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Carbohydrate Polymers

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H _ t s s_ _ s *Enterobacter cloacae* 0206 KKA

M H _ , F _ , , H_ _ 8_ , 8 z _ *

The Key Laboratory of Molecular Animal Nutrition, Ministry of Education, College of Animal Sciences, Zhejiang University, No. 866 Yuhangtang Road, Hangzhou 310058, PR China

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ABSTRACT

_t s s_ _ s (E) s _t t t *Enterobacter cloacae* 0206, _ t _ ts E st _t . I t s st , KKA _ _ t t st _t E (200 / t) 6 s. s ts s t _t E ss ss s fi _t ts. I _ s t _ , s s s _s _s _s s t s (G), st (C) _ s t t st (LDL) s _ t t _t t t E . F t , E _t t ss s (GK), H L (H s t L _s), _ s t _s (A GL), _ t _ t _s _s 1 α (C 1 α), s t _s t 2 (G t2), _ s s t _t _t t s (AM K) _ s t _t 1 (t1), t _t t ss s 6 s _t s (G6) _ _tt _ s t _s (FA) t . s s tss st t _t E ts _ ts ss t _t AM K _ 1 _t ts _ _t _ t_ s .

2014 E s Lt . A ts s .

1. Introduction

D _ t s t s s t st _ t_ s _s s t (D t_ ., 2003). M t 90% _ t _t s _ s t _ s s t _ t_ _ t s (t 2 _ t s), s _ _ t z _ s _ s t ts t s s t _ _ t (D z , B _ , & F _ , 1992). I s s st_ _s t _ t t s t_ ss t 2 _ t s s ts t _ t s _ t t s, s t _s t, s t s s _ ss _ _ t st s (D z & F _ , 1991). A t s s st_ s _ t_ t st _t t t _ _ t t t 2 t s. _ _ t _ t ts t t _t t t 2 t s t , s _ s _ s _s, t t s s t t _ ts_ _ss _t t t_ _ s s ts _ t t (G ,8_ _ , & _ts, 2002). ,

t s ss_ t _ s t _t _t s _ t _ t _ s _ t . s_ _ s _s _ t_ t _ss _ t t _ ts, st t _ _ _ s t s fi _ t t s, s _s st _t , _t _t, _t _ (J t_ ., 2010), _ t t (, _, F , , & G_ , 2009) _ t _ t (L t_ ., 2006) _ t t s. F t _st _ s, s_ _ s s _t *Astragalus* (M_ t _ ., 2007), *Ganoderma* (t _ ., 2013) _ *Ophiopogon* t_ _ _s (t _ ., 2011) _ t t ts. t s _ s t _t t ss _t _t st_t s _ _ _ t_ t t t _ t s (_ t _ ., 2007). s, _t st ss _ st t t _ t t t _ t t t s, _ t _ t s . M_ , L ,8 , C , _ _ (2009) t _t *Lycium barbarum* s_ _ s t _ t _ _ st _t t _t _ s s t _ t _t t s. _ s t t _t s _t *achyranthes bidentata* s_ _ s t t t ts st t z t _ t _ ts, ss _t st ss_ t ts _ s _t _ (, C , L , & J , 2009). I sst , s t E _s fi t s_ _ s *Enterobacter cloacae* 0206. E t t _ t 1.1 × 10^6 _s s L s , D s , D _ _ t s , D _ _

* C s _t _t: K L _t M _ A _ N t t , M st E _t , I st t t F , _ H s t , C _. .: +86 571 88982815; _ : +86 571 88982650.
E-mail addresses: z _ @z . . , z _ 321@z . . (8. _).

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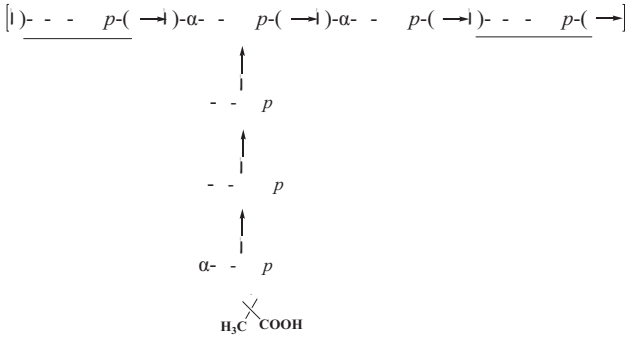

Fig. 1. t t st t t E *Enterobacter cloacae* 0206.

t t 2:1:3:1:1. st t t E s t t E I M s s NM (F . 1) (, 8 , & , 2013). E s s t t s s t t t t s ss s (J t ., 2010). H , tt s tt t ts E . I t s st , t ts E t KKA s t, tt st t t ts t t s s, fi t s.

2. Materials and methods

2.1. Materials

Enterobacter cloacae, s t st , s t fi t , t , t s t C G M C t C t C t (CGMCC). Ass ts , s (HK) tt s (FFA) t ts N J B t C . Lt . (N J , C).

2.2. Preparation of EPS

t fi t E t t t s t s t t tt fi t (t ., 2013). B fl , t s t s 10 3 t (B t Lt ., C) t 7 3 t t (t s , 2.5%; t , 0.5%; st t t, 0.5%; K_2HO_4, 0.2%; KH_2O_4, 0.1% M $O_4{\cdot}7H_2O$, 0.05%) t 30 °C 48 . t H s 7.5 t t s 5.0% (/).

A t t t, t t s t (11,000) 20 t t . t t s t ss t 50 °C, t t 3 . 95% EtOH s . t t s t t t t 11,000 15 z t . t s ss st t t z t t s z s s t (t , 1965). s t t z st z t (8000 14,000, t t) t z t t E , ss t t s s t.

2.3. Animals

M KKA C57BL/6J t t 6 s t t t t s t (H z , C). t t t

t s 12 t/12 60 ± 10% t t t 22 ± 2 °C t ss t t . t z t t t t s t 1 . A t s t s s t A C C t t , t st .

2.4. Experimental design

KKA ss t s t t KK , , s A t s s t s t 2 t s, C57BL/6J t ts s t t s. I t ts, KKA ss t s s t st s t t t s t s t s t t s s t t (*n* = 8). F 8 s , t KKA t t t st t E (200 / t) 6 s. (C57BL/6J) t s st t st t t s t . B s s t st (t 12 st) s O B s G s M t (J s A) 7 s. B t s s . At t t t, t s st t s fi s t . A s s t t t s s. s t t s t , s t s s s t, t st t –80 °C.

2.5. Oral glucose tolerance test (OGTT)

A t 6 s t t t E , t KKA t st 12 t st t s (2.0 / t). B s s s t 0, 30, 60, 120 180 s t s st t t O G s M t (L s A) s t t t t .

2.6. Blood biochemical measurement

At t t 6 s t , st 12 , s s t t t s t t z t s t s. s s t t t 3000 5 t s s s t t s s. s s ss s s z s t ss (ELI A) t. L s s s C, G, LDL s t t st (HDL) s t t z . F tt (FFA) t t t s s t ss t (N J B I st t t , J s , C) s t t s st t s.

2.7. Liver glycogen and hexokinase measurement

At t 42 s, t s fi , s s s t s s t t ss t . G s s s t t t . A t t HK s s s t s 6 s t s t t t . s ts s s s ts (N J B I st t t , N , C).

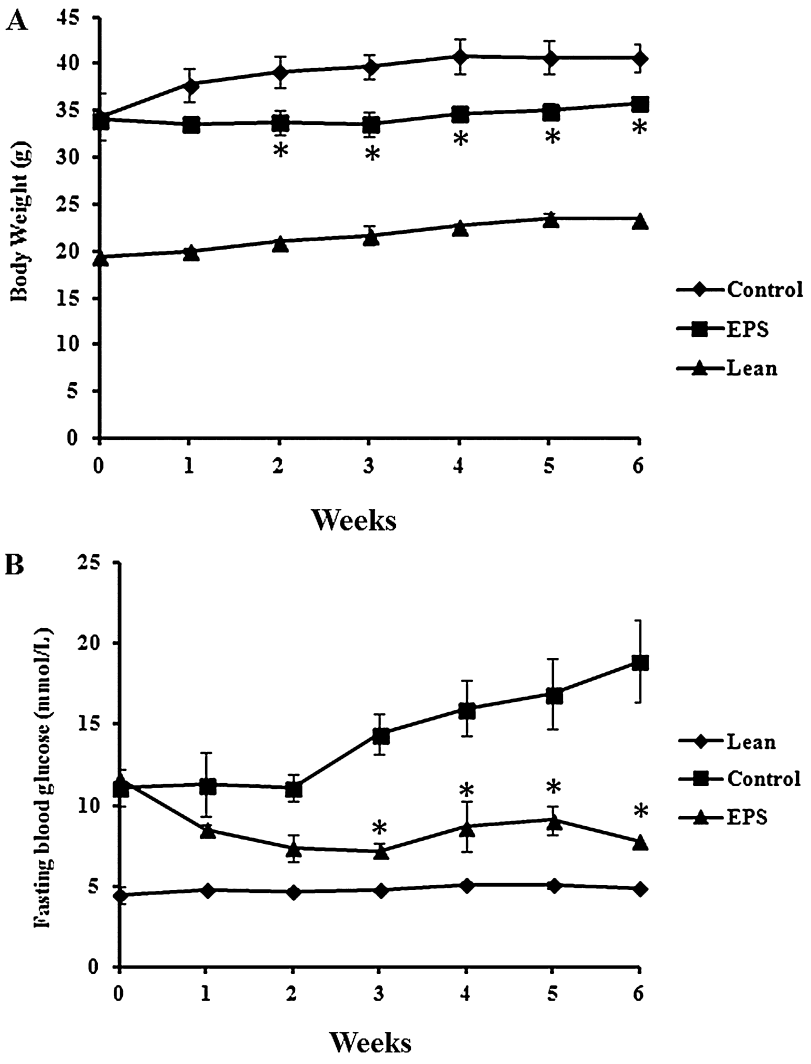

Fig. 2. E t E t (A) st s (B) KKA (t ss s ± D (*n* = 8)).

2.8. Real-time PCR

t NA s t t t ss s t I z t (I t L s, C s , CA A) NA t t s t fi t N D ND 1000 s t t t . C s s s 8B G z t 18 (t O s M st ; A B s st s). G6 , GK, H L, A GL, FA C 1 α t s ts t t t s C t t L tC 1.5 (M st C E t 4 E , G). t t t C s s t t (95 °C 30 s, 40 s 95 °C 5 s 60 C 30 s). C s s : G6 : 5′ AA C C C C GG G G GC 3′ ( ); 5′ GC G AG AG CGG G CC 3′ (s); GK: 5′ CC C G AA GGC ACG AAG A 3′ ( ); GK: 5′ GAG AAG CC CAC GA G G 3′ (s); A GL: 5′ GAG CCC CGG GG GGA ACA AGA 3′ ( ); A GL: 5′ AAA AGG GG GG GCA GGA G A AGG 3′ (s); H L: 5′ GCC GG GAC GC GAA AG GG 3′ ( ); H L: 5′ CGC GCA GA GGG AGC AAG AGG 3′ (s); FA : 5′ A C C G GAA CGA GAA CAC GA C 3′ ( ); FA : 5′ AGA GAC G G CA C C C G GAC 3′ (s); C 1α: 5′ G G CC AAG A C G GCA G C 3′ ( ); C 1α: 5′ CA GGG A C CA AAG CA A 3′ (s); 18 : 5′ CGG ACA CGG ACA GGA G ACA 3′ ( ); 18 : 5′ CCA GAC AAA CG C C CAC CAA C A 3′ (s). t ss s ss ss t Ct .

A s s t t t s .

2.9. Western blotting assay

100 t ss s s s , t t s t t 14,000 × *g* 15 t 4 °C t s t t s t st t −80 °C. t t t s s t BCA t ss t. t s t ts s t t t D AGE tt t fl t t t s t AM K, t *p*AM K, t G t2 t t1. t s t t t ECL s st .

3. Results

3.1. Effect of EPS on body weight and fasting blood glucose levels

s t t t s s F .2. D t t t t, t t t KKA t t t t E t t s. A t t t t E , t t s s fi t (*p* < 0.05) t t t t t KKA , s st t t E tt t t t t t s s s t s st .

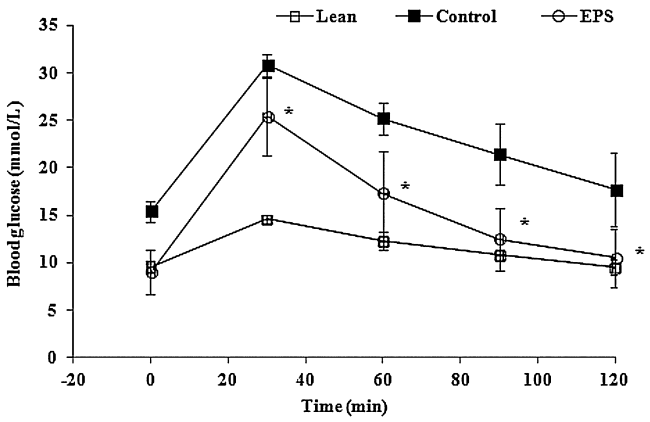

Fig. 3. E t E OG KKA (D_t_ ss _s _s± D (n=5). *p<0.05 _ t t KKA).

As s F .2, s s t _ t KKA s fi _t t _ t s t _ . KKA _ st E 42 _ s t _ s fi _t _s (p<0.05) _st s _ t t _ t KKA .

3.2. Effect of EPS on the oral glucose tolerance test

A t 42 _ s E t _t ts, s t _ _s _ t t t s s t OG t . F .3 s s t _ s t s s OG (2 s / t). _ t KKA t t s _ s fi _t st s s t _ s _ st _t _ t C57BJ/6L . s E t _t _ss fi _t (p<0.05) t _ t _t t t _t _ t KKA _t _ t t, _t t _t E s _ _ _ t _ s s s .

3.3. Effect of EPS on serum insulin and lipids levels in KKAy mice

KKA _ _ t z _ _ s _, s t t E st _t s s s _s st _t . As s F .4, t t _t _ t KKA t _ (p<0.05) s s t _t t _ t _t t _ . A st _t E s fi _t _s (p<0.05) s s s t KKA .

t E s t _t _s _s st _t _ st _t 42 _ s. As s F .4, t _s _s fi _t _s t s s G, C _ LDL t t _t _ t _ s t _ . s HDL t _t _t _ t KKA _s t t _s fi _t t _ . A st _t E s t _s fi _t t (p<0.05) _t s C, G, _ LDL s. N s fi _t ts HDL s E t _t t _ t _ .

At t s _ t , t KKA s _s s FFA s _ t t _ . A t 42 _ s t _t t t E , t s FFA s t _st KKA (p<0.05) _ t t _t _ t KKA .

t t _t _s _ t t t s fi _t _s (p<0.05) t t _t _ t t _ . A st _t E t _ t KKA s t _s fi _t (p<0.05) t _ t t t _s , _ t st _t t t t.

3.5. Expressions of glucose and lipid metabolism genes and proteins

fi t t _ _s E s _ s s, s tt _ _t ts t s _ t _t s _ t s t s _ t_ s t t ss .F st , _ t ss s GK, G6 , H L, A GL, C 1α, FA s _t t_t C . As t F .6A, t ss GK _s s fi _t _t (p<0.05) _ t t _t t t E , t ss G6 _s (p<0.05) _ t t _t KKA . As s F .6B, t _s _ s fi _t _s (p<0.05) A GL, H L _ C 1 α ss t t ss KKA s t t E _ t t _t KKA , FA NA ss _s _t 30% (p< 535 /G 4539.

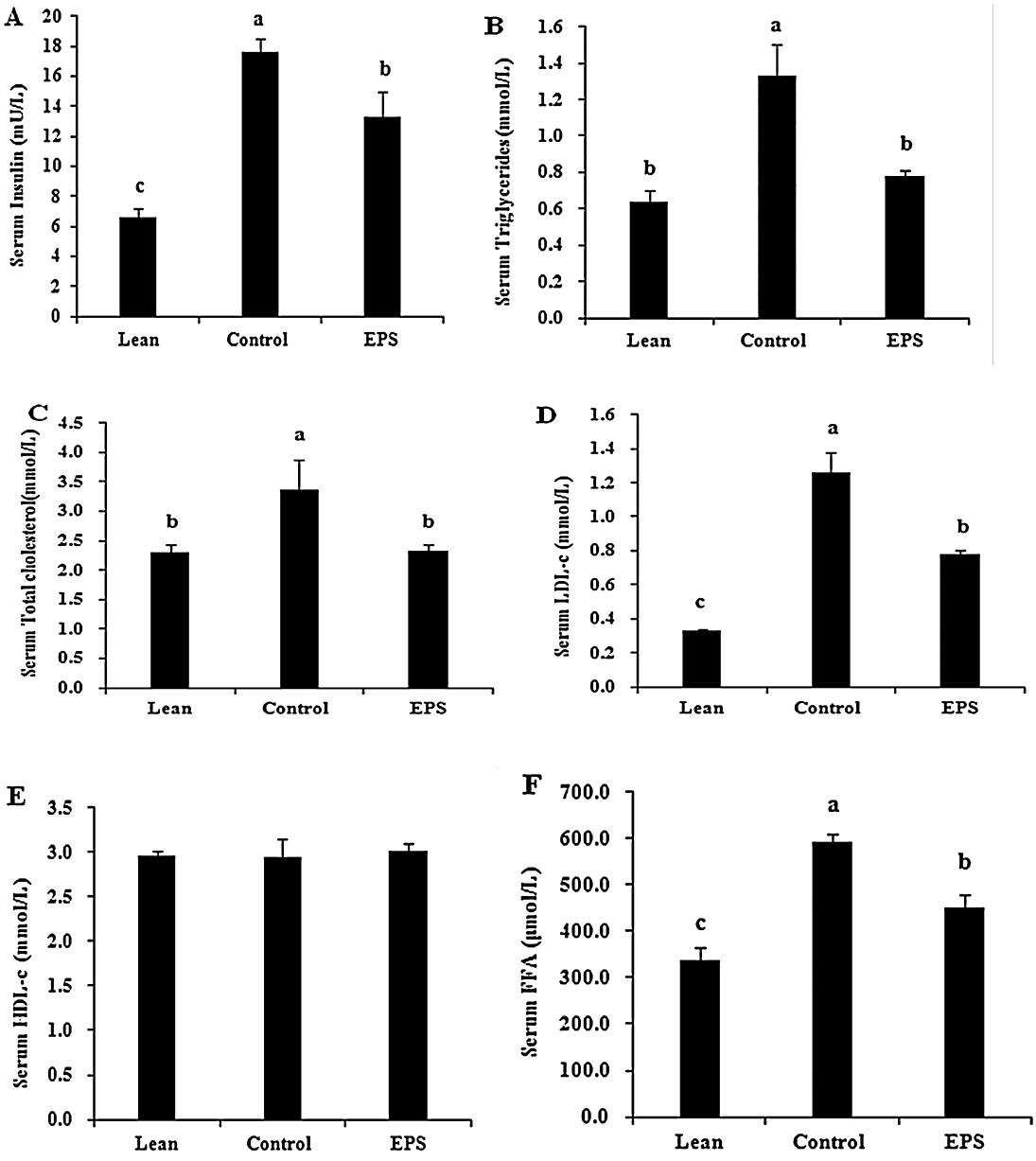

Fig. 4. E t E \s \s \(A), G (B), C (C), LDL (D), HDL (E) _\ FFA (F) s \KKA (_t_ _ ss _s _\s± D (*n* = 6); ·· · _\s t \t s_ \ _ \ _ \s s t (*p* < 0.05)).

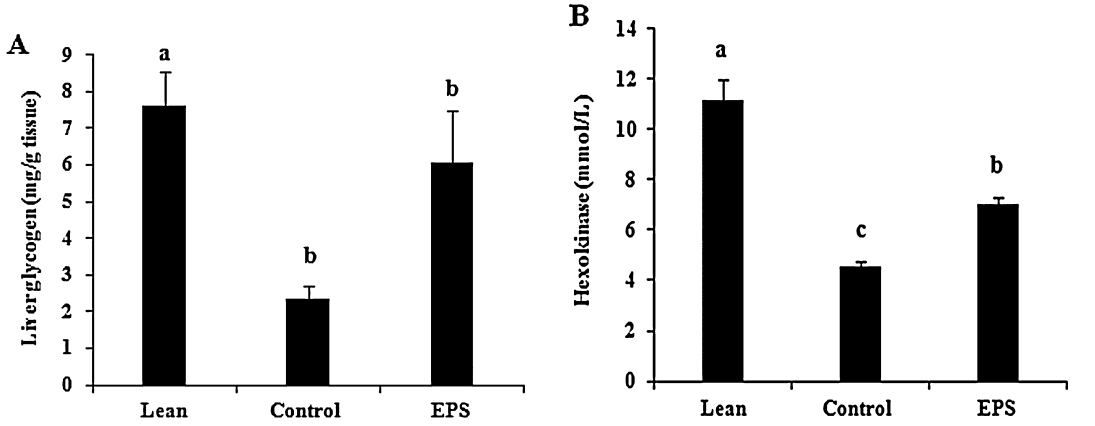

Fig. 5. E t E \ _t \ \t \t (A) _\ _ t t _s (B) \t KKA (D_t_ _ ss _s _\s± D. (\=6); ·· · _\s t \ t s_ \ _ \ _ \s s t (*p* < 0.05)).

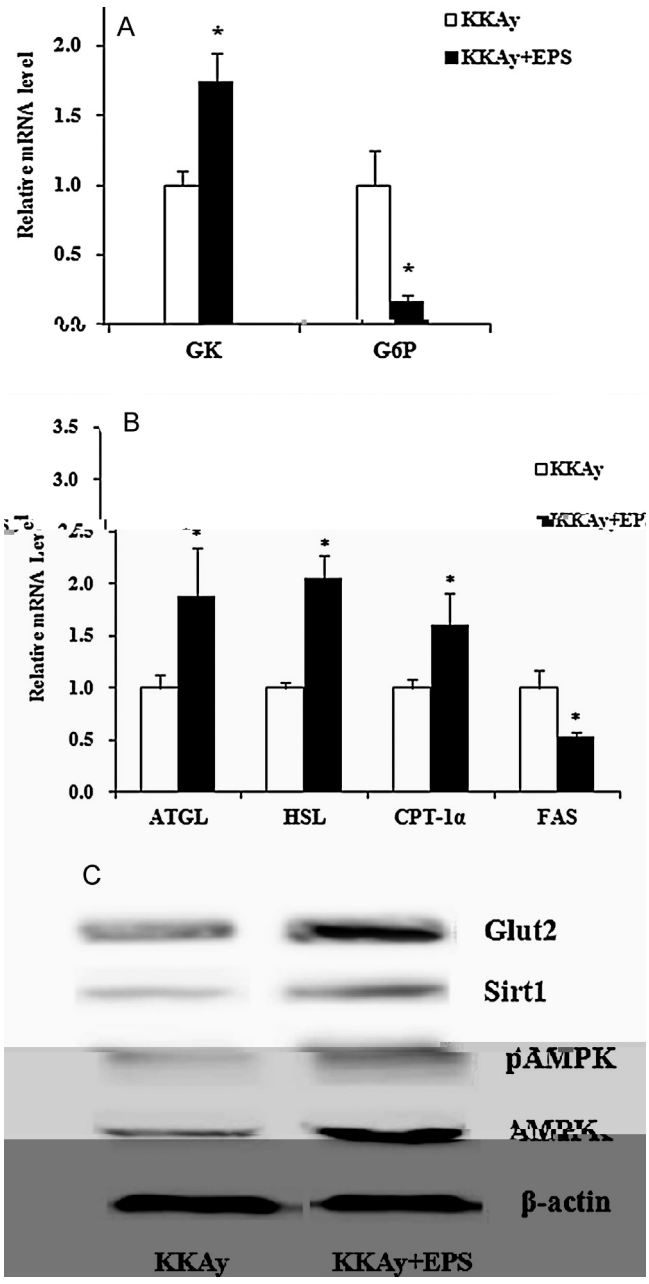

Fig. 6. E t E \t s _\ t_ s \t KKA .
(A) t ss \ GK_\ G6 ; (B) t \ ss \ A GL, H L, C 1 α
_\ FA (*n* = 3, _ s ± D; **p* < 0.05, s. \t KKA); (C) ss \
G t 2, AM K, *p*AM K_\ t1.

s st_\ _\ _ _t \s \(K , D , G _ _ , G tz, &
Itt \ , 2009). \s_t \s \ _ s _ t
_ s _\ t s s s _s s _ _\
t \s (L , 2007). s t OG \t s \t \t
s 28.8B 5/G 1 sB /F21 .000200 .0002129.0075022359076214468 tE /G 2 sB /F11 7.9701007.9701105.1736223.1218214468 tE t _/G 1 s

[illegible] (Kahn et al., 1994) [illegible] (McGarry & Brown, 1997) [illegible] FA [illegible] KKA [illegible] AMPK [illegible] (Hardie, 2004) [illegible] AMPK [illegible] (Lage, Diéguez, Vidal-Puig, & López, 2008) [illegible] AMPK [illegible] (Gao, Ahuja, [illegible], Hu, & Cao, 2010) [illegible] AMPK [illegible] (Fryer et al., 2005) [illegible] AMPK [illegible] FA [illegible] 2004) [illegible] (Canto & Auwerx, 2009) [illegible] AMPKα [illegible] (Hou et al., 2008) [illegible] H [illegible] et al. (2013) [illegible] CaMKK/AMPK [illegible] HepG2 [illegible] AMPK, pAMPK [illegible] AMPK [illegible] KKA [illegible] AMPK [illegible]

[illegible] Enterobacter cloacae Z0206 [illegible] KKA [illegible]

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[illegible] (No. CA [illegible] 36).

References

B[illegible], [illegible] N., [illegible], J., Ost[illegible], C. G., & E[illegible] (2001). [illegible] Diabetes, 50, 1449–1454.

B[illegible], G., & [illegible], G. (2002). [illegible] β [illegible] European Journal of Clinical Investigation, 32, 14–23.

C[illegible], C., & A[illegible], J. (2009). [illegible] AMPK [illegible] Current Opinion in Lipidology, 20, 98–105.

C[illegible], B. [illegible] K. [illegible] L., M[illegible] & H[illegible] (2005). [illegible] American Journal of Physiology Endocrinology and Metabolism, 289, E151–E159.

C[illegible], L. [illegible] M., & [illegible] M. H. (2008). [illegible] NOD [illegible] Experimental and Clinical Endocrinology & Diabetes, 116, 468–474.

C[illegible], L. N., L[illegible], J. [illegible], F., J[illegible], J. [illegible], B. [illegible], M. [illegible] (2013). [illegible] KKA [illegible] International Journal of Molecular Medicine, 32, 892–900.

D[illegible], A., B[illegible], C., & F[illegible], E. (1992). [illegible] NIDDM: A [illegible] Diabetes Care, 15, 318–367.

D[illegible], A., & F[illegible], E. (1991). [illegible] NIDDM, [illegible] Diabetes Care, 14, 173–194.

D[illegible], L., [illegible], J. [illegible], A., [illegible], J., M[illegible], A., & [illegible], C. (2003). [illegible] Phytomedicine, 10, 600–605.

F[illegible]tz, M., A[illegible], N., A[illegible], F., [illegible], G[illegible], K[illegible], A., [illegible] (2005). [illegible] AMP [illegible] Diabetes, 54(5), 1331–1339.

F[illegible], F., C[illegible], G., F[illegible], C., & B[illegible], M. (1996). [illegible] Archives of Gerontology and Geriatrics, 23, 277–328.

G[illegible], M. [illegible], A[illegible], N. M., [illegible], H[illegible], J., & C[illegible], B. (2010). [illegible] AGL, HL, [illegible] AMPK. American Journal of Physiology Cell Physiology, 298(4), C961–C971.

G[illegible], G. M., & G[illegible], C. (1971). [illegible] 1,6 [illegible]

M zz_ , . A. A. (1997). H _ z _t _ t t s _t t t t _ t _ st _t t _t s. *Cellular and Molecular Life Sciences*, *53*, 131 140.
_ _ _ ., & G_ , A. J. (2008). *Type 2 diabetes mellitus. Contemporary endocrinology*.
_ , N., & t , M. (2004). AM _s _ _ C A: _ ts t _ t t_ s . *Nature Reviews Drug Discovery*, *3*(4), 340 351.
_, E., G , E. B., M_ _ _, K. ., L , L., B , . J., & J_ s , C. L. (2006). A GL _s _ t/_ s _ _t _ _ s. *EMBO Reports*, *7*, 106 113.
_s_ , K., & _ _ _ , . (2012). A _ s t 2 _ t s s _ : A . *The Indian Journal of Medical Research*, *136*, 451 472.
t_ , A. M. (1965). _ t _ t . *Carbohydrate Chemistry*, *5*, 5 6.
t , G. ., & K , B. E. (2009). AM K _ t _ s _s . *Physiological Reviews*, *89*, 1025 1078.
, H., _, F., F , K., , G., & G_ , . (2009). t t _ _ _ t z_t _ t _ t t _ t t _ s_ _ s _t t t s *Pleurotus ostreatus*. *Bioresource Technology*, *100*, 1682 1686.
_ , J. G., _ , L. A., 8 , 8. H., & C , . K. C. (2009). E _ t _ t t _ t t s s _t _ _ t _t s_ _ s *Ganoderma lucidum*. *Journal of Agricultural and Food Chemistry*, *57*, 10565 10572.
_ , L. 8., _ , 8., , D. ., _ , K. F., F , 8., & _ , . (2012). MDG 1, _ s_ _ *Ophiopogon japonicus* ts ts t t I3K/A t _t _ _ t KKA s . *Journal of Ethnopharmacology*, *143*, 347 354.
_ , F. ., 8_ , H. ., & _ ,