƒ‡⁄ ƒP""¯ ⁄˘¡Cƒ ' ‹ O†£¥˝¯ ⁄˘" ‹"Oƒ " ‡ ¥ ‰Ł« h «§¶i⁄ @B"" „ Œ ⁄ "R¡C

^ ` ƒ:okadaic acid, fl ‰ Ł ,,fl " † ›M

Abstract

Several studies have shown that lipolytic hormones such as catecholamines stimulate lipolysis in visceral and subcutaneous fat cells. The mechanisms are mediated by increasing in intracellular cAMP concentrations and then activating cAMP-dependent protein kinase (PKA) which induce phosphorylation and activation of hormone-sensitive lipase (HSL). The activated HSL translocates from cytosol to the surface of intracellular lipid droplets to promote lipolysis. Okadaic acid, an inhibitor of protein phosphatases type-1 and type-2A, is also found to stimulate lipolysis in adipocytes. It does not increase

activity of HSL but induces translocation of HSL to the lipid droplets, thus the surface proteins located on lipid droplets may be closely related to this lipolytic action. Recently, visceral fat cells are found to contain higher HSL activity than the subcutaneous fat cells. There is also considerable difference in protein pattern between visceral and subcutaneous lipid droplet preparations. In this study, we found the increase of free fatty acid during isoproterenol- or okadiac acid-stimulated lipolysis by spectrophotometry. The protein pattern and protein content of lipid droplet fractions following isoproterenol- or okadiac acid-stimulated lipolysis were different from that of control by SDS-PAGE. The changed proteins could be identified in advanced studies.

Keywords¡Gokadaic acid, lipolysis, fat cells

⁄G¡B†‡Si⁄″fie

1. «e¤¥»P‹ ª¤S¥ ″″

fl ″ † ´ · ¥ D › n ¤ ƒ†(«fOp¯@f† '…ƒh″c†¸ȳ)₣ ₣¸«¥O•x″″¥ \ fl Ą.ffl ¥₣Ł ⁄ , (lipolysis)† ¥˝″″fl ″ »˜(free fatty acid)⁄] ‹ O ¤¯Øn″«fl ¶ q ¤ • ‰ S⁄f@¡″C¤Ø • ƒ ₧›⁄ØSG″″ƒ ,m¥ Dn⁄ ‹ ¤ ⁄j¡^A⁄ @ ^ ‹ O ƒ'† ¥.h»Sfl ″ † ″‹ subcutaneous type¡A¥t⁄ @ «h‹ O ƒ'⁄″¯ƒ' ₱ ″″fl ″ † ″‹ visceral type (Leibel et al., 1989)¡G¥ ' visceral type ″″fl ″ † ¤ fl ‰ Ł ⁄,† ¥˝″″fl ″ »˜⁄jf ₧g¥ ¤ x ″ ØRfl (hepatic portal vein)¶ ŧJ¤x¯ƒ¥ ₣¸· ` ¤t⁄†″¡Aƒ ƒ„ƒ† · ″ – ‰isceral type ″″fl ‰Ł ¥ Ñ´»P¿ } Sfl₣ ₣¸ °fl w˘ ¥fl fl₣ƒ†,ß⁄j″″^ þ'(Bjorntorp, 1985)¡C

L‰ ‹ Ovisceral type ' ¿‹ Osubcutaneous type ″″fl ″ † ›M¸Afb¤ ¤ †″',»X˜ ₰¤° ¿Efl ‰ Ł ⁄,fi ¡A¤ T fi S ¶ · ,‹ Ği⏐¥ † ″»₰P† › ₥⁄″W¤ „ † ₣«₣A¤ ˘o † ›M⁄″″cyclic AMP (cAMP)¿ ₰⁄W ¡A‹ ¡⁄˘† ›M‰Ł⁄″″cyclic AMP-dependent protein kinase (PKA)¡A¶if «P¤ ˘ † ›M″› ₮dfl ‰ Ł ⁄,″″» fl hormone-sensitive lipase (HSL)` O»˜⁄¥ ₣¸ ¡⁄˘(Olsson et al.,1987)¡ Ą ‹ ¡⁄˘″″HSL • ⏐¥ † ›M‰Ł⁄⁄ † ¤ fl ″ ″w″″ ›–¡A¶i⁄@¤ BNfl ‰ Ł ⁄,ƒ ¤ ¥˝(glycerol)»Pfl ″ »˜(free fatty acid)˜ ¥X¡Aƒ ƒ„fl ‰ Ł ⁄,″″„ L{⁄⁄HSL ″″^ O»˜⁄˘»P‹ ¡⁄˘¤ ƒ†›«›n″″¥ Ñ (Yeaman et al., 1994)¡C

″ æ~¤ †\ƒh″″‹ ª¤s† ~ ofk₣fl ″ † ›M⁄″″″fl ″ ″ w ″ ›ƒ††\ƒh″″† ₩ ‰Łfp perilipins¡Badipose differentiation-related protein (ADRP)»P capsular protein ¥¡A‹ ¤ ƒ† ‰ –⁄fl ‰Ł¥ Ñ´″″¥ Ñ (Londos, 1999)¡CPerilipins ‹ O† ƒ†Q ф{″″fl ″ ″ w ″ ›⁄– ₩ ‰ ₤ĄA ƒsƒ ₰ fl ″ † ›M»P' TJ^ ⁄ ″c† ›M^″″fl ″ ″ w ″ ›(Greenberg et al., 1991¡BServetnick et al., 1995)¡C• † › M ¤ ¤ † ″,•»₰¤Q†₫KEA¡` O»˜⁄˘¡A†Q»{‹ ¤ ƒ† SUHSL ″ ' fl ″ ″ w ″ ›″″¥ Ñ (Londos et al., 1995)¡CAdipose differentiation-related protein (ADRP)«h‹ O• ¶Mƒsƒ ₰ †\ƒh⁄ £ ₣″^ ″″† ›M⁄⁄¡Afp adipocytes, steroidogenic Leydig cells, fibroblasts »P hepatoma cells¡C⁄ ₩ ƒ„† ₩ ‰Ł¥i¥Hk«P¶i† ›MS l ƒ ‹ ₤fl ″ »˜ (long-chain fatty acid)¡AS ¸ƒ¤fl ″ ″w ¿n¡A¤ ¥]† ƒb″ S ¸ƒ¤″″fl ″ ″w' ₱ ¥[¥Hk‹ O@ (Brasaemle et al., 1997)¡Cƒ †Q¯n† ₩ ‰Ł(capsular protein)«h‹ OS › „ Œ « ₫'{¡Afs ƒ ₰ ' TJ^ ⁄ ″c† ›M^″″fl ″ ″ w ″ ›¡–A• † › M ¤ ¤ † ″,›₰¤¸ª ¿₰Łfi‰Ł•⏐–qfl ″ ″ w ″ ›⊢ ,¤¡A· ´ ƒb† ›M‰Ł⁄⁄¡A– ·∞»Pfl ‰ Ł ⁄,ƒ†^ ¡C(Wang and Fong, 1995¡EFong et al., 1996)¡C† ″æS › ″″‹ ª¤s ф{ƒbfl ″ † ›M»P ˙⁄W, ¢¥ ‰Ł† ›M^″″fl ″ ″ w ″ ›ƒ†¤'

¥ L « ‹ ƒ °(beta-actin)" " ƒ(Dong et al., 2001)¡ C ƒ] ‹ ƒ ‡ " ƒ·¿,g¥‡"
ƒ h fi ‰ (anti-actin antibodies)' ¸¥H^ "«cytochalasins ‡ B † z · ⁄ W , ¢ ¥‰Ł† °›M⁄z´Z
‡ ¥ « Æ ¡ Ao‡ACTH ' ⁄ o"fl ‰Ł⁄ , †{¶ H ⁄• |o¥ ˝ ¡ F ƒ‹‰Ł⁄ , '˜"¥X‡T
J(cholesterol)« o L " k „ Be¯ƒØ†⁄‰Xƒ¤ · ⁄ W ¢¥ «(cortisols)¡ C]ƒ „ – ·œ¥‰‹
ƒ ‡J¥ i fl»P ‰Łƒb› M⁄"""„ Beƒ‡^ (Mrotek and Hall, 1977; Hall et al., 1981)¡C
¥ ƒ ¥i¤£¡Afbfl ‰Ł⁄ , "„ L{⁄⁄ £P"fl " " W ‡›¥ ‰Ł‰ C " t ‡‡›» «n"¤ ƒ ¡C
Okadaic acid ‹ O⁄ @ serine/threonine phosphatase typ-1 »Ptype-2A "‰ ¤ £A¥H
ƒ„^ "«¤ ¿E† ›Mi • | ›P† ›M⁄"""‡\fh‡ ¥ ‰Ł¥X†{ " « C»"‰"†{¶H (Bialojan et
al., 1988; Haystead et al., 1989)¡C⁄ W okadaic acid ¤ ¿Efl " † M¡¥Ai¥H« ¶£ifl ‰Ł⁄ ,
"‰ ¶¿¡Af ¥ £ fl " " W "Y–perilipins ⁄]ƒ‡` C ›⁄"""†{¶H(Clifford et al., 1998)¡C
¥H⁄"¯ffl " † ´·(epididymal fat pad)‹ ‰ fi˘¡A¶i⁄@¤B⁄ "Rƒ„^ "«"‰ ¶¿ ´ fi ¡ A o†{
okadaic acid "‰¤ ¿E• |¤ ˘oHSL ` C ›⁄¡Af ‹O¤^⁄£• |¤˘† ›M⁄"total HSL "‰‹ ¡ ' …W¶¥A[
ƒ ‹O« ¶£i` C ›⁄""HSL ´ † ƒ fl " " W (Morimoto et al., 2000)¡Cƒ„¥~¡A– N H⁄fuX
ƒ¤"fl " "w(pure lipid droplets)»P` C ›⁄""HSL ƒ@ƒP ¤|¡A¤^⁄£• ¥X†{fl ‰Ł⁄ , "‰†{
¶ £F⁄˘ƒ ‹O–q† ›M⁄⁄ ´ ¥X"fl " "w(crude lipid droplets)»P` C ›⁄""HSL ƒ@ƒP
¤|¡A⁄~• | o¥ fl ‰Ł⁄ , ¡ C ·"› ƒ]ƒ „ ‰ ¡Afl " † ›M"fl ‰Ł⁄ , S ¶¿¡A £ P»›n« ¡
⁄˘"""HSL ⁄S¥~¡A¥D›n¤ Mwƒ]fl ‹Ofbfl " "w"" "c¡A¥ SYfl " " W "Y‡ ¥ ‰ŁS Ct‰
––fl ‰Ł¥ Ń´"›« › ¤⁄ƒ(Morimoto et al., 2000)¡Cƒ ‹O¥Hokadaic acid ¤ ¿E«Æfl " "
›–‡ ¥ ‰Ł"‰ ¶q»P" ^ ƒ‡ L¯ ⁄˘¡A«o' ¥..ƒ‡⁄ m̃‡Ł ¡C¥t⁄@⁄Ł›–¡A⁄wf‡‹ ª ¤ s ‡Si«
¥X¥H« ƒP¿@« "¥¿ · W,f¢ (norepinephrine)¤ ° E£fi¡A⁄"¯ffl " † ›M⁄› ¢dfl ‰Ł⁄ ,
""HSL ‹ ¦°¡A⁄æ¥ ⁄Ufl " † ›M⁄"HSL ‹ ¦°‹ "¡Ff ¥B† ›M⁄"fl " " W ‡›¥ ‰Ł
"" ^ »P‰ ¶q⁄]⁄£" ‹ ƒP(Morimoto et al., 1997)¡C‡ "æ‡\f h " ¥H»«"3T3-L1 fl
" « ¤›† ›M« ‰ fi˘¡Afb^ "«isoproterenol "‰ ¶¿U¤ ¿Efl ‰Ł⁄ , ¡A⁄æ,ß¤ ¿E«e«Æfl
" " W ‡›¥ ‰Ł"¯ ⁄˘¡C ¢{⁄£‰ ¤ ¿E«e«Æfl " " W ‹›–ƒ‡perelipin, S3-12,
vimentin ¥H⁄¸TIP47 ¥ "c'°‡ ¥ ‰Ł¡C‹ ⁄˘ƒa¡Aadipophilin, caveolin-1 ¥H⁄¸tubulin
¥ "c'°‡ ¥ ‰Ł¥u¥X†{ƒb¤ ¿E«Æ"fl " " W ¡C¥t¥~` ƒ‡‡\fh»Pfl ‰Ł¥ Ń‹ ^ "‰†J
¥ ‰ ŁAfb^ "«¤ ¿E«e«Æ]‡£ƒ‡⁄ £P"¯ ⁄˘(Brasaemle et al., 2004)¡C
' ¥H¥ » „ Œ ¯ ›p› e§¥H⁄j¥ „« ‹ ¤s„ ¶H¡A⁄ § ©¤¥X⁄"¯ƒ(epididymal fat pads)
»P¥ ⁄U(femoral subcutaneous fat tissues) ¥‡¡ƒ "fl " † ´·¡A⁄ ´ ¥ ¤ " † M¡A„'
okadaic acid ¤ ¿E¡A« ¶£ifl " † ›M⁄"""HSL »Pfl " " W ‡›¥ ‰Ł` C ›⁄¡A¤^⁄ §O ¸
·œfl " »˜"‰˜ 'æ¶qƒ‡ L¯ª fi ŧS¡Cƒ ¥Bƒb¤ ¿E«e«Æ⁄ ´ ¥X† ›M⁄"""fl " "w¡A¥H„ Ča
"k(SDS-PAGE)⁄ "R" ›–‡ ¥ ‰Ł"‰ ¶q»P" ^ ‹O§ § ¯ ¡C§ ˘ –´¥ ƒ„„ Œ ¶i⁄@¤B⁄F,
ƒ ' ⁄"¯ƒ»P¥ ⁄U"fl " † M¡A fl ‰Ł¥ Ń´"" ¤ »P" ›–‡ ¥ ‰Łƒ‡ƒ ⁄ £P¡A¥H ‹ –N
¤ ‡ fQ ‰Ł‹ ^ fl ¤lf"" ´ƒ¡C

2. ‹ ª¤S⁄Ł"k

¡]1¡^„Œ¯ °"«

¤œ250-300 § J › « §¶M¡star rat¡A „ } i ƒ b ‡ q › • ¤ } ƒ n " " „⁄U¡A¤^ „⁄'¥¿–
¥Hsodium pentobarbital (50 mg/kg body weight), g , ¡ ˜"›fl¥G‡ ´K¡A⁄ § O ¤ œX⁄"¯ƒ
"fl " † (epididymal fat pad)¥H⁄ ¥ ⁄Ufl " † (femoral subcutaneous fat tissue)¡C

¡]2¡^fl " † ›M"″⁄ ´

fi 1964 ƒ~Rodbell "″⁄ Ł " ¶ifƒ " † ›M"″⁄ ´ ¡ C § @ ƒ p ⁄ ¡G"″N⁄″j¥ „« ¯ƒfl " † ´·(epididymal fat pads)¥ H ⁄¸¥⁄Ufl " † ´·(femoral fat tissues)¡A⁄ § O ¸m' normal saline (0.85 %⁄§ NaCl)⁄ ⁄ ... ‹ «Æ¤Æ¥Xfl «««ÆNfl " † ´·, ¸m albumin-bicarbonate buffer(§t 4% BSA, 3.3 mg/ml collagenase type II⁄¸ 3£g moles glucose, pH 7.4)¡A¤ • w/v = 1:1⁄ æ ¡C¤ ^ ¥ ⁄¸p–Nfl " † ´· ¯ ƒ ¤p¶Afb 37°C ⁄⁄ ⁄ ˘ ‡1⁄pfi ¡Cƒ „ fi †‰l´·"″‰ ƒ › ¯ ″ß¡¥Afl," † ›M• ƒ fl " † ´·⁄⁄⁄ ´ ¥X¤ ¡A ¤ ˘ – o ‰ w ‰ †G¡C¥HV¿hB–Nƒ¤¥ ƒ ¶ " ¥...⁄ , ⁄§fl " † ´·¶ ¤ ¥X¥ Æ «Æ¡A¥H 400 g⁄§´ ‡t´ ⁄ 1⁄ ˜`¡A« Æi⁄ ´ ¥X⁄ Wh⁄§fl " † ›M¡A⁄U..h(¥]§t⁄W¥ † ´·,⁄″¥ † ›M, ¥ ¤‰† ›M ¥)«h¥ Æ ¡ ∈N⁄ Wh⁄ ´ ¥X⁄§fl " † ›M¥H 37°C "″‰ w ‰†.G ‹«¡Afa¥H 400g ´ ‡t´ ⁄ 1⁄ ˜`¡Af„†M‹ ~¤ BJ›«‰˘3 ƒ,«Æ¡Af ¶ ⁄ Wh¥ ƒ "″fl " † ›M¡A¶ ƒ „ Œ ¡C

¡]3¡^Okadaic acid "″¤°¿E

– N » s ‡ ˘ § · ⁄§fl " † › M ¡ A • n⁄ ^ « Æ ¥¶ Buffer, A(§t 2.5% ⁄⁄¡A¥t¥~¥[⁄J BSA, 135 mM NaCl, 5 mM KCl and 1 mM $MgCl_2$, 25 mM TES, pH 7.4)¡ A ƒ Æ[⁄J okadaic acid¡ F˘ okadaic acid ‡ † @«0¡B3¡B10¡B30 µM¡A' 37°C ⁄ ⁄ ˘ ‡2⁄ p fi¡C ¥H 400g ⁄ § ´ ‡t´¡Af ‹ ¶ ⁄ W ... h ⁄ § fl " † M¶¡Af fl ..." h″w⁄»§† ‹h¶iƒ fl " »˜(free fatty acid)⁄§ »œ¡C

¡]4¡^ ¸·œfl " »˜⁄§·œ'w⁄Ł"k

¥ » „ Œ ¯–˜1986 ƒ~ Okuda ¥ " ⁄Ł"k¡ A»·œfl " »˜" " ˜ ' æ ¶ q ¢¥ –N⁄W ›z okadaic acid ¤ ¿Efl " † › M Æƒ‹§• »G(‹ øœ 250£gl)»P 3ml chloroform-heptane 1:1(§t 2% methanol) † ¥X¡A¤ ^ ¯ ˝ '§10 min¡Af ÆH 2,000 g ´ ‡ t´ 5 ⁄ ˜`¡A¥H ' §l"″⁄Łƒ¡– NW.. h • »G.. h § l ¥¥¡Æ∈N 1 ml "″Copper reagent ¥ [ƒ⁄.Uhƒ ‡ • »G ...h¡AƒAƒ,¯ ' 10 min¡A¤ ^¥H 2,000g ´ ‡ t´ 10 min¡C¤œ⁄W..h" ‰Ł‹ ø0.5 ml »P 0.5 ml chloroform (⁄″§t 0.1% bathocuproine »P 0.05% butylated hydroxyanisole)† V ƒ §¡¡Af «Æ¥H" ¨Ł 480nm ·œ¶qfl " »˜⁄§˜ ' ¶q¡C

¡]5¡^† ›M⁄″fl " ″w⁄§⁄ ´

– N ƒ ‹ ¶ " " £ƒfP‡¡ƒ ›M»P phosphate-buffered saline (PBS: 137 mM NaCl, 2.7 mM KCl, 1.5 mM KH_2PO_4, pH 7.2)¡ A ¥ H Ø¿ 1:4æ⁄§⁄æ¤ , ¸m ⁄ƒ"ß ª ¿ i " ⁄⁄‹ ª¿i 10⁄ ˜`¡A– ¥H 13,000g "″´ ‡t´ ⁄ 30⁄ ˜`¡A¤ œ ⁄ W h¥fl ƒ" ...h(⁄ S "‹ fat cake)¡A ¤ ^»P§ ƒ‡ 0.025 % Triton-X 100⁄§PBS † ¥X¡A• nœ10⁄ ˜`¥H ¥h† ›M‰⁄⁄§fl ‰ ƒ¤ ¥ ¡Af Æ,¥H 13,000g "″´ ‡t´ ⁄ 30 min¡A¤ œ ⁄ W h¥fl ƒ" ...h¡A§ Y ¥ i " ¤B† ›M⁄" "″fl " ″w¡A¶ i ⁄ @ ¤ B §¥⁄¸„qRa¤ " ›¥ ‡‰Ł¡C

¡]6¡^„q"a(SDS-PAGE)

⁄ W › ¥¿(¥`... , g ^ "«)¤°¿Eg ^ ˜ ‹‹¤¿Æ⁄ ´ ' – o " " fl " ″w⁄ ´ ' –o"″fl " ″w ¡ Æ 10£gl »P Bio-Rad protein assay dye † V ƒ §@«¥Æ¥H⁄ ¥ œ ¥ œ « p ƒ595nm ⁄ Uœ–o‡ J ¥ ‰Ł¢@ C ¤ l ¥ [ƒ ¥¯ample buffer (§t 2% SDS, 15% glycerol, 1.5

M β-mercaptoethanol ／STris-HCl, pH 6.8)¡ Æb95°C ／ ／ ¥…5 ／ ˜`¡A¥H10,000 rpm ´ ＋ ť ｲ／ ˜`¤ ˇ fl " ‰／¡Œfl Bæ«Æ U … h S t ＋ ¥ ¡æŒH»"＄ ¡＃¥ ˇ ƒ "0.$t% SDS "" 10 % polyacrylaminde gel ¶ i ƒ „q¡"Æ– N £ƒP ／ ¶q" " ＋ ¥ ‰Ł´ ¡AƒA¥H Commassie Blue R-250 ‹ ¥ƒ ¡A‹ æß／ £P＋¡ƒ " " fl"＋ ›Mƒb^ ˜ " « ®¿Æ«Æefl " ‴w»– ＋ J ¥‰ŁS†SƒP¡C

3. †"G»P Q‰

– q ／ j ¥ „ « ˜ , A ／ W ／ Ł ¤ œ ¥ X " " ／ " ¯ ƒ fl " „ ¡A／ ´ ¥X¿W¥ ""fl " isoproterenol ¤ ¿E¡A o † {H^ "«¿ ® –q0¡B3¡Bl0¡B30 µM ＋ v " ¥W¥Æ¤ C † @ ／＋ ƒ (ml)" " fl "†¤O›Mƒi (h)˜ 'æ¥ X"· ´ fl"» ˜ S t ¶ｲ–]q•0.275¡B0.300¡B0.325¡Bl.075 mM ＋ v " ¥W¥¡[ƒ„1 ' ¥¡C^ "«isoproterenol ¥ D › n • » , ' ¥†¡ZÆƒ„ƒ]Q‰S¥／ ¤ˇ^ "«¿ ® ‹ 0¡ A † ›M · ƒ ＋ ‴¥» ´ fl"» ˜ ˜ 'æ¡¶C¥ ¥~¡ A ¥ Ł˜Ùokadaic acid ¤° ¿Efi ¡A／] o † { H ¤°¿Ł0¡®3¡Bl0¡B30 µM ＋ ¥／W／ fi ¡A¤ C † @ ／＋(mƒ)" fl " † ›MC／ ƒi (h)˜ 'æ¥ X"· ´ fl"» ˜ S ¶q–q0.100¡B0.900¡Bl.000¡Bl.075 mM ＋v " ¥ W¥¡[ƒ„2 ' ¥¡C¥ ' ^ "«okadaic acid ‹ O• » , 'DMSO ／¡Aƒ ƒ„• ^ "«¿ ® ‹ 0fi ¡A‹ O¥HDMSO ¥ N ¡A¥i ф{DMSO ＋B†zƒ ¥G• |S ¤ fl " † ›M"¥»· ´ fl " »˜"˜ 'æ¡O› L ＋ ¥ fŁU " „ ¸／ ´ ¥ X"ƒŁg^""＋¤ ¿Æ«Æ˜ 'æ" " fl"» ˜ S¶q , ß ¡]data not shown ¡ˆC／æ, ß„ˇ／®»P„ˇ／Gfi ／]¥i¥H ф{okadaic acid SC¿ ® ""¤ ¿E«K ¥i¥H„ Æ , ß／ j ‴fŁ‰Ł／ , ¡C

, g–˜¤ œ ／ ´fl"w" "Łƒ¡ ÆHSDS-PAGE „ ¤a／Łƒ¡／ " R " ›–¥ƒ‰Ł"˜ ／ˇ¡A¯ª ¥ ／ " ¯ ﬂ ‴w" →＋¥J ‰Łƒbˆ "«isoproterenol »Pokadaic acid fi ‰ T ŒS‡e{／ £P"˜"＋J¥ ‰Ł«‹ƒ¡] ƒ„3 ' ¥¡C¥ ¿ ¬ Ł "«¤ ¿E"˜„ •†¡ A – q fl " ／＋／›M ´ ¥ X"˜ﬂw¤" " →＋¥J ‰ŁS ¶q„ ß ƒh"˜ƒ＋72¡B56 »P32 kDa ¥＋J¥ ‰Ł¡]lane 1¡ˆF• ¥H30 µM "" isoproterenol ¤ ¿E«Æ ф{¤ ／／56 kDa "˜"＋J¥ ‰Ł« ‰œ¯ª• ／¡]lane 2¡ˆƒ • ¥H30 µM "" okadaic acid ¤ ¿E«Æ«h ф{¤ ／／56 kDa "˜"＋J¥ ‰Ł'œ¯ª... W [¡Aƒ 72 »P32 kDa ¥＋J¥ ‰Ł« ‰œ¯ª• ／ ¡]lane 3¡ˆC

¥ «／ƒw＋＋\ƒh／ m̃ˆ„Œ¡ A ˆ"¤« ¿E«e«Æ＋\ƒh" " fl"˜"w" →＋¥J ‰Ł"˜S ¶q›P" ^ • |†£¥˝S ¯ (Brasaemle et al., 2004)¡ŒPerilipin " ″ ／l¶q‹ ø« 56 kDa¡Aƒbepinephrine ¤ ¿E«Æƒ＋＋ ´ fl"˜"w" ›–"˜– $¸¡Aƒ„†{¶H»PS › "˜" ф{"˜"56 kD ＋J¥ ‰Ł' °‰Ł‹ "æ¡C ‹ OS ·N‹ Operelipin ¥H／ ¸¥ ¥~"˜"72 »P32 kDa "˜"＋J¥ ‰Ł«h» S¶ｲi@¤B„Œ ^ 'œ¡C

ƒ ／ ˜m¡G

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1

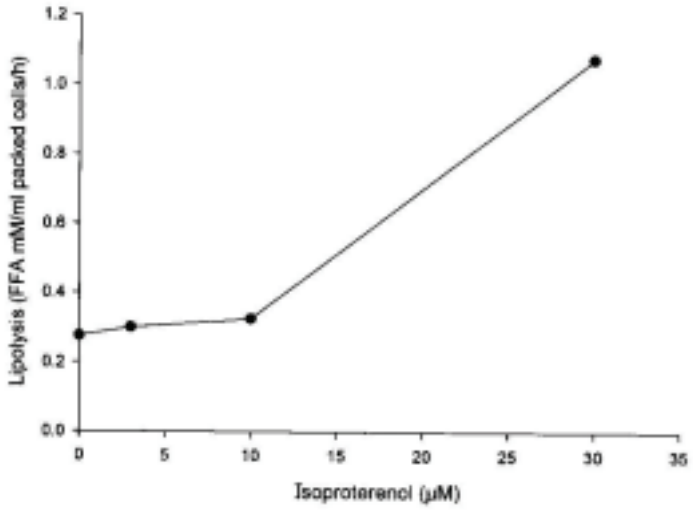

Fig.1 Effect of isoproterenol on lipolysis in rat fat cells.

2

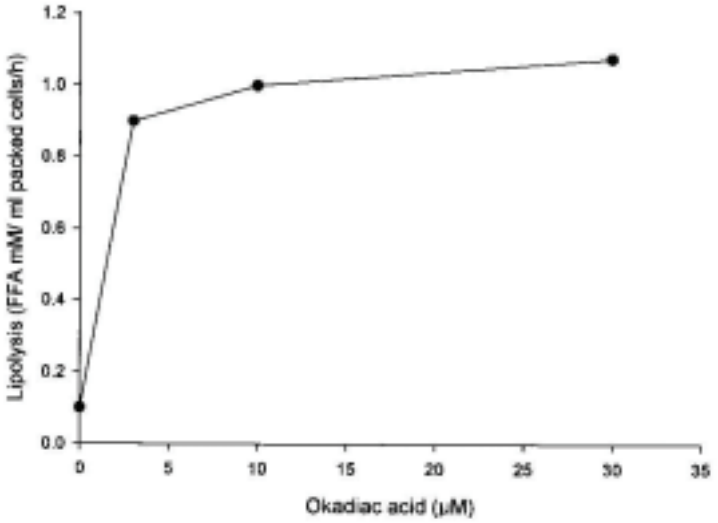

Fig. 2 Effect of okadaic acid on lipolysis in rat fat cells.

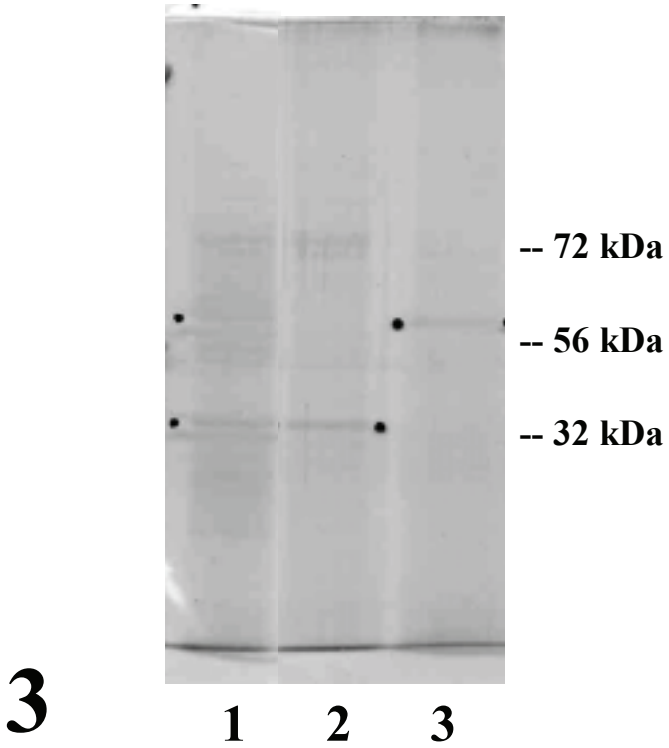

Fig. 3 SDS-PAGE of proteins of lipid droplet fraction from rat fat cells. Lane 1: normal fat cells. Lane 2: isoproterenol-stimulated fat cells. Lane 3: okadaic acid-stimulated fat cells. Note that the 56 kDa protein decreased during isoproterenol treatment but it increased during okadaic acid treatment.