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Suppressive effects of saponin-enriched extracts from quinoa on 3T3-L1 adipocyte differentiation

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This study was performed to investigate the effect of quinoa saponins (QS) on the differentiation of 3T3-L1 preadipocytes. QS inhibited triglyceride (TG) accumulation in the mature adipocytes, evidenced by oil-red O staining and intracellular quantification. Real time-PCR analysis and western blot analysis showed that QS significantly down-regulated the mRNA and protein expression of key adipogenic transcription factors, peroxisome proliferator-activated receptor γ (PPARγ), and CCAAT/enhancer-binding protein alpha (C/EBPα), however, they had no significant effect on CCAAT/enhancer-binding protein beta (C/EBPβ) and CCAAT/enhancer-binding protein delta (C/EBPδ) which are the upstream regulators for adipogenesis compared with mature adipocytes. QS also reduced mRNA and protein expression of sterol regulatory element-binding protein-1c (SREBP-1c) related to the late stage of adipogenesis. Furthermore, lipoprotein lipase (LPL), adipocyte protein 2 (aP2) and glucose transporter 4 (Glut4), as adipocyte specific genes, were decreased in mature adipocytes by QS treatment. These findings indicate that QS are capable of suppressing adipogenesis and therefore they seem to be natural bioactive factors effective in adipose tissue mass modulation.

1. Introduction

Obesity has reached epidemic proportions worldwide, leading to the development of many common medical conditions, such as type 2 diabetes and metabolic syndrome with a high increasing rate, accelerated cardiovascular diseases and increased cancer risk.[1] Intraabdominal visceral fat accumulation, which depends on adipocyte proliferation and differentiation of pre-adipocytes, would directly contribute to the occurrence of obesity.[2] During adipocyte differentiation, adipogenic transcription factors, such as peroxisome proliferator-activated receptor γ (PPARγ), CCAAT/enhancer-binding protein alpha (C/EBPα), and sterol regulatory element-binding protein-1c (SREBP-1c), are considered to be key regulators of adipogenesis, which govern the expression of genes involved in the induction of adipocyte phenotypes.[3–5] Several studies suggest that suppression of transcription factors such as PPARγ, C/EBPα, and SREBP-1c, which are necessary for adipogenesis to occur, can inhibit preadipocyte differentiation.[6–8]

Quinoa (*Chenopodium quinoa* Willd.) saponins have been found in all parts of the quinoa plant such as leaves, flowers, fruits, seeds, and seed coats.[9] The saponins of quinoa are bitter and represent the major antinutritional factor in the grain. In Ecuador, the seeds of quinoa are commonly soaked in water to remove the bitter saponins before cooking.[10] There is now much interest in the saponins in quinoa for their physiological functionalities, such as anti-inflammatory activity,[11] antifungal activity,[12] antitumor activity,[13] and antioxidant activity.[14] However, little is known about quinoa saponins on preadipocyte differentiation and the formation of mature adipose cells. In this study, we investigated the effects of QS on 3T3-L1 cell preadipocyte differentiation. Furthermore, we investigated the regulatory effects of QS on the mRNA expression of transcriptional factors, PPARγ, C/EBPα and SREBP1c, lipoprotein lipase (LPL), adipocyte protein 2 (aP2) and glucose transporter 4 (Glut4).

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2. Materials and methods

2.1. Materials

3T3-L1 cells were obtained from the Institutes for Biological Sciences, Chinese Academy of Sciences (Shanghai, China). Fetal bovine serum (FBS), Dulbecco's modified Eagle's

medium (DMEM), insulin, dexamethasone (DEX) and 1-methyl-3-isobutylxanthine (IBMX) were purchased from Sigma Chemical Co. (St Louis, MO).

2.2. Extraction of QS

QS was prepared as we previously described.[11] Quinoa seeds were finely powdered and defatted at room temperature with petroleum ether and extracted with MeOH by exhaustive maceration. The residue was successively dissolved in water and partitioned with EtOAc and *n*-BuOH, respectively. After filtration, the extracts were loaded onto a macroreticular resin column (type: AB-8) and eluted with 30, 50, 70, and 90% of methanol–ethanol solution. The 50% resultant QS was concentrated and spray-dried. Individual components in QS were determined using high-performance liquid chromatography (HPLC). The QS sample was analyzed by an Agilent 1200 series liquid chromatograph containing an autosampler coupled with a 6300 series ion-trap mass spectrometer (Agilent, Santa Clara, CA). On the basis of peak areas, QS contained 14.52 g kg^{-1} 3-*O*-hexose–pentose–pentose phytolaccagenic acid 28-*O*-hexose, 98.25 g kg^{-1} 3-*O*-α-L-arabinopyranosyl phytolaccagenic acid 28-*O*-β-D-glucopyranosyl ester, 84.28 g kg^{-1} 3-*O*-β-D-glucopyranosyl-(1→3)-α-L-arabinopyranosyl phytolaccagenic acid 28-*O*-β-D-glucopyranosyl ester, 142.68 g kg^{-1} 3-*O*-α-L-arabinopyranosyl-(1→3)-β-D-glucuronopyranosyl serjanic acid 28-*O*-β-D-glucopyranosyl ester, 35.54 g kg^{-1} 3-*O*-β-D-xylopyranosyl-(1→3)-β-D-glucuronopyranosyl hederagenin 28-*O*-β-D-glucopyranosyl ester, 149.25 g kg^{-1} 3-*O*-β-D-glucopyranosyl-(1→3)-α-L-arabinopyranosyl hederagenin 28-*O*-β-D-glucopyranosyl ester, 135.48 g kg^{-1} 3-*O*-β-D-glucopyranosyl-(1→3)-α-L-arabinopyranosyl serjanic acid 28-*O*-β-D-glucopyranosyl ester, and 32.44 g kg^{-1} 3-*O*-β-D-glucuronopyranosyl oleanolic acid 28-*O*-β-D-glucopyranosyl ester.

2.3. Cell culture

3T3-L1 cells were cultured according to the provider's established protocol. 3T3-L1 cells were grown in DMEM containing 4.5 g L^{-1} D-glucose with 10% heat-inactivated FBS, 1% penicillin and 1% HEPES buffer at 37 °C under a humidified atmosphere of 5% CO_2.

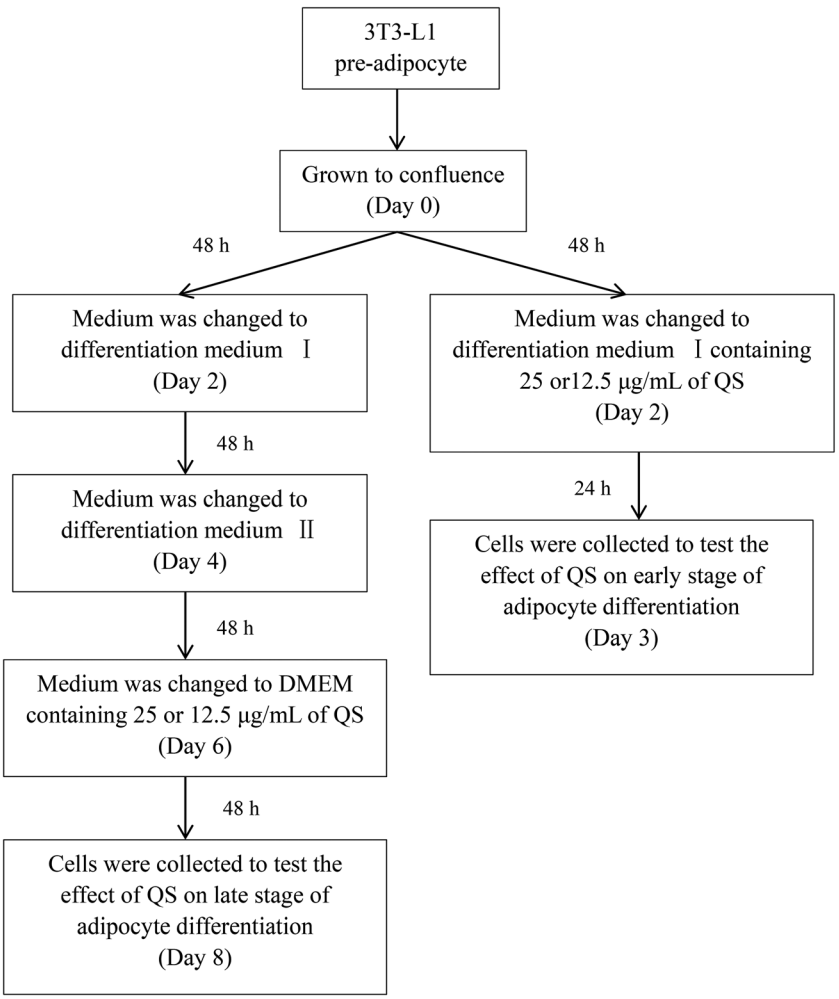

Fig. 1 Procedure for QS treatment during 3T3-L1 differentiation.

Table 1 The primer sequence used for real-time PCR

Gene name	Forward primer	Reverse primer	Accession no.
PPARγ	TTTTCAAGGGTGCCAGTTTC	AATCCTTGGCCCTCTGAGAT	NM_011146
C/EBPα	TTACAACAGGCCAGGTTTCC	GGCTGGCGACATACAGTACA	NM_007678
C/EBPβ	CCTTTAAATCCATGGAAGTGG	GGGCTGAAGTCGATGGC	NM_005194.2
C/EBPδ	ACGACGAGAGCGCCATC	TCGCCGTCGCCCCAGTC	TRCN0000013697
SREBP1c	TGTTGGCATCCTGCTATCTG	AGGGAA AGCTTTGGGGTCTA	NM_011480
LPL	CATCGAGAGGATCCGAGTGAA	TGCTGAGTCCTTTCCCTTCTG	NM_008509
ap2	GGCCAAGCCCAACATGATC	CACGCCCAGTTTGAAGGAAA	NM_024406
Glut4	ACGACGGACACTCCATCTGTTG	GGAGACATAGCTCATGGCTGGAA	NM_009204
β-actin	CCACAGCTGAGAGGGAAATC	AAGGAAGGCTGGAAAAGAGC	X03672

2.4. MTT viability assay

Cell viability was assessed by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.[15] 3T3-L1 cells were grown in 96-well plates (10 000 cells per well) and allowed to adhere overnight. After 24 h, culture media containing QS was added to each well. The control culture was treated in DMEM medium. The cells were incubated for 48 h at 37 °C under a humidified atmosphere of 5% CO_2. After 48 h, the culture medium and test samples were replaced with 20 μL of MTT (1 mg mL^{-1}) in a culture incubator in the dark for 4 h. Then the MTT reagent was removed, and formazan crystals were solubilized in 200 μL of DMSO. The absorbance of each well was read at 570 nm using a microplate reader (Bio-Rad, MA, USA).

2.5. 3T3-L1 cell pre-adipocyte differentiation

A pre-adipocyte differentiation assay was assessed according to the modified method of Yim, Hosokawa, Yoshida, Saito, & Miyashita.[16] 3T3-L1 pre-adipocytes were cultured in DMEM with 10% FBS, 100 U mL^{-1} penicillin, and 100 μg mL^{-1} streptomycin at 37 °C under an atmosphere of 5% CO_2 and grown to confluence. 48 h after confluence, the medium was changed to differentiation medium I containing 10 μg mL^{-1} insulin, 0.5 mM IBMX, and 0.1 μM DEX in DMEM and was incubated for an additional 48 h. Cells were then incubated in differentiation medium II containing 5 μg mL^{-1} insulin in DMEM for 48 h and further cultured in DMEM without insulin, which was replaced with fresh medium for 48 h. QS were added to DMEM after a 48 h incubation period with differentiation medium II. The final QS concentration in the medium was adjusted to 25 and 12.5 μg mL^{-1}, respectively. QS was firstly dissolved in DMSO and diluted to 1000 μg mL^{-1} with DMEM medium. To test the effect of QS on the early stage of adipocyte differentiation, the QS stock solution was added to differentiation medium I after a 48 h confluence. Cells were collected after 24 h of DM induction. On the other hand, to test the effect of QS on the late stage of adipocyte differentiation, the QS stock solution was added to the DMEM medium after a 48 h incubation period with differentiation medium II. Cells were collected at day 8. The final QS concentration in the medium was adjusted to 25 and 12.5 μg mL^{-1} without cytotoxicity, respectively (Fig. 1).

2.6. Oil-red O staining and OD measurement

After induced for 8 days, the cells were washed with PBS twice, fixed with 10% formalin at room temperature for 10 min, and stained with oil red O (Sigma, St. Louis, MO, USA) at 60 °C for 10 min.[7] The pictures were taken using an Olympus (Tokyo, Japan) microscope, then they were extracted with isopropanol and O.D. absorbance measured at 492 nm using a microplate reader (Bio-Rad, MA, USA).

2.7. Quantification of intracellular triglyceride (TG)

TG was measured according to the method previously described with slight modifications.[17] The mature adipocytes (day 8) were washed twice and collected in 1 ml of ice-cold PBS (0.1 mol L^{-1}, pH 7.4) using a cell scraper, and then sonicated (300 W, 15 min, repeated 3 times). The cell was re-suspended by vortex (60 s) before sonicating each time. The suspension was used to measure the intracellular TG content, which was then analysed with the enzymatic kit (Nanjing Jiancheng Bioengineering Institute, China) at 510 nm. The TG content was normalized to the cell protein content.

2.8. RNA extraction and real-time reverse transcription polymerase chain reaction (RT-PCR)

Total mRNA was extracted and isolated from 3T3-L1 cells collected at 24 h or day 8 using the Trizol Reagent (Invitrogen,

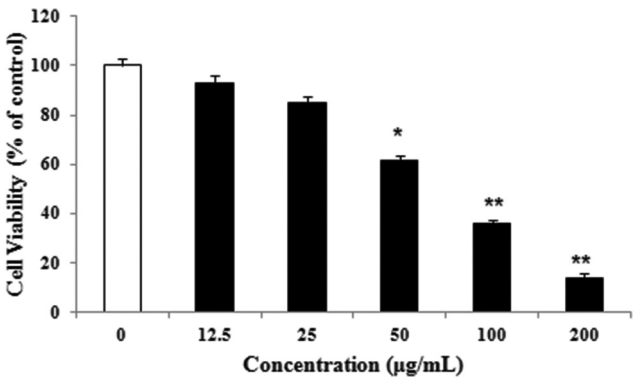

Fig. 2 MTT cell viability of QS. Cultures in basal medium served as control. Three independent experiments were performed and the data are shown as mean ± SD. *$p < 0.05$ *vs.* control; **$p < 0.005$ *vs.* control.

Carlsbad, CA, USA) according to the manufacturer's instruction. Total RNA was converted to complementary DNA (cDNA) using the High Capacity cDNA Reverse Transcription kit (Applied Biosystems, Foster City, CA, USA). Quantification of gene expression in 3T3-L1 cells treated with QS was measured using a 7300 Real Time PCR System (Applied Biosystem). All primers used for real-time PCR for these genes are listed in Table 1. Real-time PCR was performed using a TaqMan Fast Universal PCR Master Mix (Applied Biosystems). The relative amount of each gene was calculated using the $2^{-\Delta\Delta CT}$ method. The level of transcripts was normalised using β-actin as an internal standard. Levels of each different mRNA in the control cells were designated as 1, and the relative levels of the gene transcripts of the samples were expressed as the fold change. Analysis was carried out in triplicates.

2.9. Western blot analysis

Western blot analysis was performed according to the method previously described by Yim *et al.*[16] Ice-cold lysis RIPA buffer (20 mM Tris-HCl, pH 7.4), 150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate (SDS), 0.1 mg mL^{-1} phenylmethanesulfonyl fluoride (PMSF), 50 μg mL^{-1} aprotinin, and 1 mM sodium orthovanadate were used to lyse cells. Cell lysates were centrifuged at 12 000*g* for 20 min at 4 °C. The supernatant was collected and estimated for protein measurement. Ten micrograms of protein was separated

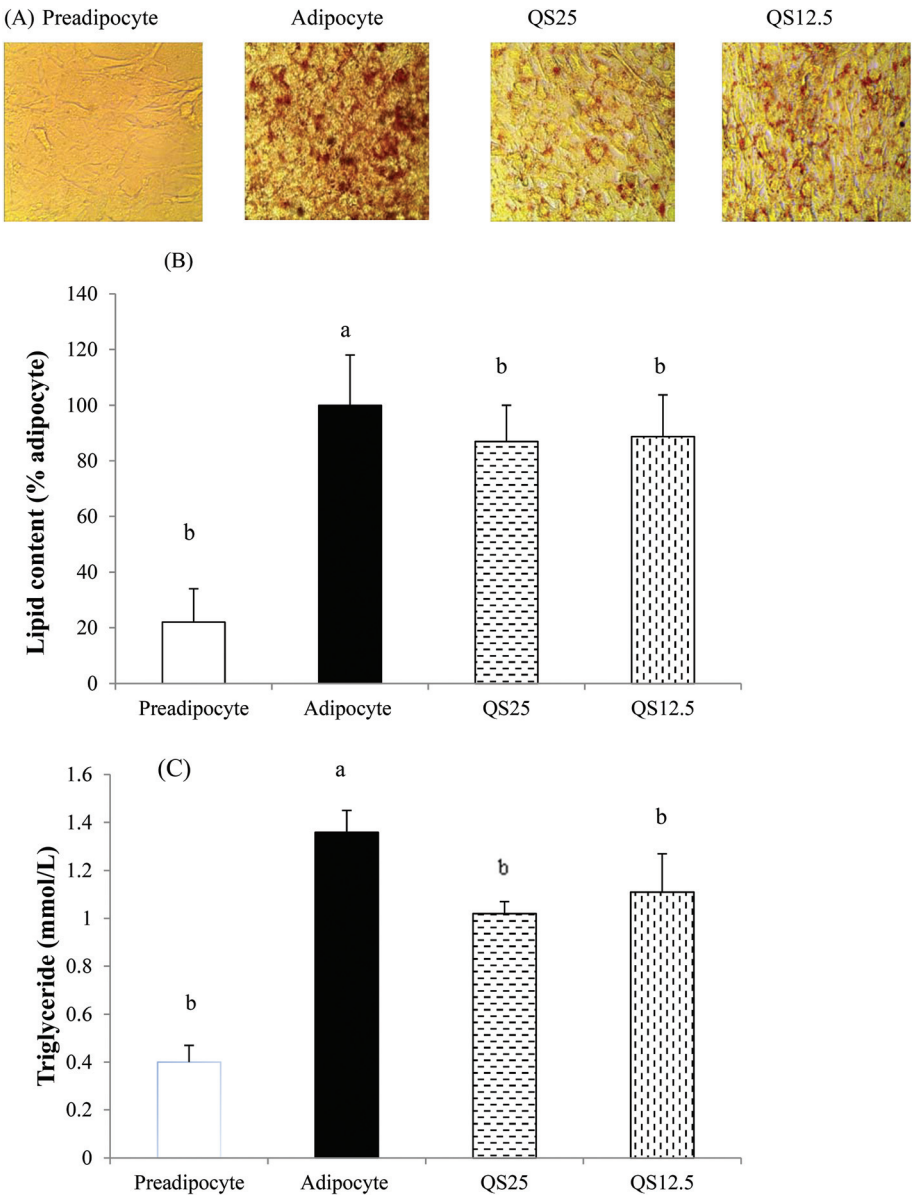

Fig. 3 Effect of QS on intracellular lipid accumulation in 3T3-L1 cells. 3T3-L1 cells were treated with QS (50 or 25 μg mL^{-1}) for day 8. (A) The mature adipocytes were stained with oil-red O, and the (B) OD value (% adipocyte) and (C) TG content (mmol L^{-1}) were measured to quantify intracellular lipid content. Three independent experiments were performed and the data are shown as mean ± SD. Values which do not share the same letter are significantly different ($p < 0.05$).

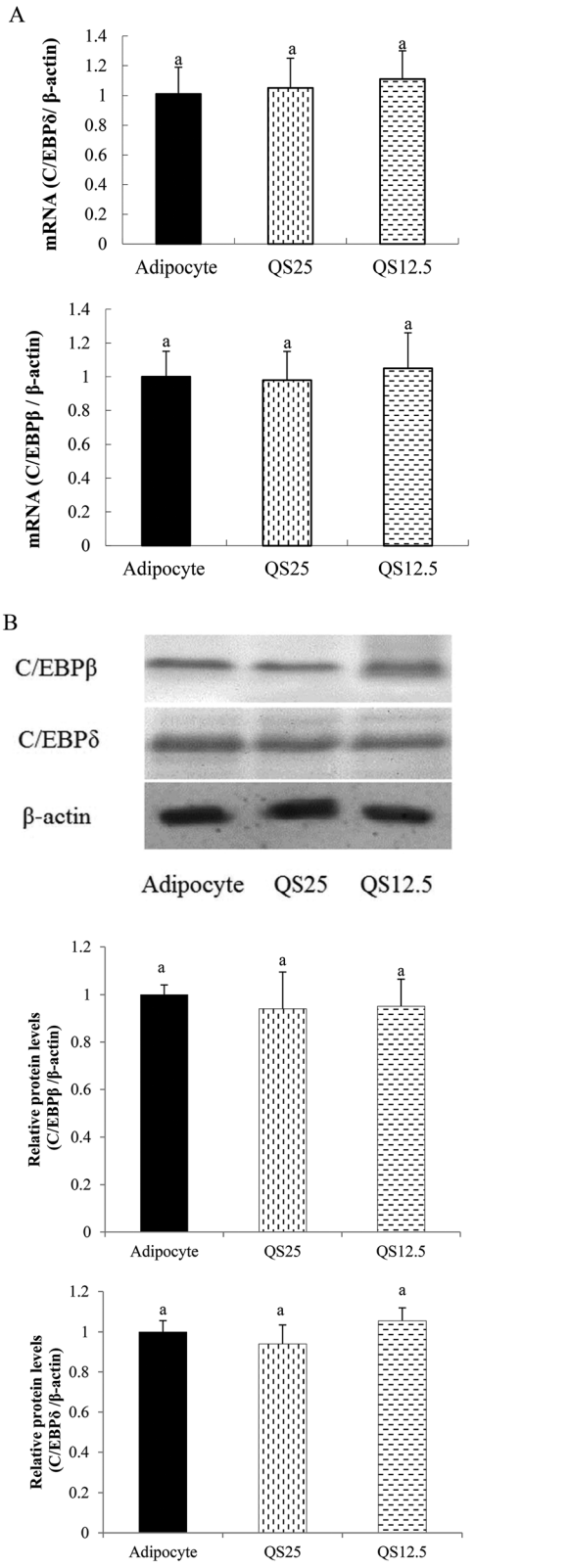

Fig. 4 Effect of QS on the mRNA expression (A) and protein levels (B) of C/EBPδ and C/EBPβ in 3T3-L1 cells. Three independent experiments were performed and the data are shown as mean ± SD. Values which do not share the same letter are significantly different ($p < 0.05$).

by 10% SDS-polyacrylamide gel electrophoresis (PAGE) and transferred to the polyvinylidene difluoride (PVDF) membrane. After being blocked for 1 h at room temperature in 5% skim milk in tris-buffer salt containing 0.1% Tween-20 (TBST), anti-PPARγ, C/EBPα, C/EBPβ, C/EBPδ, SREB1c, LPL, aP2, Glut4 and β-actin antibody (Santa Cruz, CA) were respectively added and incubated for 2 h at room temperature. The membrane was then washed with TBST three times for 5 min each time, incubated with a HRP-conjugated secondary antibody for 1 h at room temperature, and washed with TBST three times for 10 min each time. The signal was detected using a SuperSignal ELISA Pico Chemiluminescent Substrate (Thermo Fisher Scientific, USA).

2.10. Statistics

Data, which were expressed as the mean ± SD, included at least three replicates per sample. ANOVA and Tukey's test were performed using SPSS (Statistics for Social Science) version 17.0. All graphical representations were performed using Sigma plot version 11.0 (SPSS, USA). Statistical significance was set at $p < 0.05$.

3. Results and discussion

3.1. Cell viability analysis

Toxic concentrations of QS were assessed *via* the MTT viability assay. There were no significant ($p > 0.05$) differences in the viability of 3T3-L1 cells at a concentration of up to 25 μg mL^{-1} for QS compared with the control (Fig. 2). The results showed that the viability of 3T3-L1 cells significantly ($p < 0.05$) decreased at a concentration of 50 μg mL^{-1} and higher for QS compared with the control (Fig. 2). A 27% decrease in viability was observed at a concentration of 50 μg mL^{-1} of QS, suggesting that QS exerts a toxic effect on 3T3-L1 cells at a concentration higher than 50 μg mL^{-1}. It was therefore decided that the non-cytotoxic concentrations of 25 and 12.5 μg mL^{-1} of QS will be used in the following experiments.

3.2. Effect of QS on intracellular lipid accumulation in 3T3-L1 cells

To determine the effects of QS on pre-adipocyte differentiation, we treated confluent 3T3-L1 cells with or without QS for 8 days. Following various treatments, differentiated 3T3-L1 cells in plates were subjected to oil-red O staining. Representative images of oil-red O staining demonstrate that QS suppressed lipid accumulation. This result was consistent with a previous study, which reported that the ginsenoside Rh2, an active component of *Panax ginseng*, effectively blocked adipocyte differentiation.[18] This result was supported by quantitative data obtained by spectrophotometric analysis of oil-red O-stained cells eluted with isopropanol (Fig. 3A), which revealed that QS significantly inhibited adipogenic differentiation compared with control 3T3-L1 cells ($p < 0.05$, Fig. 3B). These observations were confirmed by the TG content assay (Fig. 3C). The TG contents of QS-treated 3T3-L1 cells were lowered by 25.01% and 18.38% respectively, compared with control ($p < 0.05$). Thus,

treatment of 3T3-L1 cells with QS during induction resulted in inhibition of adipocyte differentiation.

3.3. Effect of QS on mRNA expression and protein levels of PPARγ, C/EBPα, C/EBPβ, C/EBPδ and SREBP1c

The mRNA levels of PPARγ, C/EBPα, C/EBPβ, C/EBPδ and SREBP1c were measured after the induction of the differentiation process (day 8) in the 3T3-L1 cells, in the presence of QS, at concentrations of 12.5 and 25 mg mL^{-1}. Consistent with previous studies on saponins from *Panax notoginseng*,[19] ginseng[18] and platycodin,[20] our results indicated that QS suppressed the expression of PPARγ, C/EBPα and SREBP1c compared with control adipocytes ($p < 0.05$) (Fig. 4A and 5A). These results were consistent with a study of Foucault *et al.*,[21] who observed that quinoa extract reduced adipose tissue development of diet-induced obesity in mice by regulating the

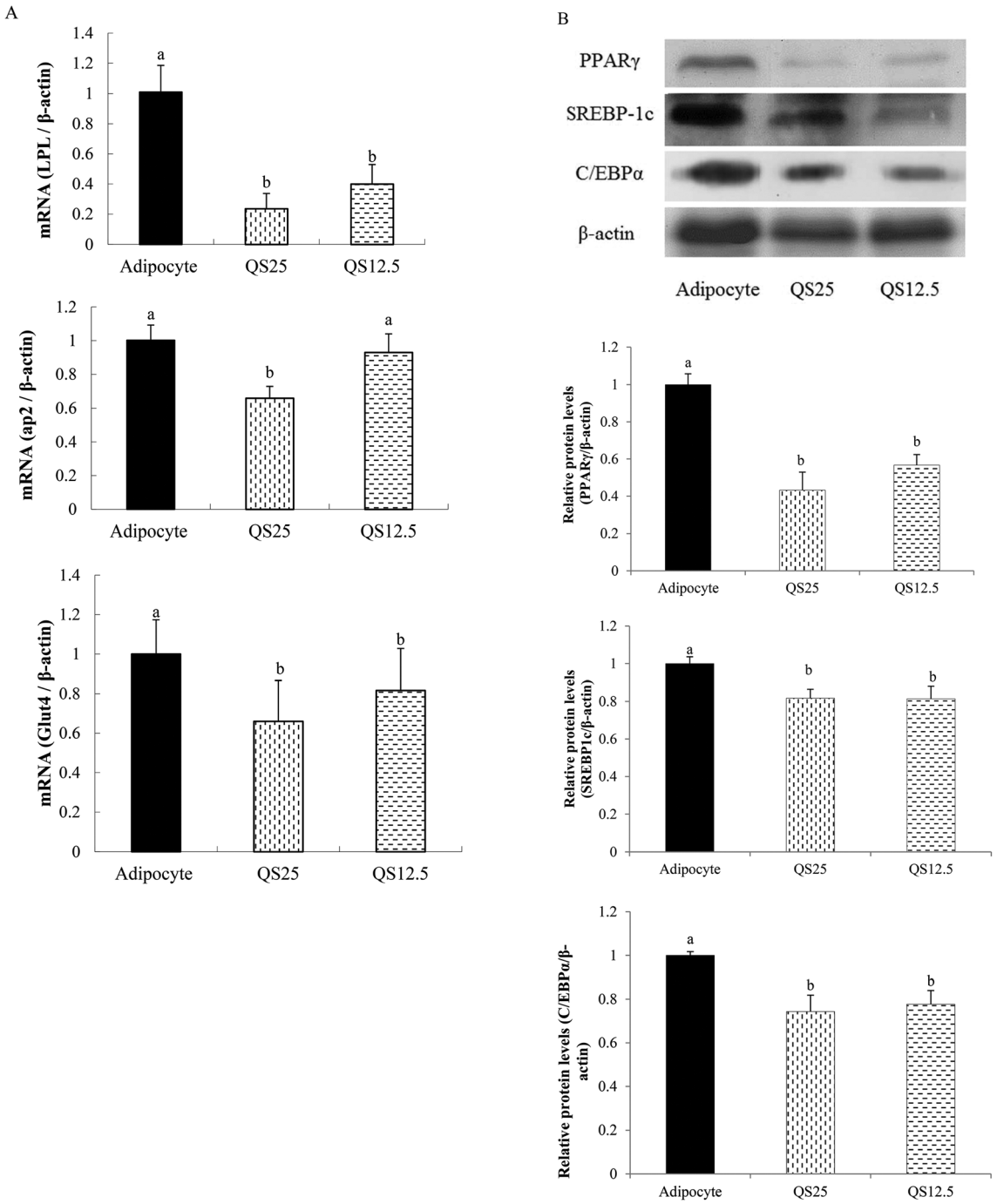

Fig. 5 Effect of QS on the mRNA (A) and protein (B) expression of PPARγ, C/EBPα and SREBP1c in 3T3-L1 cells. Three independent experiments were performed and the data are shown as mean ± SD. Values which do not share the same letter are significantly different ($p < 0.05$).

expression of adipocyte-specific genes in PPARγ, C/EBPα and SREBP1c. However, QS didn't significantly affect the expression of C/EBPβ and C/EBPδ, which are the upstream regulators for adipogenesis, compared to the control ($p > 0.05$) (Fig. 4A). QS significantly down regulated mRNA expression of PPARγ and C/EBPα, which are the central determinants of the transcriptional factor during adipocyte differentiation. These two major transcription factors work sequentially and cooperatively in stimulating fat cell differentiation. PPARγ is necessary to promote adipogenesis and C/EBPα is influential in maintaining the expression of PPARγ.[22] Hwang *et al.*[18,23] have reported that ginsenoside Rg3 and Rh2 from ginseng reduced preadipocyte viability and the differentiation from preadipocyte to adipocyte through a down-regulation of the PPARγ. Kim *et al.*[24] have also observed that saponins isolated from *Cheonggukjang* inhibited adipocyte differentiation and triglyceride accumulation in 3T3-L1 preadipocyte cells.

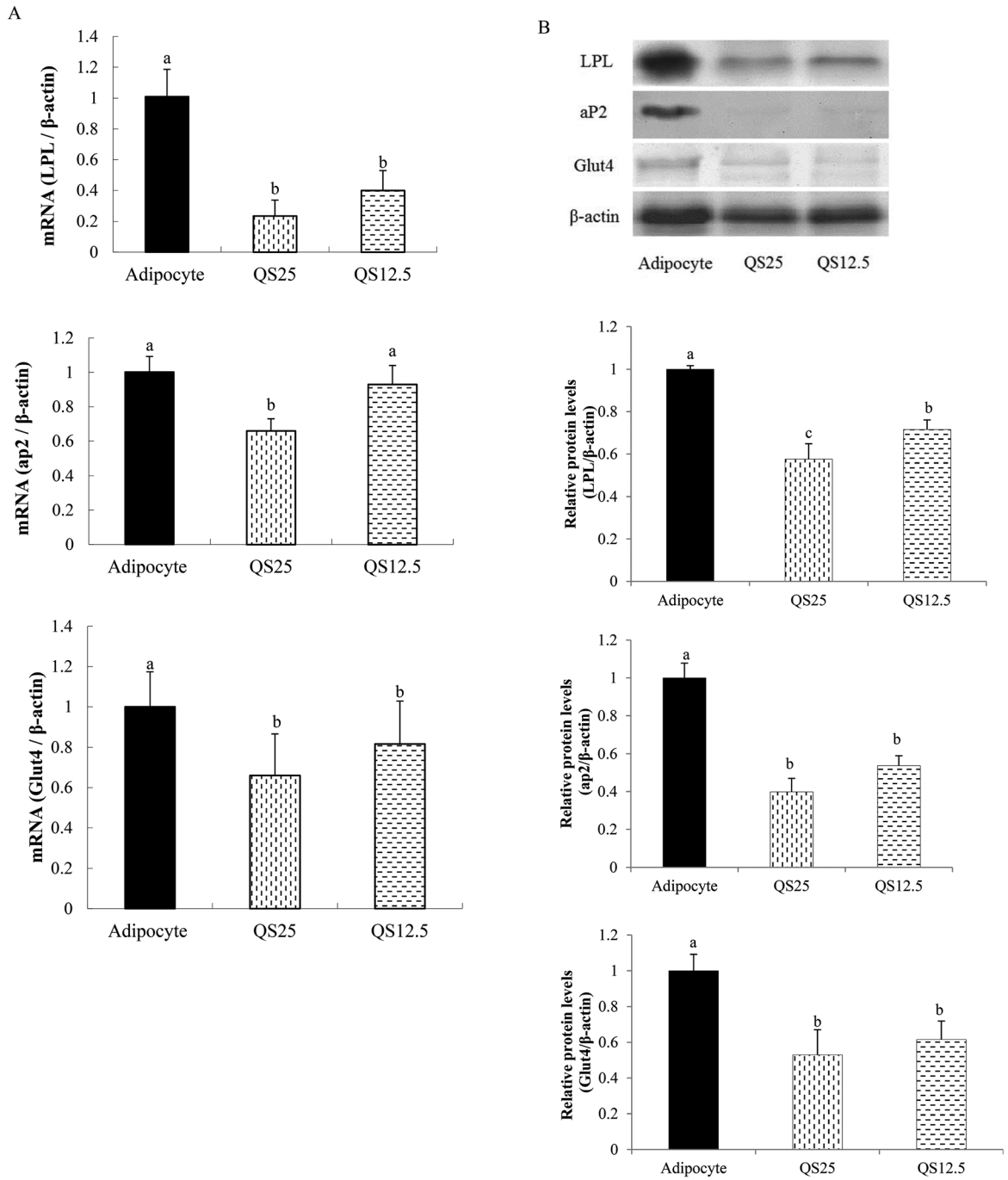

Fig. 6 Effect of QS on the mRNA (A) and protein (B) expression of LPL, ap2 and Glut4 in 3T3-L1 cells. Three independent experiments were performed and the data are shown as mean ± SD. Values which do not share the same letter are significantly different ($p < 0.05$).

PPARγ and C/EBPα transcription factors activate the expression of genes involved in adipogenesis, such as aP2, LPL, fatty acid transporter and others, which participate in creating the adipocyte phenotype.[25] SREBP1c increases the expression of many lipogenic genes, including fatty acid synthase (FAS).[25] Inhibited SREBP1c and normal C/EBPβ and C/EBPδ compared to the control indicate that QS regulates the intermediate stage of adipocyte differentiation by inhibiting the expression of genes critical for adipogenesis without affecting the expression of upstream regulators such as C/EBPβ and δ.[17]

Besides, we further examined PPARγ, C/EBPα and SREBP1c expression levels by western blotting. Consistent with mRNA expression, the PPARγ, C/EBPα, C/EBPβ, C/EBPδ and SREBP1c protein expression levels showed a similar pattern (Fig. 4B and 5B). These results suggested that QS suppresses PPARγ, C/EBPα, C/EBPβ, C/EBPδ and SREBP1c protein expression by down-regulating their mRNA transcription.

3.4. Effect of QS on mRNA expression and protein levels of LPL, aP2 and Glut4

The mRNA expression levels of LPL, aP2 and Glut4 were down-regulated by QS ($p < 0.05$, Fig. 6A). Western blot analysis confirmed the reduction, and QS markedly inhibited the protein expression levels of LPL, aP2 and Glut4 at concentrations of 25 and 12.5 μg mL^{-1} (Fig. 6B). LPL and aP2 mRNA are adipocyte specific genes involved in fatty acid metabolism regulated by PPARγ and C/EBPα.[26–28] From these results, the suppressive effects on adipocyte specific mRNA expression are suggested to be mediated by the down regulation of PPARγ and C/EBPα mRNA expression. Glut4 creates and maintains the adipocyte phenotype and is a key regulator of whole-body glucose homeostasis.[29] Sim, Xu, Wong, Sim, & Lee[30] reported that increased Glut4 translocation improved hyperglycaemia and insulin resistance.

4. Conclusion

Our data demonstrate that QS suppressed 3T3-L1 adipocyte differentiation and decreased cell viability in mature adipocyte cultures. QS reduced the lipid accumulation during the differentiation process and down-regulated the adipogenesis-related transcriptional factors, PPARγ, C/EBPα, C/EBPβ, C/EBPδ and SREBP1c, and adipocyte-specific molecules, LPL, aP2 and Glut4. These findings are helpful in understanding the anti-adipogenic properties of QS, however further *in vivo* studies should also be conducted in order to confirm the anti-obesity effect of QS.

Acknowledgements

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References

1 D. W. Haslam and W. P. James, Obesity, *Lancet*, 2005, **366**, 1197–1209.
2 S. Fujioka, Y. Matsuzawa, K. Tokunaga and S. Tarui, Contribution of intra-abdominal fat accumulation to the impairment of glucose and lipid metabolism in human obesity, *Metabolism*, 1987, **36**, 54–59.
3 F. M. Gregoire, C. M. Smas and H. S. Sul, Understanding adipocyte differentiation, *Physiol. Rev.*, 1998, **78**, 783–809.
4 C. Huang, Y. Zhang, Z. Gong, X. Sheng, Z. Li, W. Zhang and Y. Qin, Berberine inhibits 3T3-L1 adipocyte differentiation through the PPARγ pathway, *Biochem. Biophys. Res. Commun.*, 2006, **348**, 571–578.
5 P. Tontonoz, E. Hu and B. M. Spiegelman, Stimulation of adipogenesis in fibroblasts by PPAR gamma 2, a lipid activated transcription factor, *Cell*, 1994, **79**, 1147–1156.
6 Y. Y. Chen, M. H. Lee, C. C. Hsu, C. L. Wei and Y. C. Tsai, Methyl cinnamate inhibits adipocyte differentiation via activation of the CaMKK2–AMPK pathway in 3T3-L1 pre-adipocytes, *J. Agric. Food Chem.*, 2012, **60**, 955–963.
7 B. Huang, H. D. Yuan, D. Y. Kim, H. Y. Quan and S. H. Chung, Cinnamaldehyde prevents adipocyte differentiation and adipogenesis via regulation of peroxisome proliferator-activated receptor-γ (PPARγ) and AMP-activated protein kinase (AMPK) pathways, *J. Agric. Food Chem.*, 2011, **59**, 3666–3673.
8 S. W. Kang, S. I. Kang, H. S. Shin, S. A. Yoon, J. H. Kim, H. C. Ko and S. J. Kim, Sasa quelpaertensis Nakai extract and its constituent p-coumaric acid inhibit adipogenesis in 3T3-L1 cells through activation of the AMPK pathway, *Food Chem. Toxicol.*, 2013, **59**, 380–385.
9 T. Kuljanabhagavad, P. Thongphasuk, W. Chamulitrat and M. Wink, Triterpene saponins from Chenopodium quinoa Willd, *Phytochemistry*, 2008, **69**, 1919–1926.
10 J. Ruales and B. M. Nair, Saponins, phytic acid, tannins and protease inhibitors in quinoa (Chenopodium quinoa, Willd) seeds, *Food Chem.*, 1993, **48**, 137–143.
11 Y. Yao, X. S. Yang and G. X. Ren, Anti-inflammatory activity of saponins from quinoa (*Chenopodium quinoa* Willd.) seeds in lipopolysaccharide-stimulated RAW 264.7 macrophages cells, *J. Food Sci.*, 2014, **79**, H1018–H1023.
12 G. M. Woldemichael and M. Wink, Identification and biological activities of triterpenoid saponins from Chenopodium quinoa, *J. Agric. Food Chem.*, 2001, **49**, 2327–2332.
13 J. Li, W. J. Guo and Q. Y. Yang, Effects of ursolic acid and oleanolic acid on human colon carcinoma cell line HCT15, *World J. Gastroenterol.*, 2002, **8**, 493–495.
14 I. Gulcin, V. Mshvildadze, A. Gepdiremen and R. Elias, The antioxidant activity of a triterpenoid glycoside isolated

from the berries of Hedera colchica: 3-O-(β-d-glucopyranosyl)-hederagenin, *Phytother. Res.*, 2006, **20**, 130–134.

15 H. Vaidya, R. K. Goyal and S. K. Cheema, Anti-diabetic activity of swertiamarin is due to an active metabolite, gentianine, that upregulates PPAR-γ gene expression in 3T3-L1 cells, *Cell*, 2012, **40**, 60.

16 M. J. Yim, M. Hosokawa, Y. Mizushina, H. Yoshida, Y. Saito and K. Miyashita, Suppressive effects of amarouciaxanthin A on 3T3-L1 adipocyte differentiation through down-regulation of PPARγ and C/EBPα mRNA expression, *J. Agric. Food Chem.*, 2011, **59**, 1646–1652.

17 J. R. Noh, Y. H. Kim, J. H. Hwang, G. T. Gang, S. H. Yeo, K. S. Kim and C. H. Lee, Scoparone inhibits adipocyte differentiation through down-regulation of peroxisome proliferators-activated receptor γ in 3T3-L1 preadipocytes, *Food Chem.*, 2013, **141**, 723–730.

18 J. T. Hwang, S. H. Kim, M. S. Lee, S. H. Kim, H. J. Yang, M. J. Kim, H. S. Kim, J. Ha, M. S. Kim and D. Y. Kwon, Anti-obesity effects of ginsenoside Rh2 are associated with the activation of AMPK signaling pathway in 3T3–L1 adipocyte, *Biochem. Biophys. Res. Commun.*, 2007, **364**, 1002–1008.

19 J. J. Kim, H. Xiao, Y. Tan, Z. Z. Wang, J. Paul Seale and X. Qu, The effects and mechanism of saponins of Panax notoginseng on glucose metabolism in 3T3-L1 cells, *Am. J. Chin. Med.*, 2009, **37**, 1179–1189.

20 H. Lee, R. Kang, Y. S. Kim, S. I. Chung and Y. Yoon, Platycodin D inhibits adipogenesis of 3T3-L1 cells by modulating kruppel-like factor 2 and peroxisome proliferator-activated receptor ero, *Phytother. Res.*, 2010, **24**, S161–S167.

21 A. S. Foucault, V. Mathe, R. Lafont, P. Even, W. Dioh, S. Veillet, D. Tome, J. F. Huneau, D. Hermier and A. Quignard-Boulange, Quinoa extract enriched in 20-hydroxyecdysone protects mice from diet-induced obesity and modulates adipokines expression, *Obesity*, 2012, **20**, 270–277.

22 Z. Wu, E. D. Rosen, R. Brun, S. Hauser, G. Adelmant, A. E. Troy and B. M. Spiegelman, Cross-regulation of C/EBPα and PPARγ controls the transcriptional pathway of adipogenesis and insulin sensitivity, *Mol. Cell*, 1999, **3**, 151–158.

23 J. T. Hwang, M. S. Lee, H. J. Kim, M. J. Sung, H. Y. Kim, M. S. Kim and D. Y. Kwon, Antiobesity effect of ginsenoside Rg3 involves the AMPK and PPAR-gamma signal pathways, *Phytother. Res.*, 2009, **23**, 262–266.

24 H. J. Kim, J. T. Hwang, M. J. Kim, H. J. Yang, M. J. Sung, S. H. Kim, S. Park, E. J. Gu, Y. Park and D. Y. Kwon, The inhibitory effect of saponin derived from Cheonggukjang on adipocyte differentiation in vitro, *Food Sci. Biotechnol.*, 2014, **23**, 1273–1278.

25 E. D. Rosen, C. J. Walkey, P. Puigserver and B. M. Spiegelman, Transcriptional regulation of adipogenesis, *Genes Dev.*, 2000, **14**, 1293–1307.

26 R. J. Christy, V. W. Yang, J. M. Ntambi, D. E. Geiman, W. H. Landschulz, A. D. Friedman and M. D. Lane, Differentiation-induced gene expression in 3T3-L1 preadipocytes: CCAAT/enhancer binding protein interacts with and activates the promoters of two adipocyte-specific genes, *Genes Dev.*, 1989, **3**, 1323–1335.

27 K. Schoonjans, J. Peinado-Onsurbe, A. M. Lefebvre, R. A. Heyman, M. Briggs, S. Deeb and J. Auwerx, PPARalpha and PPARgamma activators direct a distinct tissue-specific transcriptional response via a PPRE in the lipoprotein lipase gene, *EMBO J.*, 1996, **15**, 5336.

28 B. Gustafson, M. M. Jack, S. W. Cushman and U. Smith, Adiponectin gene activation by thiazolidinediones requires PPARγ2, but not C/EBPα—evidence for differential regulation of the aP2 and adiponectin genes, *Biochem. Biophys. Res. Commun.*, 2003, **308**, 933–939.

29 A. D. Kohn, S. A. Summers, M. J. Birnbaum and R. A. Roth, Expression of constitutively active Akt Ser/Thr kinase in 3T3-L1 adipocytes stimulates glucose uptake and glucose transporter 4 translocation, *J. Biol. Chem.*, 1996, **271**, 31372–31378.

30 M. K. Sim, X. G. Xu, Y. C. Wong, S. Z. Sim and K. O. Lee, Des-aspartate-angiotensin I exerts hyperglycemic action via glucose transporter-4 translocation in type 2 diabetic KKA^{y} mice and GK rats, *Endocrinology*, 2007, **148**, 5925–5932.

