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ARTICLE

Pentamethylquercetin generates beneficial effects in monosodium glutamate-induced obese mice and C2C12 myotubes by activating AMP-activated protein kinase

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Abstract
Aims/hypothesis Pentamethylquercetin (PMQ) has recently been shown to have glucose-lowering properties. Here, we aimed to characterise the effectiveness and underlying mechanisms of PMQ for ameliorating metabolic disorders in vivo and vitro.
Methods We generated a mouse model of obesity by neonatal administration of monosodium glutamate (MSG) and used it to assess the properties of PMQ as a treatment for metabolic disorders. We also investigated the possible underlying mechanisms of PMQ in the prevention of metabolic disorders.
Results Compared with normal mice, MSG mice had metabolic disorders, including central obesity, hyperinsulinaemia, insulin resistance, hyperglycaemia, hyperlipidaemia, decreased phosphorylation of AMP-activated protein kinase (AMPK) and acetyl-CoA carboxylase (ACC), and downregulated levels of GLUT4 in gastrocnemius muscles. In MSG mice, PMQ treatment (5, 10, 20 mg/kg daily) reduced body weight gain, waist circumference, adipose tissue mass, serum glucose, triacylglycerol and total cholesterol, while improving insulin resistance, activating AMPK and increasing ACC phosphorylation and GLUT4 abundance. In C2C12 myotubes, PMQ (10 μmol/l) increased glucose consumption by ~65%. PMQ treatment (1–10 μmol/l) also activated AMPK, increased ACC phosphorylation and GLUT4 abundance, and upregulated the expression of some key genes involved in fatty acid oxidation.
Conclusions/interpretation These findings suggest that PMQ can ameliorate metabolic disorders at least in part via stimulation of AMPK activity.

Keywords AMP-activated protein kinase · Fatty acid oxidation · Glucose transporter type 4 · Insulin resistance · Metabolic syndrome · Monosodium glutamate · Pentamethylquercetin

Abbreviations

ACC	Acetyl-CoA carboxylase
ACO	Acyl-CoA oxidase
AMPK	AMP-activated protein kinase
FER	Food efficiency ratio
GAPDH	Glyceraldehyde phosphate dehydrogenase
GM	Growth medium
HOMA-IR	HOMA of insulin resistance
MSG	Monosodium glutamate
MTT	3-(4,5-Dimethylthiazol-2-yl)-2, 5-diphenyltetraz-olium bromide
PGC-1α	PPAR gamma, coactivator 1α
PMQ	Pentamethylquercetin
PPAR	Peroxisome proliferator-activated receptor

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Introduction

The metabolic syndrome is a constellation of interrelated metabolic risk factors, including obesity, insulin resistance, hyperglycaemia, dyslipidaemia and hypertension. At present the underlying pathophysiology remains unclear, but it has been closely linked to insulin resistance and obesity [1, 2]. For this reason, research into so-called insulin-sensitising agents has received considerable attention and several major insulin-sensitising agents have been developed in recent years, including metformin [3] and thiazolidinediones [4]. Some studies have indicated that metformin and thiazolidinediones exert beneficial effects, at least in part, by activating AMP-activated protein kinase (AMPK) [5, 6]. AMPK is a major regulator of cellular and whole-body energy homeostasis, and is activated by decreases in a cell's energy state as reflected by an increased AMP:ATP ratio. As far as we know, AMPK upregulates catabolic pathways that generate ATP, while inhibiting anabolic pathways that consume ATP [7]. When activated, AMPK acts to maintain cellular energy stores, stimulates fatty acid oxidation, enhances insulin sensitivity, ameliorates hyperglycaemia and hyperlipidaemia, and inhibits proinflammatory changes. Thus, AMPK is a well accepted therapeutic target for the metabolic syndrome and type 2 diabetes [8, 9].

The use of natural products for the treatment of metabolic diseases has not been explored in depth, although several modern oral hypoglycaemic agents, e.g. metformin, are derivatives of natural plant products [10, 11]. Flavonoids have several biological effects, such as anti-inflammatory, antioxidant, anti-cancer, anti-obesity and anti-human immunodeficiency virus type 1 (HIV-1) pharmacological properties [12]. One of the most widely distributed flavonoids in human dietary sources is the flavonol, quercetin. In animal models and cellular lines, this molecule has been reported to have cardioprotective, anti-inflammatory and anti-obesity effects [13, 14]. In vitro experiments have revealed that quercetin can improve glucose metabolism and obesity by activating AMPK [14, 15]. Compared with flavonoid aglycones, methoxylated flavonoids have higher intestinal absorption and greater resistance to hepatic metabolism [16]. Polymethoxylated flavones are a group of highly methoxylated phenolic compounds existing widely in the natural world. Previous studies have demonstrated that an experimental diet supplemented with citrus polymethoxylated flavones ameliorated insulin resistance in a fructose-induced hamster model of insulin-resistance [17]. Moreover, nobiletin has been shown to significantly improve hyperglycaemia and insulin resistance in obese diabetic *ob*/*ob* mice [18].

Pentamethylquercetin (PMQ), which is present in sea buckthorn (*Hippophae rhamnoides*) [19] and the rhizome of *Kaempferia parviflora* [20], is a typical member of the polymethoxylated flavone family in the natural world. We have previously reported that PMQ upregulated adiponectin levels in 3T3-L1 adipocytes [21] and had glucose-lowering effects in a neonatally streptozotocin-induced rat model of diabetes [22]. Although PMQ has been reported to have glucose-lowering effects, the molecular targets of this compound have not been revealed and a more comprehensive analysis of its role in metabolic disorders has not been undertaken. We therefore investigated the effects of PMQ, and the mechanisms involved, on metabolic disorders, using a neonatal monosodium glutamate (MSG)-induced mouse model [23].

Methods

Animal studies PMQ was synthesised by the Food and Drug Evaluation Center of Tongji Medical College at Huazhong University of Science and Technology at a purity of 99.5% as examined by HPLC [21]. Kunming mice were purchased from the Animal Center of Tongji Medical College at Huazhong University of Science and Technology at 10 weeks of age. All mice were housed at 22±2°C and 55±5% relative humidity, with a light/dark cycle of 12 h and free access to food and water. All experiments were approved by the Ethics Committee of Animal Use for Teaching and Research of Tongji Medical College at Huazhong University of Science and Technology. After a 2-week acclimatisation period, 12-week-old virgin female mice were mated with male mice at a ratio of 1:1. Once daily from day 2 to day 8 after delivery, pups were given a solution of MSG dissolved in saline (300 g/l) or equipotent vehicle (10 μl/g body weight); administration was subcutaneous via a microsyringe. At 5 weeks of age, all treated male mice were randomly divided into six different groups (n=10 mice per group) as follows: Control, Vehicle, PMQ 5 mg/kg, PMQ 10 mg/kg, PMQ 20 mg/kg and rosiglitazone 5 mg/kg. PMQ and rosiglitazone were administrated by gastric gavage for 13 weeks. Control and Vehicle groups were administered an equipotent volume of vehicle. Body weight, body length and food consumption were monitored, and the Lee index and food efficiency ratio (FER) were calculated at the end of experiment. The Lee index, which is a similar tool to BMI as used in humans to assess obesity, was calculated by dividing the cubic root of body weight by the ano-nasal length. FER was calculated as FER = total body weight gain (g)/total food intake (g) [24, 25]. At 18 weeks of age, after a 12 h fast, the body weight and waist circumference were measured, and blood samples were collected for separating serum. Then all mice were killed, and organs and adipose tissue weighed, frozen and prepared for testing.

Serum analyses Fasting serum levels of glucose, triacylglycerol and total cholesterol in each group were detected using corresponding commercial kits (Biosino, Beijing, China).

Fasting serum insulin levels were measured by commercial radioimmunoassay kit (Beijing north, Beijing, China) performed in duplicate. The HOMA of insulin resistance (HOMA-IR) was used to assess insulin resistance [26].

Cell culture Mouse C2C12 skeletal muscle cells were purchased from boster (Wuhan, China) and grown in DMEM, supplemented with 10% (vol./vol.) FBS (referred to as growth medium [GM]) in an atmosphere of 5% CO_2. After 24 h in GM, muscle differentiation was induced by incubating cells in DMEM supplemented with 2% (vol./vol.) horse serum (referred to as differentiation medium). C2C12 skeletal muscle cells were considered to be C2C12 myotubes after 96 h of differentiation in differentiation medium [27].

Cell viability assay C2C12 myotubes were incubated with DMSO or test compounds for 24 h. PBS-buffered 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (20 μl, 5 mg/ml) solution was added to each well and the plates were incubated at 37°C for another 4 h. Then the medium was discarded and the formazan blue that formed in the cells was dissolved in DMSO. The optical density was measured using a microplate spectrophotometer (Tecan, Männedorf, Switzerland) at 570 nm.

Glucose consumption assay C2C12 myotubes were cultured in 96-well culture plates. When experiments were conducted, the differentiation culture medium was replaced by DMEM supplemented with 0.25% (wt/vol.) BSA containing DMSO (vehicle), PMQ 1, 3 and 10 μmol/l, and insulin 0.1 μmol/l (as a positive control). The glucose concentration in medium was determined by the glucose oxidase method after 24 h of treatment. The glucose concentration of the wells with cells was subtracted from the glucose concentration of the blank wells to obtain the amount of glucose consumption [28].

RNA preparation and RT-PCR Total RNA was prepared with Trizol (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. RT-PCR was conducted in a previously described manner [21]. The primers used are listed in the electronic supplementary material (ESM) Table 1.

Western blot analysis C2C12 myotubes were collected by scraping into ice-cold RIPA buffer containing: 150 mmol/l NaCl, 1% (vol./vol.) NP-40, 0.5% (wt/vol.) sodium deoxycholate, 0.1% (wt/vol.) sodium dodecyl sulphate, 50 mmol/l Tris-HCl

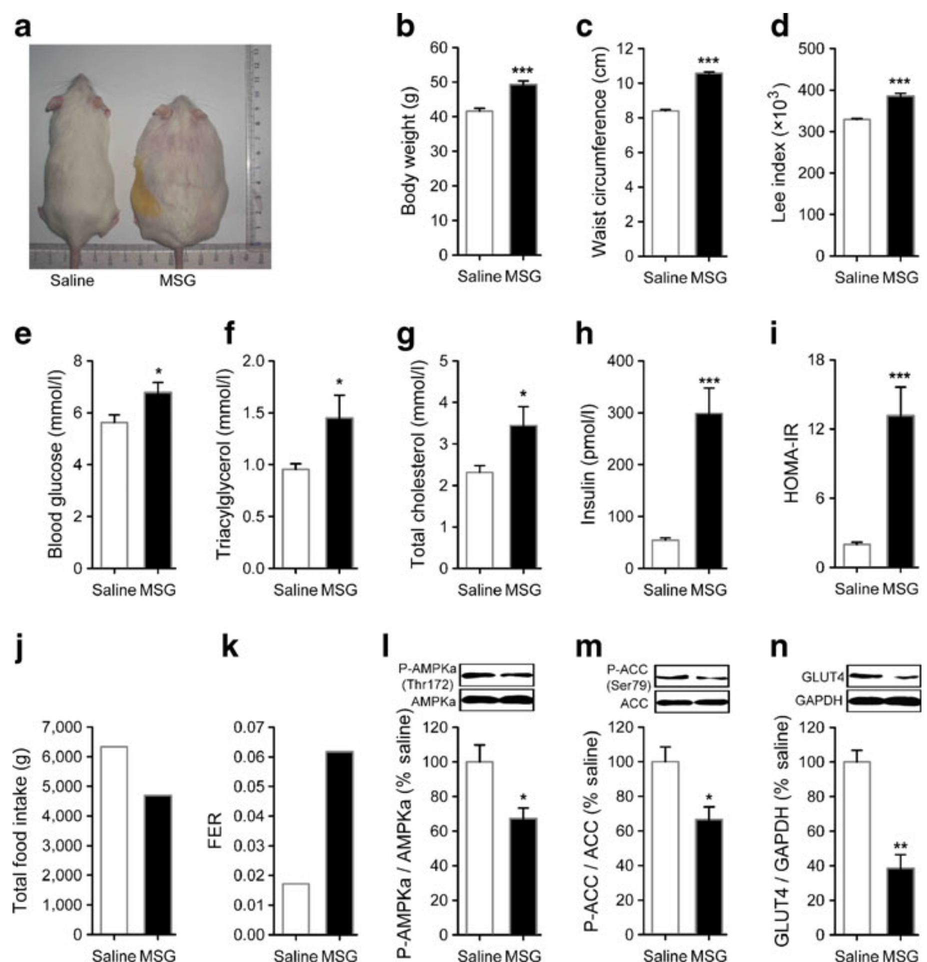

Fig. 1 Multiple metabolic disorders in MSG mice. **a** Representative image of saline-alone (Saline) and MSG-treated mice at 18 weeks of age. **b** Body weight, (**c**) waist circumference, (**d**) Lee index, (**e**) serum glucose, (**f**) triacylglycerol, (**g**) total cholesterol, (**h**) insulin and (**i**) HOMA-IR were measured to confirm the multiple metabolic disorders in MSG mice at 18 weeks of age. Total food intake (**j**) and FER (**k**) of each group were also monitored and calculated during the experimental period. **l** In gastrocnemius muscles of mice, phospho (P)-AMPKα (Thr172), (**m**) phospho-ACC (Ser79) and (**n**) GLUT4 levels were examined by western blot. Data are expressed as means ± SE; $n=10$ and (western blot) $n=3$; *$p<0.05$, **$p<0.01$ and ***$p<0.001$ vs saline alone

(pH 7.4), 50 mmol/l glycerophosphate, 20 mmol/l NaF, 20 mmol/l EGTA, 1 mmol/l dithiothreitol, 1 mmol/l Na_3VO_4, 1 mmol/l phenylmethylsulfonyl fluoride and protease inhibitors. Isolated gastrocnemius muscles were homogenised on ice in RIPA buffer. Homogenates were rotated for 40 min and centrifuged (12,000 *g* for 10 min at 4°C). The total protein concentration was measured by the Bradford method. Denatured protein samples (50 μg) were subjected to SDS-PAGE and blotted on to nitrocellulose membranes. Blotted membranes were blocked for 1 h with 5% (wt/vol.) skimmed milk at room temperature and then hybridised overnight at 4°C with primary antibodies against AMPKα (dilution 1:1,000), phospho-AMPKα (Thr172) (dilution 1:1,000), acetyl-CoA carboxylase (ACC) (dilution 1:1,000), phospho-ACC (Ser79) (dilution 1:1,000), GLUT4 (dilution 1:1,000) and glyceraldehyde phosphate dehydrogenase (GAPDH) (dilution 1:10,000). Phospho-AMPKα (Thr172), AMPKα, Phospho-ACC (Ser79) and ACC antibodies were purchased from Cell Signaling Technology (Danvers, MA, USA). GAPDH and GLUT4 antibodies were purchased from Abcam (Cambridge, UK). After incubation with horseradish peroxidase-conjugated secondary antibody (dilution 1:5,000; Cell Signaling Technology)

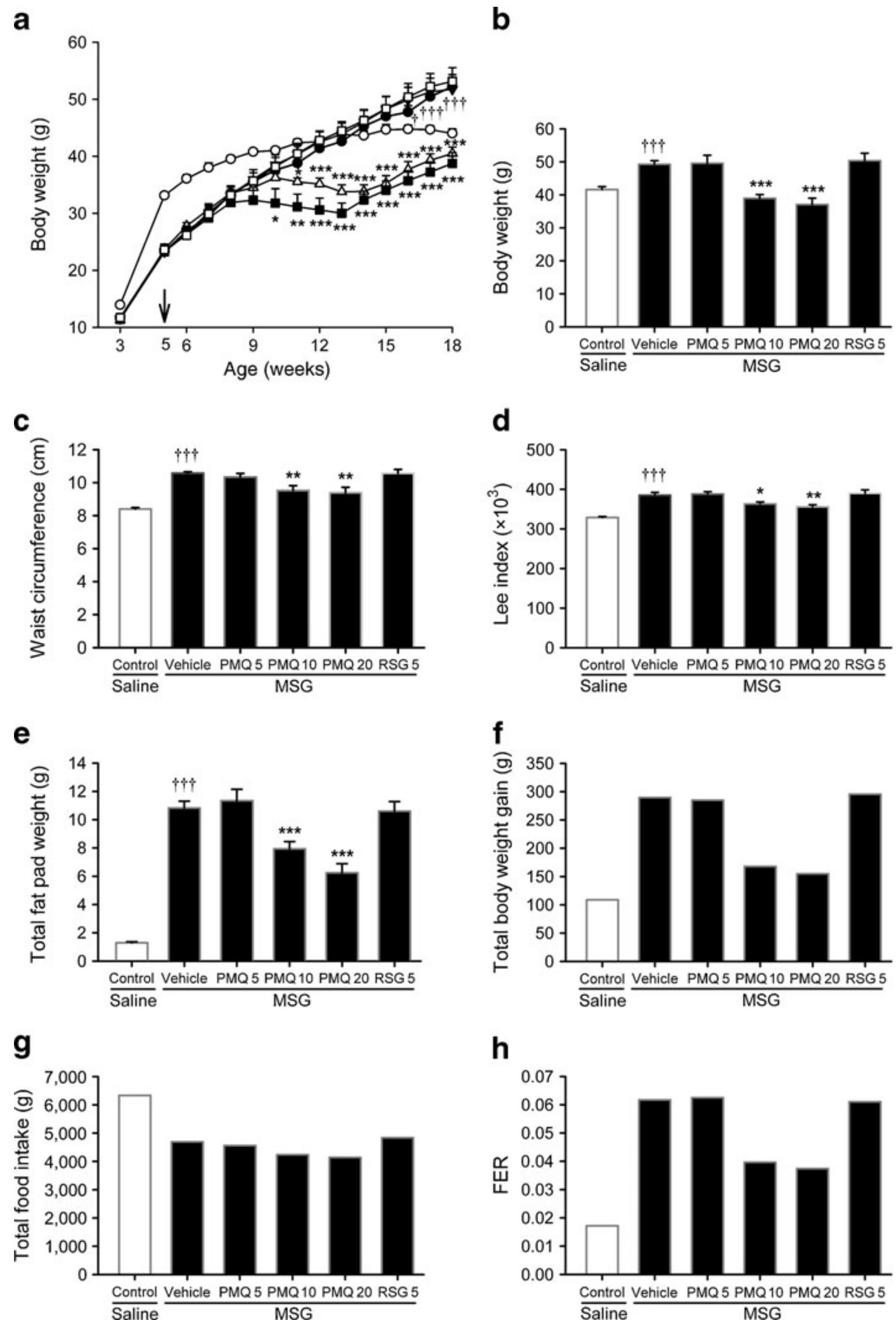

Fig. 2 Effects of PMQ on body weight, adipose tissue mass and FER. **a** Evolution of the body weights of each mouse group. White circles, Control; black circles, Vehicle; black triangles, PMQ 5 mg/kg; white triangles, PMQ 10 mg/kg; black squares, PMQ 20 mg/kg; white squares, rosiglitazone (RSG) 5 mg/kg; downward arrow, start of intervention. **b** Body weight and (**c**) waist circumference of each group of mice at the end of experiment. **d** The Lee index was used to assess the degree of obesity in each group of mice. Mice were then killed and multi-regional adipose tissue removed and weighed. **e** Total fat pad weight per group. **f** Total body weight gain and (**g**) total food intake of each group were monitored during the experimental period. The FER (**h**) was calculated at the end of experiment. Data are expressed as means ± SE; $n=10$; †$p<0.05$ and †††$p<0.001$ vs control; *$p<0.05$, **$p<0.01$ and ***$p<0.001$ vs vehicle

for 1 h at room temperature, immunoreactive signals were detected with an enhanced chemiluminescent reagent (Amersham Pharmacia Biotech, Piscataway, NJ, USA) and quantified with Image J 1.37V (developed at the US National Institutes of Health, available at http://rsb.info.nih.gov/ij/, accessed 21 February 2012). More details on reagents are provided in the ESM Methods.

Statistical analysis Values are expressed as means ± SE. One-way ANOVA was used to test homogeneity for variance and Tukey's test was applied to test the significance of differences between multiple groups. Values of $p<0.05$ were considered statistically significant.

Results

Multiple metabolic disorders in MSG mice As indicated in Fig. 1, at 18 weeks of age MSG mice had significant obesity, hyperglycaemia, hyperlipidaemia, hyperinsulinaemia and insulin resistance, as well as low food intake and high FER compared with saline-alone mice. Moreover, in gastrocnemius muscles of MSG mice phosphorylation levels of AMPK and ACC were decreased, and GLUT4 abundance downregulated. These results suggest that neonatal administration of MSG can induce multiple metabolic disorders in mice.

Anti-obesity effect of PMQ in MSG mice Young MSG mice that had not yet developed significant obesity were treated with PMQ at a dose of 5, 10 and 20 mg/kg, and with rosiglitazone at 5 mg/kg. Treatment was daily by gastric gavage from 5 to 18 weeks of age. PMQ treatment resulted in a loss of body weight over the same period (Fig. 2a). Correspondingly, at the end of experiment, the body weight and waist circumference were clearly reduced by PMQ treatment (Fig. 2b, c). The Lee index in PMQ-treated mice was also substantially reduced (Fig. 2d). Quantitative analysis of adipose tissue mass demonstrated that the total fat pad weight was reduced by ~42% at 20 mg/kg PMQ treatment (Fig. 2e). In addition, a diet consumption monitor indicated

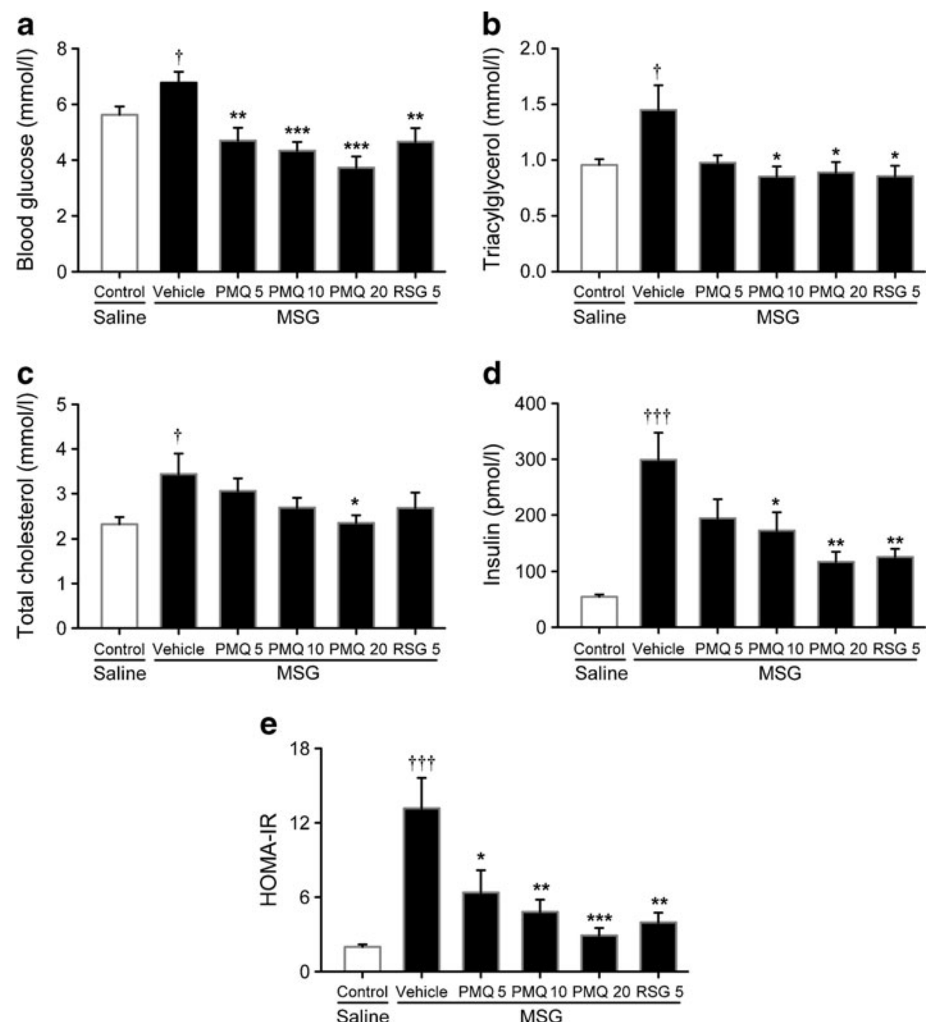

Fig. 3 PMQ improved glucose and lipid metabolism, and insulin resistance. **a** PMQ treatment resulted in significant reductions of serum glucose, (**b**) triacylglycerol and (**c**) total cholesterol. Hyperinsulinaemia (**d**) and insulin resistance (**e**) were significantly improved after PMQ treatment compared with the corresponding vehicle-treated mice. Data are expressed as means ± SE; $n=10$; †$p<0.05$ and †††$p<0.001$ vs control; *$p<0.05$, **$p<0.01$ and ***$p<0.001$ vs vehicle

that PMQ-treated mice had low food intake and FER (Fig. 2f–h). These results suggest that PMQ has a clear anti-obesity effect, while rosiglitazone (5 mg/kg) had none in MSG mice (Fig. 2).

PMQ improved glucose and lipid metabolism, and insulin resistance in vivo PMQ administration resulted in a significant reduction of serum glucose, triacylglycerol and total cholesterol levels (Fig. 3a–c). Hyperinsulinaemia and insulin resistance were also significantly improved after PMQ treatment compared with the corresponding vehicle-treated mice (Fig. 3d, e). As a positive control, rosiglitazone (5 mg/kg) achieved similar improvements in glucose, lipid and insulin metabolism (Fig. 3).

PMQ improved glucose metabolism and had no cytotoxicity in vitro To investigate the mechanisms of PMQ action, we examined glucose consumption in C2C12 myotubes after PMQ treatment. Compared with vehicle control, PMQ treatment increased glucose consumption in a dose-dependent manner. At concentrations between 1 and 10 μmol/l, PMQ increased glucose consumption by ~19 to 65%. As a positive control, insulin (0.1 μmol/l) also caused a clear increase in glucose consumption (Fig. 4a). In this study, most of the cellular data were obtained with PMQ treatment for 24 h. To examine whether PMQ exerts toxic effects in C2C12 myotubes, MTT assays were conducted. The MTT data suggested that PMQ had no cytotoxicity in our experimental system, as it did not influence cell viability at concentrations ranging from 1 to 10 μmol/l (Fig. 4b).

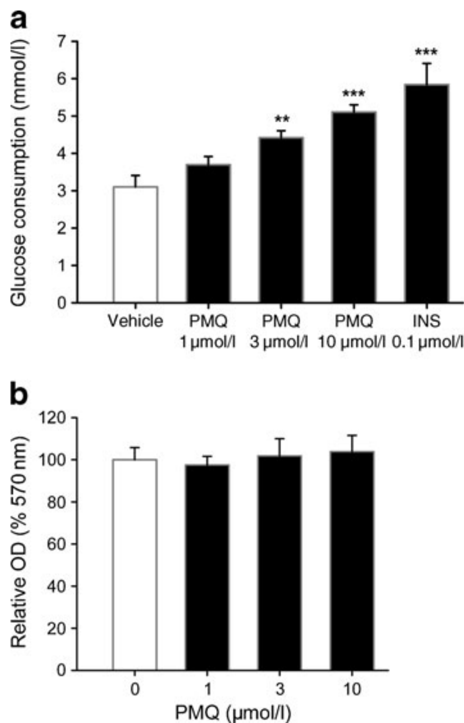

Fig. 4 PMQ increased glucose consumption in C2C12 myotubes. C2C12 myotubes were cultured in a 96-well plate and prepared as indicated in the Methods. Cells were then treated with PMQ and insulin (as a positive control) for 24 h. **a** Glucose consumption in C2C12 myotubes after PMQ treatment. **b** An MTT assay conducted to assess the cytotoxicity of PMQ in vitro did not reveal any cytotoxic effect of PMQ on cell viability in C2C12 myotubes. Data are expressed as means ± SE; n=8; ** p<0.01 and *** p<0.001 vs vehicle

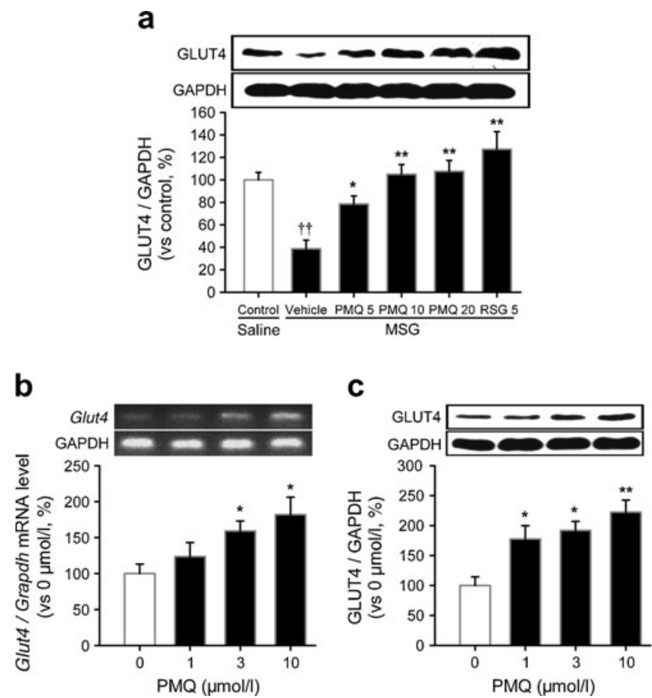

Fig. 5 PMQ upregulated GLUT4 in skeletal muscle of MSG mice and C2C12 myotubes. **a** Isolated gastrocnemius muscles were homogenised on ice in RIPA buffer to obtain total protein and GLUT4 abundance was detected by western blot. **b** C2C12 myotubes were treated with PMQ as indicated in the Methods, followed by RT-PCR and (**c**) western blot to detect the respective mRNA and protein levels of GLUT4. Data are expressed as means ± SE; n=4 (RT-PCR), n=3 (western blot); †† p<0.01 vs control; * p<0.05 and ** p<0.01 vs vehicle or 0 μmol/l PMQ

PMQ upregulated GLUT4 abundance in MSG mice and C2C12 myotubes In view of the changes in glucose metabolism observed in vivo and in vitro with PMQ treatment, we examined the effect of PMQ on GLUT4 production in skeletal muscle of MSG mice and in C2C12 myotubes. As shown in Fig. 5, PMQ upregulated GLUT4 abundance in a dose-dependent manner in vivo and vitro.

PMQ increased fatty acid oxidation To investigate the mechanism of PMQ action on lipid metabolism and body weight, we next assessed the effects of PMQ on the expression of certain genes that are known to play a critical role in fatty acid oxidation in C2C12 myotubes. As shown in Fig. 6a–e, the mRNA expression of several genes specific to fatty acid oxidation, including *Pgc-1α* (also known as *Ppargc1a*), *Ppara*,

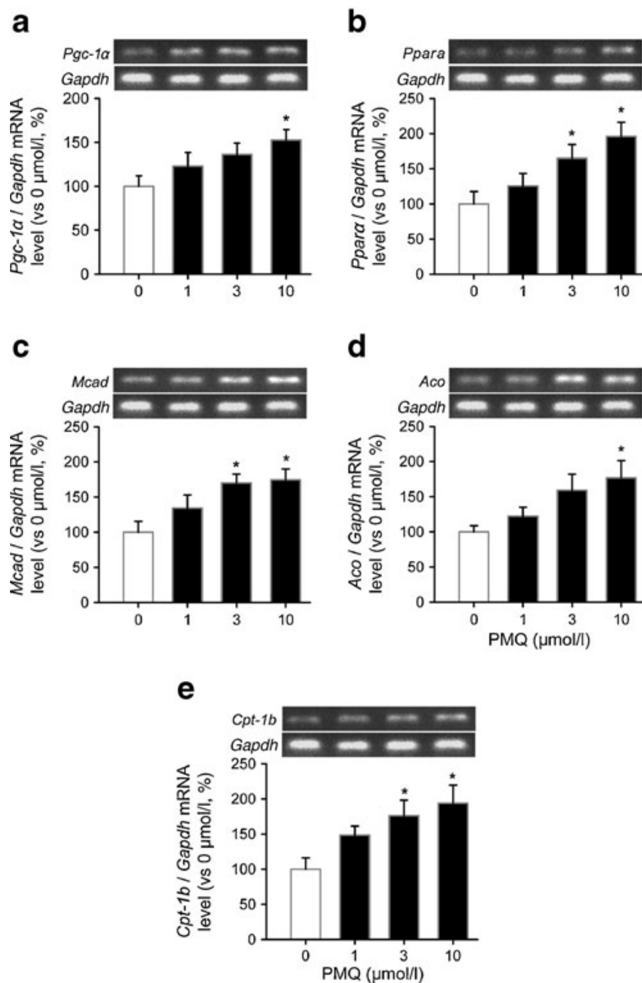

Fig. 6 Effects of PMQ on mRNA expression of genes related to fatty acid oxidation. **a** C2C12 myotubes were treated by PMQ as indicated in the Methods and relative mRNA levels of *Pgc-1α*, (**b**) *Ppara*, (**c**) *Mcad*, (**d**) *Aco* and (**e**) *Cpt1b* were analysed by RT-PCR. Each mRNA measurement was normalised to *Gapdh*. Data are expressed as means ± SE; n=4; *p<0.05 vs 0 μmol/l PMQ

Mcad (also known as *Acadm*), *Aco* (also known as *Acox1*) and *Cpt1b*, were increased in C2C12 myotubes after PMQ treatment for 24 h.

PMQ-induced phosphorylation of AMPK Based on the above results, we hypothesised that the properties of PMQ as a treatment for metabolic disorders might be effected via activation of AMPK, since AMPK is a major regulator of cellular and whole-body energy homeostasis. Consequently, we examined the effect of PMQ on AMPK phosphorylation in skeletal muscle of MSG mice and in C2C12 myotubes. We also examined phosphorylation of ACC, which is a major substrate of AMPK and a key regulator of fatty acid oxidation. As expected, AMPK phosphorylation and ACC phosphorylation were increased in MSG mice and C2C12 myotubes after PMQ treatment. As a positive control, 5 mg/kg rosiglitazone and 1 mmol/l metformin were also seen to activate phosphorylation of AMPK and ACC (Fig. 7a–d). Time course experiments showed that the effect of PMQ on phosphorylation of AMPK began at 8 h after exposure and lasted for at least 16 h in C2C12 myotubes (Fig. 7e).

Discussion

The prevalence of the metabolic syndrome is high and increasing throughout the world [29]. So far, the criteria for identifying the metabolic syndrome are not unanimously agreed, but all the definitions identify obesity and insulin resistance as core components [30, 31]. The metabolic syndrome is associated with an increased risk of cardiovascular disease events in general populations [29]. Therefore, study of the prevention and treatment of the metabolic syndrome has received considerable attention, with animal models being helpful in further understanding the potential pathophysiology of the disorder. The present data indicate that MSG mice exhibit multiple metabolic disorders, including significant obesity, insulin resistance, hyperglycaemia, hyperlipidaemia and hyperinsulinaemia, and are therefore an appropriate animal model to mimic human metabolic syndrome.

PMQ has been shown to have anti-cancer, anti-cardiac hypertrophy and anti-apoptosis pharmacological properties [32, 33]. Very recently, several studies described the metabolism-regulating properties of PMQ, including glucose-lowering effects and upregulation of adiponectin production [21, 22]. In the present study, we show for the first time that PMQ reduces body weight, serum glucose, lipid and insulin levels, as well as improving insulin resistance in MSG mice. Most importantly, with regard to possible mechanisms, we show that PMQ clearly activates AMPK activity in vivo and vitro. Moreover, PMQ induces several metabolic effects consistent with AMPK activation, e.g. increased phosphorylation of ACC, upregulation of GLUT4 (at protein and gene levels) and enhanced expression of genes involved in fatty acid oxidation.

AMPK is a major regulator of cellular and whole-body energy homeostasis, and is activated under stress events such as hypoxia, ischaemia, glucose deprivation and exercise [7]. Activation of AMPK stimulates fatty acid oxidation to generate more ATP to cope with acute energy demand and inhibits anabolic processes that consume ATP. In addition, AMPK activation enhances insulin sensitivity, inhibits hepatic glucose production, stimulates glucose uptake in muscle and inhibits fatty acid synthesis [34]. Thus, AMPK is a well accepted target for the treatment of the metabolic syndrome. The present data show that AMPK is involved in facilitating the beneficial effects of PMQ. Thus PMQ activated the phosphorylation of AMPK and ACC in skeletal muscle of MSG mice. In C2C12 myotubes, after treatment with PMQ for 24 h, AMPK phosphorylation and ACC phosphorylation were markedly increased

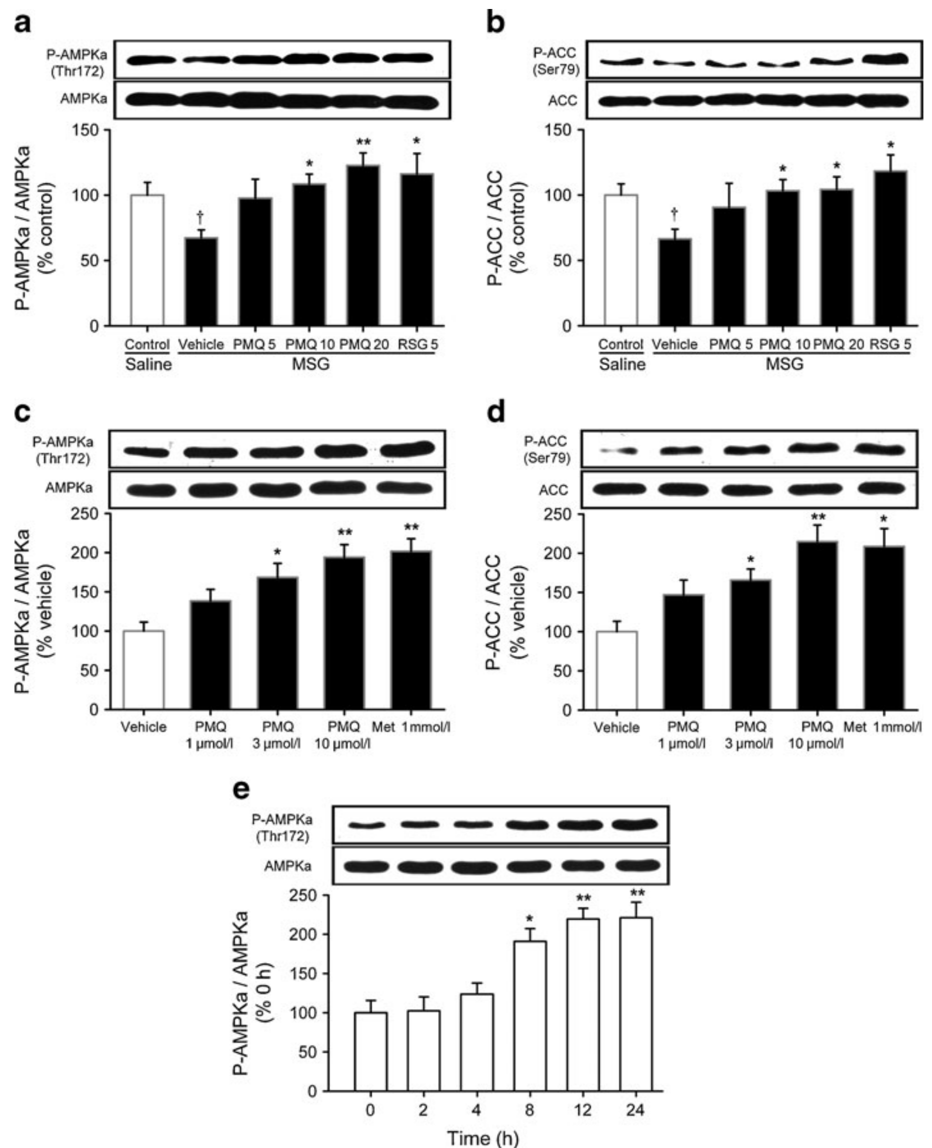

Fig. 7 Effects of PMQ on the AMPK signalling pathway. Gastrocnemius muscle of mice and C2C12 myotubes were examined by western blot. Rosiglitazone (RSG) (5 mg/kg) and metformin (Met) (1 mmol/l) were used as positive control for activation of AMPK. **a** PMQ increased phosphorylation levels of AMPK (P-AMPK) and (**b**) ACC (P-ACC) in MSG mice, and (**c, d**) in C2C12 myotubes. **e** C2C12 myotubes were treated with PMQ (10 μmol/l) for various time periods in 0.25% (wt/vol.) BSA-DMEM and western blot performed to test AMPK phosphorylation and total protein levels. Data are expressed as means ± SE; $n=3$; †$p<0.05$ vs control; *$p<0.05$ and **$p<0.01$ vs vehicle or 0 h PMQ

in a dose-dependent manner. The time course study suggests that PMQ can induce long-lasting phosphorylation of AMPK (at least 16 h).

The major cellular mechanism for disposal of an exogenous glucose load is insulin-stimulated glucose transport into skeletal muscle. Skeletal muscle stores glucose as glycogen and oxidises it to produce energy following the transport step. GLUT4, as a principal glucose transporter protein that mediates glucose uptake, is thus a major mediator of glucose removal from the circulation and a key regulator of whole-body glucose homeostasis [35]. Since regulation of glucose uptake is critical for treatment of the metabolic syndrome as well as diabetes, upregulation of GLUT4 at the protein and gene level is an important mechanism in the enhancement of glucose uptake in animal cells. It has been reported that AMPK controlled the capacity of skeletal muscle carbohydrate metabolism by regulating the abundance of GLUT4, which increased the maximal rate of glucose transport into skeletal muscle [36, 37]. Eid et al. have shown that quercetin and quercetin glycosides stimulated AMPK and enhanced basal glucose uptake in C2C12 cells

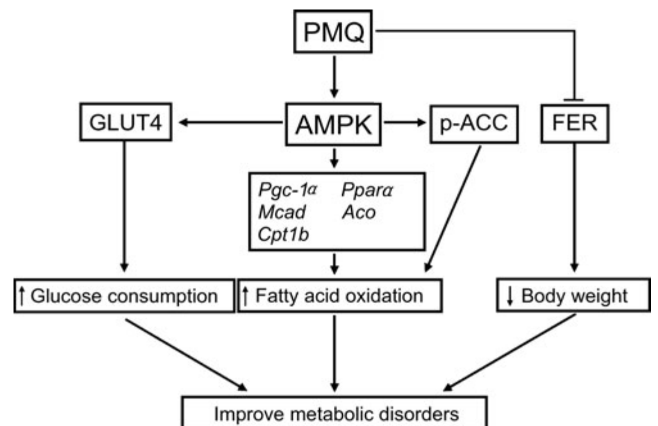

Fig. 8 Schematic model of how PMQ might protect against metabolic disorders

[15]. These results prompted us to hypothesise that PMQ may also enhance glucose uptake and thus improve glucose metabolism. In this study, we measured glucose consumption to reflect glucose uptake levels in C2C12 myotubes. The data suggest that PMQ was able to increase glucose consumption in a dose-dependent manner. Further studies found that PMQ upregulated *Glut4* (also known as *Slc2a4*) expression at the mRNA level and at the protein level in C2C12 myotubes. PMQ also upregulated GLUT4 protein levels in skeletal muscle of MSG mice. These results prompted us to postulate that the improvement of glucose metabolism effected by PMQ probably involves increased GLUT4 abundance.

It is generally believed that AMPK activation increases fatty acid oxidation by inhibiting ACC, which decreases intracellular malonyl CoA levels [36]. Peroxisome proliferator-activated receptor (PPAR)α plays an important role in the transcriptional control of mitochondrial fatty acid oxidation by upregulating the expression of genes involved in fatty acid oxidation in skeletal muscle [38, 39]. The enhancement of fatty acid oxidation contributes to the effects of PPARα activation on hyperlipidaemia. Many groups have reported that activation of PPARα may suppress weight gain of adipose tissues [40, 41]. In the present study, PMQ increased mRNA expression of *Ppara* and its target genes, including *Aco*, *Mcad* and *Cpt1b*, in C2C12 myotubes. PPAR gamma, coactivator 1α (PGC-1α) is involved in numerous biological responses related to energy homeostasis, thermal regulation and glucose metabolism [42]. Although PGC-1α was originally identified as a transcriptional coactivator of the nuclear receptor PPARγ, it is now known that PGC-1α coactivates PPARα in the transcriptional control of mitochondrial fatty acid oxidation [43]. We also observed that *Pgc-1α* mRNA expression was increased in response to PMQ treatment in C2C12 myotubes. Therefore, we consider that the anti-obesity and lipid-lowering effects of PMQ in part probably involve increased trafficking of NEFA into mitochondria for oxidation reaction via increased ACC phosphorylation and expression of genes related to fatty acid oxidation.

It is intriguing to compare the metabolic effects of PMQ with other insulin-sensitising agents such as rosiglitazone. Based on the data presented here, PMQ and rosiglitazone share a number of beneficial features, e.g. the improvement of serum glucose, lipid metabolism and insulin resistance. One of the major disadvantages of thiazolidinediones is that they lead to increased adiposity due to their stimulatory effects on adipocyte differentiation [44]. In this study, we also observed that rosiglitazone led to a slight increase of body weight, while PMQ had the opposite effects. These results suggest that PMQ may have a different action mechanism from that of rosiglitazone when used to treat metabolic disorders in MSG mice.

AMPK belongs to a family of serine/threonine protein kinases and is highly conserved from yeast through to humans. It consists of three subunits: a catalytic subunit (α) and two regulatory subunits (β and γ). In mammals, each subunit of AMPK contains two to three isoforms (α1, α2; β1, β2; γ1, γ2 and γ3) [7]. Thus far, several major regulating mechanisms involved in the activation AMPK are known, including the AMP:ATP ratio, upstream kinases, hormones and cytokines. Any metabolic stress that inhibits ATP production or accelerates ATP consumption will tend to increase the AMP:ATP ratio, with subsequent activation of AMPK. Several kinases have been shown to phosphorylate threonine 172 on the catalytic α-subunit of AMPK, leading to its activation. The first kinase is liver kinase B1 (LKB1), which is also known as serine/threonine kinase 11 (STK11), and is responsible for AMPK activation in most scenarios. The second kinase is calmodulin-dependent protein kinase-β (CaMKK-β), which phosphorylates AMPK, instead of AMP, in response to increases in intracellular Ca^{2+} levels. Other regulatory enzymes include TGF-β-activating kinase 1 (TAK1), ataxia-telangiectasia mutated (ATM) and protein phosphatase 2C (PP2C). AMPK can also be activated by hormones and cytokines, including leptin, adiponectin, interleukin-6 and ciliary neurotrophic factor (CNTF) [7, 45]. As a structural analogue of PMQ, quercetin was able to inhibit mitochondrial F0F1-ATPase activity and increase the AMP:ATP ratio, further activating AMPK [46]. Another study showed that quercetin activated AMPK by AMP-dependent and AMP-independent mechanisms [47]. Additionally, quercetin has been shown to activate AMPK via activation of reactive oxygen species generation and inositol-requiring enzyme 1α (IRE1α) RNase [48, 49]. The present data also show that PMQ can activate AMPK. The potential mechanisms involved in the activation of AMPK by PMQ remain to be further investigated.

In conclusion, we present for the first time evidence that PMQ displays beneficial effects in the treatment of metabolic disorders, at least in part via stimulation of AMPK activity (Fig. 8). Nevertheless, further mechanistic studies will need to be performed in order to understand how PMQ affects AMPK and FER, and hence metabolic disorders in MSG mice. It will be of future interest to clarify whether PMQ also improves metabolic disorders in other animal models or humans, and whether PMQ affects other metabolism-related signalling pathways.

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Duality of interest The authors declare that there is no duality of interest associated with this manuscript.

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