

Energy-regulated molecules maintain young status in the trophocytes and fat cells of old queen honeybees

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Abstract Queen honeybees (*Apis mellifera*) have much longer lifespans than worker bees. Energy-regulated molecules in the trophocytes and fat cells of workers during aging have been determined, but are unknown in queen bees. In the present study, energy-regulated molecules were evaluated in the trophocytes and fat cells of young and old queen bees. Adenosine monophosphate-activated protein kinase $\alpha 2$ (AMPK- $\alpha 2$), phosphorylated AMPK- $\alpha 2$ (pAMPK- $\alpha 2$), and cAMP-specific phosphodiesterases activity increased with aging. The pAMPK- $\alpha 2$ /AMPK- $\alpha 2$ ratio and AMPK activity; adenosine triphosphate (ATP), adenosine diphosphate (ADP), and adenosine monophosphate (AMP) concentrations; the ADP/ATP ratio and the AMP/ATP ratio; the cyclic adenosine monophosphate concentration; forkhead box protein O expression; Silent information regulator T1 (SirT1) expression and activity; and peroxisome proliferator-activated receptor- α (PPAR- α) expression were not significantly different between young and old queen bees. These results

show that energy-regulated molecules maintain a youthful status in the trophocytes and fat cells of queen bees during aging. These cells seem to have longevity-promoting mechanisms and may clarify the secret of longevity in queen bees.

Keywords Aging · AMPK · SirT1 · ATP · ADP · AMP · PPAR- α · Honeybee

Abbreviations

AMPK	AMP-activated protein kinase
pAMPK	Phosphorylated AMP-activated protein kinase
cAMP	Cyclic adenosine monophosphate
PDE	cAMP-specific phosphodiesterases
ATP	Adenosine triphosphate
ADP	Adenosine diphosphate
AMP	Adenosine monophosphate
FoxO	Forkhead box protein O
SirT1	Silent information regulator T1
PPAR- α	Peroxisome proliferator-activated receptor- α
$\Delta\psi_m$	Mitochondrial membrane potential
NAD ⁺	Nicotinamide adenine dinucleotide oxidized form
NADH	Nicotinamide adenine dinucleotide reduced form
ND1	NADH dehydrogenase 1
ATR	Ambient temperature reduction
CR	Caloric restriction
LKB1	Liver kinase B1

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Introduction

Honeybees (*Apis mellifera*) are a unique model system for studying aging and longevity because queen bees have a much longer lifespan than worker bees, although they develop from the same genome. Their trophocytes and fat cells are suitable for cellular senescence and longevity studies due to lack of cell division during adulthood, ease of isolation from the abdomen, and convenient manipulation (Hsieh and Hsu 2011a, b; Nilsen et al. 2011; Chan et al. 2011; Hsu and Chan 2013; Chuang and Hsu 2013; Hsu and Chuang 2013; Hsieh and Hsu 2013; Hsu and Hsieh 2014; Hsu et al. 2014). Trophocytes, which are large and irregularly shaped, and fat cells, which are small and spherical, attach to each other to construct a single layer of cells around each abdominal segment.

The study of age-related molecules in queen bees revealed that trophocytes and fat cells seem to have longevity-promoting mechanisms to maintain the longevity of queen bees when compared to worker bees (Hsieh and Hsu 2011a, b). The studies of oxidative stress and antioxidant enzyme activity in queen bees indicated that an increase in oxidative stress and a consequent increase in stress defense mechanisms were associated with the longevity of queen bees (Hsieh and Hsu 2013). The increase in oxidative stress and antioxidant enzyme activity was proposed to be concomitant with an increase in energy metabolism. Recently, a study of mitochondrial energy utilization in queen bees showed that mitochondrial density, mitochondrial membrane potential ($\Delta\psi_m$); nicotinamide adenine dinucleotide (NAD^+), nicotinamide adenine dinucleotide reduced form (NADH), and ATP concentration; NAD^+/NADH ratio; and relative expression of NADH dehydrogenase 1 (ND1) and ATP synthase normalized against mitochondrial density were not significantly different between young and old queen bees (Hsu and Lu, unpublished data). These results showed that mitochondrial energy utilization maintained a young status in the trophocytes and fat cells of old queen bees, and supported the inferences of previous studies (Hsieh and Hsu 2011b, 2013).

Mitochondrial energy utilization is associated with cellular energy metabolism. In particular, the NAD^+ and ATP concentrations and the NAD^+/NADH ratio are correlated with the activity of AMPK and SirT1. To further explore cellular energy metabolism in the

trophocytes and fat cells of queen bees, we evaluated the changes in cellular energy-regulated molecules in the trophocytes and fat cells of young and old queen bees. The expression of AMPK- $\alpha 2$, pAMPK- $\alpha 2$, FoxO, SirT1, and PPAR- α ; the activity of AMPK, cAMP-specific phosphodiesterases (PDE), and SirT1; and the concentration of ATP, ADP, AMP, and cAMP were examined in the trophocytes and fat cells of young and old queen bees to clarify the relationship between energy-regulated molecules and longevity in queen bees. In addition, young worker bees have higher cellular energy status and express higher levels of energy-regulated molecules than old worker bees (Hsu and Chuang 2013). A comparison of the differences of expression in energy-regulated molecules between worker bees and queen bees will help us to understand the mechanisms of longevity in queen bees.

Materials and methods

Queen honeybees

Queen bees were purchased from a single commercial breeder (Hsinchu, Taiwan). As described in our previous studies (Hsieh and Hsu 2011b, 2013), young (2-month-old) and old (16-month-old) queen bees were collected from different hives on the same dates for use in the following studies. The young and old queen bees were mated with drones and were able to lay eggs. Young queen bees had dense, light yellowish-brown fuzz and a light yellowish-brown epidermis, whereas old queen bees had sparse, dark brown fuzz and a dark blackish-brown epidermis.

Western blotting

The supernatant of trophocytes and fat cells of queen bees was obtained as described previously (Hsu and Chuang 2013). The proteins (30 μg) from the supernatants were electrophoresed using 10–15 % acrylamide SDS/PAGE gels and transferred to polyvinylidene fluoride membranes. After blocking for 1 h at 25 °C, the membranes were incubated with antibodies against AMPK- $\alpha 2$ (1:1,000) (07-181, Millipore, MA, USA), pAMPK- $\alpha 2$ (1:1,000) (07-626; Millipore), FoxO (1:1,000) (ARP38094_T100, AVIVA Systems Biology, CA, USA), SirT1 (1:1,000) (ARP32386_T100,

AVIVA Systems Biology), PPAR- α (1:3,000) (GTX101098; GeneTex, Irvine, CA, USA), or tubulin (1:10,000) (ab6046; Abcam, MA, USA). The membranes were then probed with the appropriate secondary antibody labeled with horseradish peroxidase (1:10,000). Immunolabeled proteins were detected using chemiluminescence (PerkinElmer, Covina, CA, USA) and analyzed with ImageJ software (NIH, Bethesda, MA, USA). The protein expression levels were normalized to tubulin. This experiment was performed with five biological replicates and used a total of five young and five old queen bees.

AMPK activity

AMPK activity was evaluated using the CycLex AMPK Kinase Assay Kit (CY-1182, CycLex, Nagano, Japan). Assays were carried out as described previously (Hsu and Chuang 2013). The AMPK activity was expressed as the absorbance at 450 nm mg^{-1} of protein. This experiment was performed with five biological replicates and used a total of five young and five old queen bees.

ATP, ADP, and AMP concentrations

ATP, ADP, and AMP concentration assays were carried out as described previously (Hsu and Chuang 2013). The ATP values were expressed as nmol mg^{-1} of protein. This experiment was performed with five biological replicates and used a total of five young and five old queen bees.

Sirt1 activity

Sirt1 activity was evaluated using the CycLex SIRT1/Sirt2 Deacetylase Fluorometric Assay Kit (CY-1151, CycLex, Nagano, Japan). Assays were carried out as described previously (Hsu and Chuang 2013). The specific activity was expressed as the relative fluorescence mg^{-1} of protein. This experiment was performed with five biological replicates and used a total of five young and five old queen bees.

cAMP concentration

The cAMP concentration was assayed using the cAMP Direct Immunoassay Kit (ab65355, Abcam, MA, USA). Assays were carried out as described previously

(Hsu and Chuang 2013). The cAMP values were expressed as pmol ng^{-1} protein. This experiment was performed with five biological replicates and used a total of five young and five old queen bees.

PDE activity

The PDE activity was assayed using the PDE-Glo™ Phosphodiesterase Assay Kit (V1361, Promega, WI, USA). Assays were carried out as described previously (Hsu and Chuang 2013). The PDE activity was expressed as fluorescence ng^{-1} of protein. This experiment was performed with five biological replicates and used a total of five young and five old queen bees.

Statistical analysis

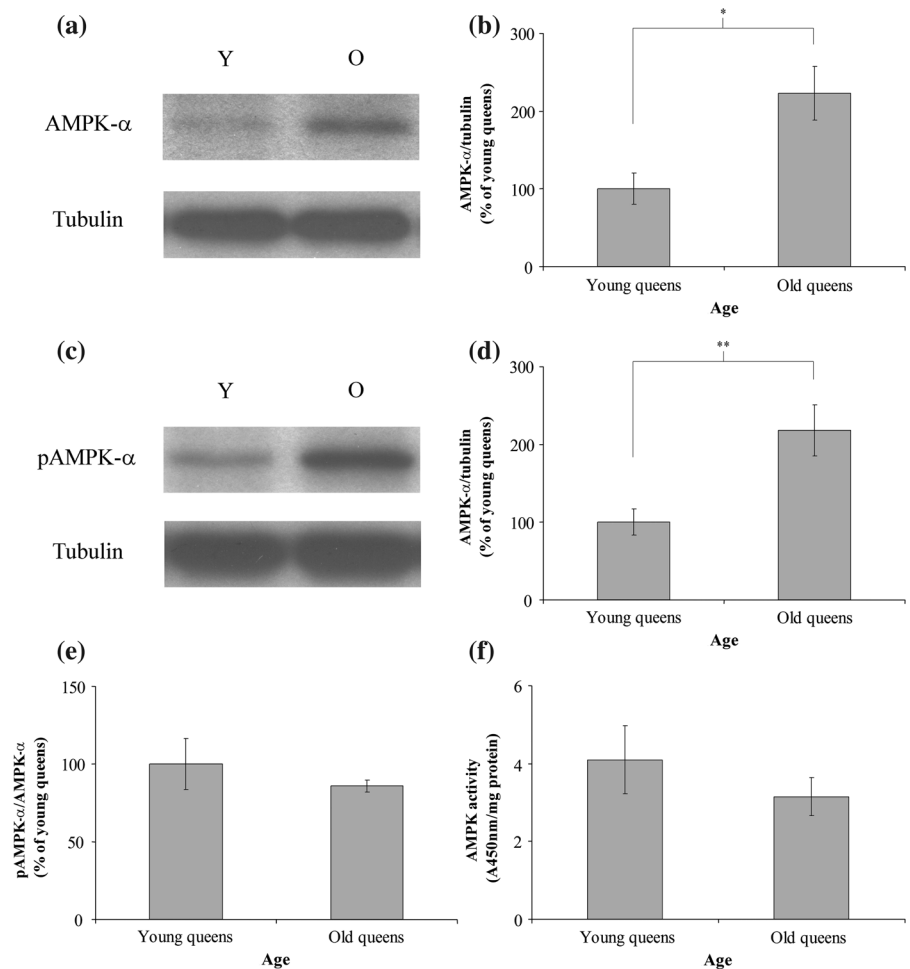
The differences in the mean values between the two age groups were examined using two-sample *t* tests. A *P* value of <0.05 was considered statistically significant.

Results

AMPK expression and activity

To determine the activity of energy-regulated molecules of trophocytes and fat cells with aging, we examined the expression of AMPK- $\alpha 2$ and pAMPK- $\alpha 2$ and the activity of AMPK in young and old queen bees. The trophocytes and fat cells of young queen bees expressed lower levels of AMPK- $\alpha 2$ than old queen bees (Fig. 1a). The statistical analysis showed that the expression levels of AMPK- $\alpha 2$ were significantly increased with aging in the trophocytes and fat cells of queen bees ($n = 5$, $P < 0.05$; Fig. 1b). Similarly, the trophocytes and fat cells of young queen bees expressed lower levels of pAMPK- $\alpha 2$ than old queen bees (Fig. 1c). The statistical analysis showed that the expression levels of pAMPK- $\alpha 2$ were significantly increased with aging in the trophocytes and fat cells of queen bees ($n = 5$, $P < 0.01$; Fig. 1d). The pAMPK/AMPK ratio was used to represent the AMPK activity. The pAMPK/AMPK ratio showed that AMPK activity was not significantly different between young and old queen bees ($n = 5$, $P > 0.05$; Fig. 1e). Likewise, AMPK activity analyzed by Kit

Fig. 1 The expression of AMPK- α 2 and pAMPK- α 2, the pAMPK/AMPK ratio, and the AMPK activity in the trophocytes and fat cells of young and old queen bees. AMPK- α 2 (a) and pAMPK- α 2 (c) were analyzed by Western blotting. Tubulin served as the loading control. *Y* young queen bees, *O* old queen bees, *P* positive control (rat muscle). The expression levels of AMPK- α 2 (b) and pAMPK- α 2 (d) were normalized to those in young queen bees. Bars are presented as the mean \pm standard error of the means (SEMs) and are expressed as percentages ($n = 5$). e The pAMPK/AMPK ratio was normalized to that in young queen bees. The results are presented as the means \pm SEMs and are expressed as percentages ($n = 5$). f The AMPK activity results are presented as the means \pm SEMs ($n = 5$). *Asterisk* indicates a statistically significant difference (* $P < 0.05$; ** $P < 0.01$; two-sample *t* test)



was not significantly different between young and old queen bees with 4.10 ± 0.88 and 3.15 ± 0.49 450 nm mg^{-1} protein in the trophocytes and fat cells of young and old queen bees, respectively ($n = 5$, $P > 0.05$; Fig. 1f). These results showed that AMPK activity maintained young status in the trophocytes and fat cells of queen bees during aging.

FoxO expression

AMPK regulates FoxO transcription factors (Barthel et al. 2002; Greer et al. 2007; Cantó et al. 2009). We assayed FoxO expressions in the trophocytes and fat cells of young and old queen bees. Old queen bees expressed similar levels of FoxO as did young queen bees (Fig. 2a). The statistical analysis showed that FoxO expression levels were not significantly

different between young and old queen bees ($n = 5$, $P > 0.05$; Fig. 2b).

ATP, ADP, and AMP concentrations and the ADP/ATP and AMP/ATP ratios

The AMPK activity was associated with cellular energy metabolism. We assayed the ATP, ADP, and AMP concentrations in the trophocytes and fat cells of young and old queen bees to determine cellular energy status of trophocytes and fat cells with aging. The mean ATP concentration in trophocytes and fat cells was 28.42 ± 7.05 and 29.93 ± 4.41 nmol mg^{-1} of protein in young and old queen bees, respectively ($n = 5$, $P > 0.05$; Fig. 3a). The mean ADP concentration in trophocytes and fat cells was 7.89 ± 2.14 and 6.61 ± 1.65 nmol mg^{-1} of protein in young and old

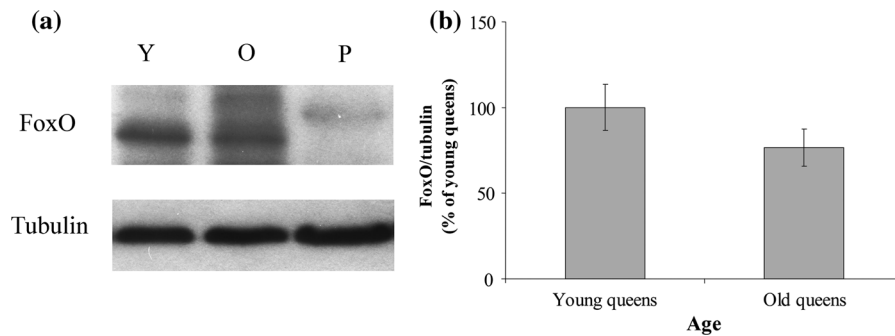


Fig. 2 FoxO expression in the trophocytes and fat cells of young and old queen bees. **a** FoxO expression was analyzed by Western blotting. Tubulin served as the loading control. *Y* young queen bees, *O* old queen bees, *P* positive control (mice liver). **b** FoxO expression was normalized to expression in young workers. The results are presented as the means \pm SEMs and

are expressed as percentages ($n = 5$; $P > 0.05$; two-sample *t* test). According to sequences, FoxO1 of mice (Q9R1E0.2) show higher molecular weight than that of honeybees (XP_001122804.2). The results of Western blotting were consistent with this prediction

queen bees, respectively ($n = 5$, $P > 0.05$; Fig. 3b). The mean AMP concentration in trophocytes and fat cells was 3.43 ± 0.79 and 2.16 ± 0.67 nmol mg⁻¹ of protein in the young and old queen bees, respectively ($n = 5$, $P > 0.05$; Fig. 3c). We also calculated the ADP/ATP ratio and the AMP/ATP ratio because fluctuation in the AMP/ATP ratio and the ADP/ATP ratio can influence AMPK activity to regulate cellular metabolism. The AMP/ATP and ADP/ATP ratio was not significantly different between the young and old queen bees ($n = 5$, $P > 0.05$; Fig. 3d, e). These results indicated that energy status maintained young status in the trophocytes and fat cells of queen bees during aging.

cAMP and PDE

We further analyzed the cAMP concentration and PDE activity because cAMP was derived from ATP, used for intracellular signal transduction, and decomposed into AMP by PDE. The mean cAMP concentration in trophocytes and fat cells was 3.93 ± 0.82 and 2.62 ± 0.29 pmol ng⁻¹ of protein in young and old queen bees, respectively ($n = 5$, $P > 0.05$; Fig. 4a), indicating that the cAMP concentrations were not significantly different between young and old queen bees. The mean PDE activity in trophocytes and fat cells was 0.22 ± 0.04 and 0.453 ± 0.07 fluorescence (Fu) ng⁻¹ of protein in young and old queen bees, respectively ($n = 5$, $P < 0.05$; Fig. 4b). These results indicated that the cAMP concentration maintained young status in the trophocytes and fat cells of queen bees

during aging and that PDE activity increased with aging in the trophocytes and fat cells of queen bees.

Sirt1 expression and activity

In addition to AMPK, Sirt1 also regulated cellular energy metabolism. Therefore, the expression and activity of Sirt1 were examined in the trophocytes and fat cells of young and old queen bees. The trophocytes and fat cells of old queen bees expressed similar levels of Sirt1 as did young queen bees (Fig. 5a). The statistical analysis showed that Sirt1 expression levels were not significantly different between young and old queen bees ($n = 5$, $P > 0.05$; Fig. 5b). We also assessed the Sirt1 activity. The mean Sirt1 activity was 51.76 ± 1.41 and 45.68 ± 2.10 fluorescence (Fu) μg^{-1} of protein in the trophocytes and fat cells of the young and old queen bees, respectively ($n = 5$, $P > 0.05$; Fig. 5c), indicating that Sirt1 activity was not significantly different between young and old queen bees. These results revealed that Sirt1 activity maintained young status in the trophocytes and fat cells of queen bees during aging.

PPAR- α expression

To confirm that Sirt1 regulated cellular energy metabolism, we assayed PPAR- α expression in the trophocytes and fat cells of young and old queen bees. The trophocytes and fat cells of old queen bees expressed similar levels of PPAR- α as did young

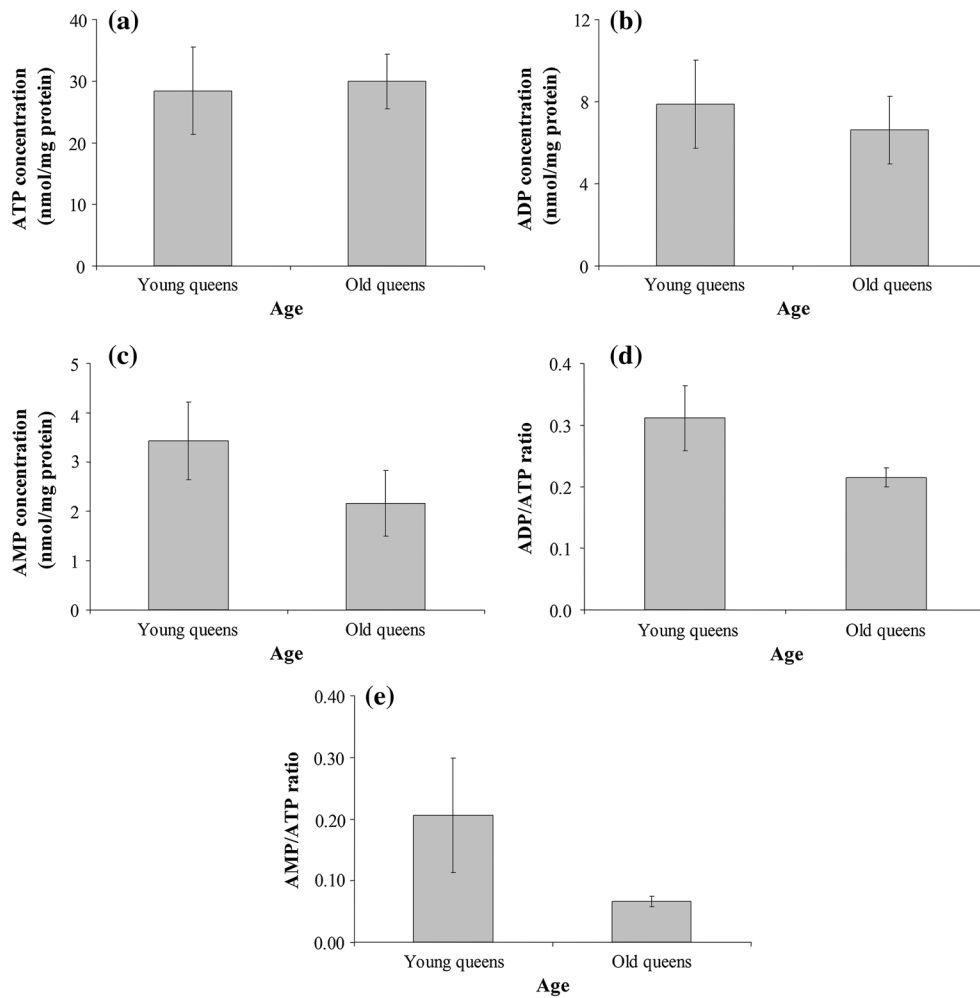


Fig. 3 The concentration of ATP (a), ADP (b), and AMP (c) and the ratio of ADP/ATP (d) and AMP/ATP (e) in the trophocytes and fat cells of queen bees. The values are expressed as the means \pm SEMs ($n = 5$; $P > 0.05$; two-sample t test)

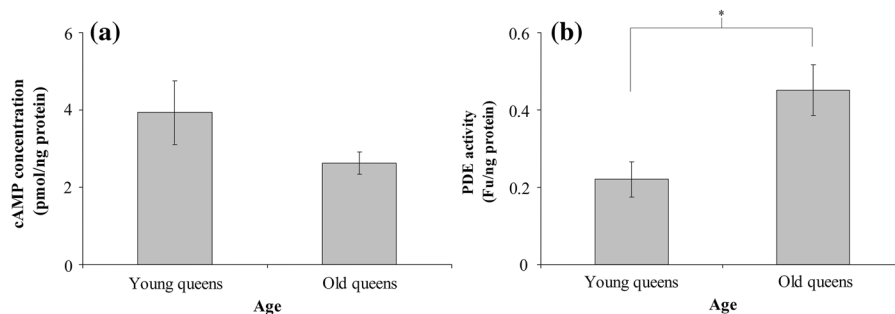


Fig. 4 The cAMP concentration (a) and PDE activity (b) in the trophocytes and fat cells of queen bees. The values are means \pm SEMs ($n = 5$). Asterisk indicates a statistically significant difference ($*P < 0.05$; two-sample t test)

queen bees (Fig. 6a). The statistical analysis showed that the PPAR- α expression was not significantly different between young and old queens ($n = 5$,

$P > 0.01$; Fig. 6b), indicating that the PPAR- α expression maintained young status in the trophocytes and fat cells of queen bees during aging.

Fig. 5 SirT1 expression and activity in the trophocytes and fat cells of young and old queen bees. **a** SirT1 expression was analyzed by Western blotting. Tubulin served as the loading control. *Y* young queen bees, *O* old queen bees. **b** SirT1 expression was normalized to expression in young workers. The results are presented as the means \pm SEMs and are expressed as percentages ($n = 5$). **c** The SirT1 activity results are presented as the means \pm SEMs ($n = 5$; $P > 0.05$; two-sample *t* test)

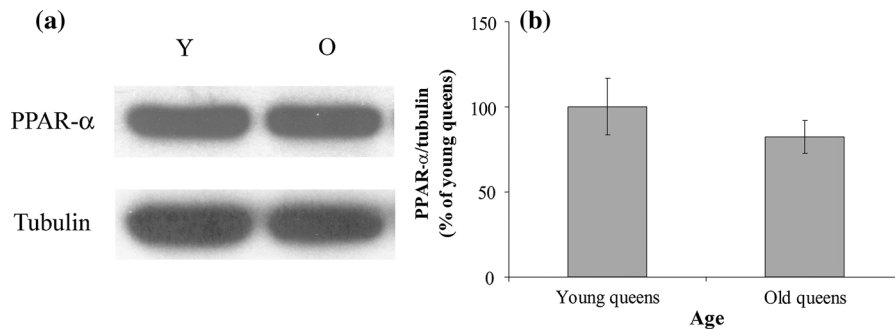
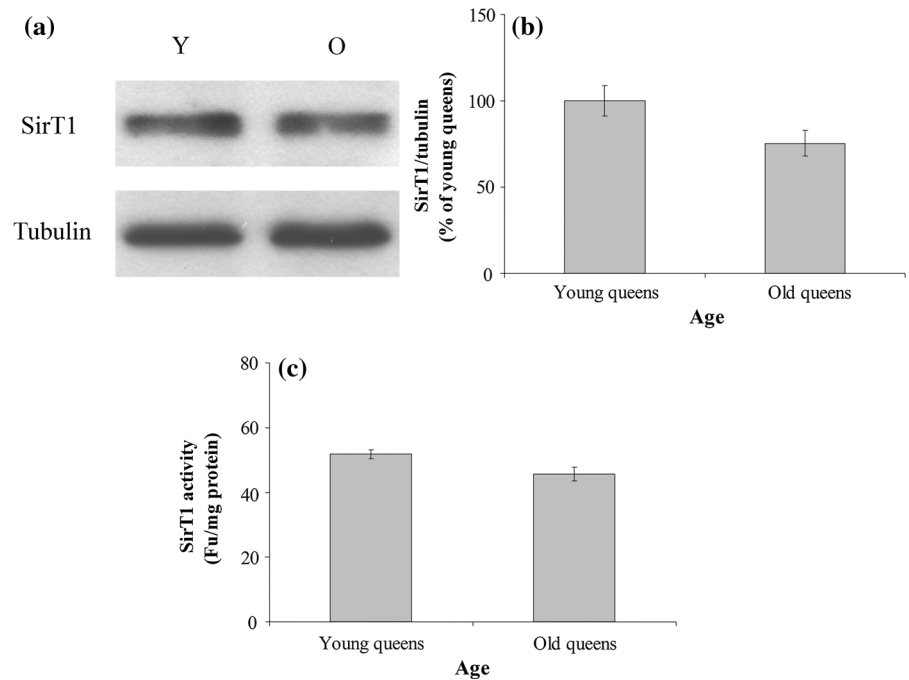


Fig. 6 PPAR- α expression in the trophocytes and fat cells of young and old queen bees. **a** PPAR- α expression was analyzed by Western blotting. Tubulin served as the loading control. *Y* young queen bees, *O* old queen bees, *P* positive control (rat

muscle). **b** PPAR- α expression was normalized to the expression in young queen bees. The results are presented as the means \pm SEMs and are expressed as percentages ($n = 5$; $P > 0.05$; two-sample *t* test)

Discussion

In this study, we evaluated the expression of AMPK- $\alpha 2$, pAMPK- $\alpha 2$, FoxO, SirT1, and PPAR- α ; the activity of AMPK, SirT1, and PDE; and the concentration of ATP, ADP, AMP, and cAMP in the trophocytes and fat cells of young and old queen bees. The activity of AMPK and SirT1; the expression of FoxO, SirT1 and PPAR- α ; and the concentration of ATP, ADP, AMP, and cAMP in trophocytes and fat cells were not significantly different between young

and old queen bees. However, PDE activity decreased with aging. These results show that energy-regulated molecules maintain young status in the trophocytes and fat cells of queen bees during aging. These cells seem to have longevity-promoting mechanisms and may clarify the secret of longevity in queen bees.

AMPK expression and activity

AMPK is a metabolic energy gauge and regulates cellular metabolism (Hardie 2011; Hardie et al. 2012).

This enzyme is a heterotrimeric Ser/Thr kinase that contains α , β , and γ subunits (Hardie 2007). The phosphorylation of the Thr¹⁷² residue in α subunits is required for full enzymatic activity (Hawley et al. 1996). In this study, AMPK- α expression increased with aging in the trophocytes and fat cells of queen bees, which corresponds with previous studies (Ljubic and Hood 2009; Hsu and Chuang 2013). However, pAMPK- α expression also increased with aging, which is not consistent with previous studies showing that AMPK- α expression decreased with aging (Qiang et al. 2007; Hsu and Chuang 2013). Caloric restriction (CR), which extends the lifespan of organisms, is associated with an increase in AMPK- α phosphorylation (Shinmura et al. 2007; Edwards et al. 2010). Maintaining high pAMPK- α level in old queen bees might play a role similar to the role observed in CR and might be associated with the longevity of queen bees.

The pAMPK/AMPK ratio and AMPK activity were similar between young and old queen bees, indicating that AMPK activity maintained young status in the trophocytes and fat cells of old queen bees. This phenomenon is not in agreement with previous studies showing the pAMPK/AMPK ratio and AMPK activity decreased with aging (Qiang et al. 2007; Reznick et al. 2007; Turdi et al. 2010; Hsu and Chuang 2013). Maintaining high AMPK activity in old queen bees might be associated with the longevity of queen bees. This finding indicates that the trophocytes and fat cells of queen bees seem to have longevity-promoting mechanisms when compared with worker bee AMPK activity and lifespan (Hsu and Chuang 2013). Although AMPK activity was not significantly different between young and old queen bees, AMPK activity had a decreased trend with aging.

Activated AMPK can activate glycolysis, fatty acid oxidation, mitochondrial biogenesis, and autophagy (Hardie et al. 2012; Reznick et al. 2007; Jornayvaz and Shulman 2010). Previous studies showed that AMPK activated mitochondrial function in the trophocytes and fat cells of worker bees (Chuang and Hsu 2013; Hsu and Chuang 2013). Similar AMPK activity in young and old queen bees reflected that trophocytes and fat cells maintained young status, which represented high cellular functions. In this study, parallel constants in AMPK activity and mitochondrial function (Hsu and Lu, unpublished data) with aging suggest that AMPK might also activate mitochondrial

function in the trophocytes and fat cells of queen bees during aging.

In addition, AMPK regulates FoxO transcription factors that regulate gene expressions in metabolism, cell cycle arrest, stress resistance, and longevity (Barthel et al. 2002; Greer et al. 2007; Cantó et al. 2009). In this study, parallel constants in AMPK activity and FoxO expression show that young and old queen bees might have similar gene regulation, which could lead to longevity.

ATP, ADP, and AMP concentrations and the AMP/ATP and ADP/ATP ratios

ATP concentrations were similar between young and old queen bees, which is not in accord with previous studies showing that ATP concentrations decreased with aging (Hsu and Chan 2013; Chuang and Hsu 2013; Brys et al. 2010). This finding supports a previous perspective that the trophocytes and fat cells of old queen bees maintain young status. Ambient temperature reduction (ATR), which can extend the lifespan of organisms, is associated with an increase in ATP concentration (Hsu and Chiu 2009). In addition, the *daf-2 Caenorhabditis elegans* mutant (*e1370*), which has an extended lifespan, also has a higher ATP concentration than the wild-type nematodes (Brys et al. 2010). These studies indicate that a high ATP concentration is associated with lifespan extension. Maintaining a youthful status in ATP concentration might play a role similar to that observed in ATR and the *daf-2 (e1370)* mutant.

Previous studies showed that AMPK might activate glycolysis or fatty acid oxidation in the trophocytes and fat cells of workers to generate NADH. NADH could be converted to NAD⁺ by ND1 to establish $\Delta\psi_m$. Subsequently, ATP was synthesized from ADP by ATP synthase (Chuang and Hsu 2013; Hsu and Chuang 2013). In this study, parallel constants in AMPK activity, ATP and ADP concentrations, NAD⁺ concentration, ND1 and ATP synthase expression (Hsu and Lu unpublished data) show that queen bees might use similar processes to produce ATP.

The fact that ADP concentrations were similar between young and old queen bees is not in agreement with previous studies showing that ADP concentration decreased with aging (Brys et al. 2010; Chuang and Hsu 2013). This finding also reveals that the trophocytes and fat cells of old queen bees maintain young

status. AMP concentrations were similar between young and old queen bees showing that the AMP concentrations did not significantly differ with aging. Further, in our study the AMP/ATP ratio was similar between young and old queen bees, which was not consistent with previous studies showing that the AMP/ATP ratio increased with aging (Wang et al. 2003; Apfeld et al. 2004). Previous studies showed that AMPK activity depended on the AMP/ATP ratio, that a very small rise in the AMP level can induce a strong increase in AMPK activity (Hardie 2004), and that the AMP/ATP and ADP/ATP ratios control AMPK activity to regulate cellular metabolism (Hardie 2011; Hardie et al. 2012). However, in this study, the AMP/ATP and ADP/ATP ratios do not seem to be key mediators of AMPK activity in the trophocytes and fat cells of queen bees. The most likely explanation is that the cellular energy requirement, not the energy deficiency, induces AMPK activity. This phenomenon is also observed in the worker bee study (Chuang and Hsu 2013).

cAMP and PDE

In addition to its role in energy storage, ATP can be transformed into cAMP by adenylate cyclase. cAMP is involved in triggering calcium signals by releasing calcium from intracellular stores. In general, the ATP concentrations is higher than the ADP concentrations, indicating that ATP may have some biological functions, most likely transforming into cAMP as a second messenger in signal transduction (Chuang and Hsu 2013). In this study, cAMP concentrations were similar between young and old queen bees, showing that cAMP might trigger similar signal transduction to maintain young status. This phenomenon is not consistent with previous studies showing that cAMP concentrations decreased with aging (Schoeffter and Stoclet 1990; Hsu and Chuang 2013). Maintaining high cAMP concentrations in old queen bees induces signal transduction to activate cellular metabolism, which might be associated with the longevity of queen bees (Chuang and Hsu 2013; Park et al. 2012). Although cAMP concentrations were not significantly different between young and old queen bees, cAMP concentrations also showed a decreased trend with aging. If the age of old queen bees was older, cAMP concentrations might decrease with aging.

In contrast, PDE hydrolyzes cAMP to AMP and inhibits the signal transduction of cAMP. In this study, the PDE activity of young queen bees was lower than that of old queen bees, which corresponds with previous studies (Forn et al. 1970; Stancheva and Alova 1991; Hsu and Chuang 2013). Lower PDE activity leads to higher cAMP concentrations, which could induce higher levels of signal transduction to activate cellular metabolism in the trophocytes and fat cells of young queen bees and vice versa. This interpretation is supported by previous studies (Park et al. 2012; Chuang and Hsu 2013). In addition, this finding supports previous postulations that if the age of old queen bees was older, then cAMP activity might decrease with aging. The facts that PDE activity decreases with aging and cAMP concentrations show a decreased trend with aging manifest that the age of old queen bees was not old enough.

SirT1 expression and activity

SirT1, a NAD⁺-dependent deacetylase, regulates a variety of cellular functions, including cellular stress responses and energy metabolism. In this study, we found that SirT1 expression and activity were similar between young and old queen bees, which is not consistent with previous studies showing that SirT1 activity decreased with aging (Braidly et al. 2011; Ferrara et al. 2008; Hsu and Chuang 2013). This finding supports previous perspectives that the trophocytes and fat cells of old queen bees maintain young status and that the trophocytes and fat cells of queen bees have longevity-promoting mechanisms.

SirT1 activity is activated by NAD⁺, which derives from the induction of AMPK (Park et al. 2012; Cantó et al. 2009). In this study, parallel constants in AMPK activity, SirT1 activity, and NAD⁺ concentration (Hsu and Lu unpublished data) with aging indicated that activated AMPK induced NAD⁺ generation, which consequently increased SirT1 activity. This speculation was supported by previous studies (Cantó et al. 2009; Hsu and Chuang 2013). CR is associated with an increase in SirT1 activity (Cantó and Auwerx 2009). Maintaining a youthful status in SirT1 activity might play a role similar to that observed in CR. In addition, SirT1 deacetylates liver kinase B1 (LKB1), which subsequently increases LKB1 activity. The reason for this is that LKB1 is an upstream activator of AMPK

and this signaling pathway might stimulate AMPK activation (Lan et al. 2008).

Although SirT1 expression and activity were not significantly different between young and old queen bees, SirT1 expression and activity also showed a decreased trend with aging. If the age of old queen bees was older, SirT1 expression and activity might decrease with aging.

SirT1 induces PPAR α expression

PPAR- α , a nuclear receptor transcription factor, increases fatty acid levels by lipase and stimulates β -oxidation (Schoonjans et al. 1996; Masternak and Bartke 2007; Yang and Li 2007). In this study, we found that PPAR- α expression was similar between young and old queen bees, which is not consistent with previous studies showing that PPAR- α expression decreased with aging (Iemitsu et al. 2002; Sanguino et al. 2005; Ye et al. 2005; Hsu and Chuang 2013). This finding supports previous perspectives that the trophocytes and fat cells of old queen bees maintain young status and that the trophocytes and fat cells of queen bees have longevity-promoting mechanisms. Although PPAR- α expression was not significantly different between young and old queen bees, PPAR- α expression showed a decreased trend with aging. The most likely reason is that the age of old queen bees was not old enough.

Previous studies showed that SirT1 regulates lipolysis by positively regulating PPAR- α (Purushotham et al. 2009; Planavila et al. 2011). In this study, parallel constants in SirT1 activity, PPAR- α expression, and NAD⁺ concentration (Hsu and Lu unpublished data) with aging reflected that NAD⁺ activated SirT1, which consequently induced PPAR- α in the trophocytes and fat cells of queen bees. This inference is supported by previous studies (Purushotham et al. 2009; Planavila et al. 2011; Hayashida et al. 2010; Hsu and Chuang 2013). CR is associated with an increase in PPAR- α expression (Masternak and Bartke 2007; Hayashida et al. 2010; Kersten et al. 1999). Maintaining a youthful status in PPAR- α expression might play a role similar to that observed in CR. In addition, parallel constants in PPAR- α expression and ATP concentration indicate that PPAR- α might increase fatty acid levels to increase ATP concentration by β -oxidation (Hsu and Chuang 2013).

Taken together, these results indicate that energy-regulated molecules maintain young status in the trophocytes and fat cells of queen bees during aging, these cells seem to have longevity-promoting mechanisms, and they may be used to clarify the secret of longevity in queen bees.

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Conflict of interests We have no conflicts of interest or disclosures.

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