

REVIEW

Chlorogenic acid improves food allergy through the AMPK/ACC/CPT-1 pathway

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Abstract

Chlorogenic acid (CGA) is a polyphenol prevalent in daily food and plants. Food allergy (FA) can lead to metabolic disorders of the immune system. The objective of this study was to investigate CGA therapeutic effect on FA and regulatory mechanism through shrimp food allergy in mice models. Here, 24 female BALB/C mice were randomly allocated into the (I) Control group, (II) Food allergy group, (III) Chlorogenic acid low (50 mg/kg), and (IV) high group (200 mg/kg). Enzyme-linked immunosorbent assay revealed that CGA decreased levels of IgE and IgG induced by food allergy significantly. Real-time PCR demonstrated that high-dose chlorogenic acid significantly reduced Acetyl-CoA carboxylase (ACC) mRNA expression and increased Carnitine palmitoyltransferase-1 (CPT-1) mRNA expression. Western blot indicated that CGA promoted a noticeable increase at the levels of AMP-activated protein kinase (AMPK) and ACC phosphorylation, resulting in a significant activation in AMPK and inhibition in ACC, and increased CPT-1 expression. Consequently, CGA improves FA by the regulation of the AMPK/ACC/CPT-1 signaling pathway in the spleen.

Practical applications

Chlorogenic acid is a water-soluble polyphenolic substance that is widely distributed in natural plants that show a variety of pharmacological effects. At present, CGA has been developed as a weight-reducing tonic in western countries. As one of the most widely found and most easily obtained phenolic acids from food, the diverse physiological effects of CGA (such as anti-inflammatory, antioxidant, metabolic regulation, intestinal microbial regulation, etc.) imply its potential for application in functional foods, food additives, and clinical medicine. However, the basic molecular mechanisms of its effects have not been elucidated. In this study, CGA reduced allergy in a mouse model, likely by interacting with the AMPK/ACC/CPT-1 pathway.

KEYWORDS

acetyl-CoA carboxylase, AMP-activated protein kinase, carnitine palmitoyltransferase-1, chlorogenic acid, food allergy

Abbreviations: ACC, Acetyl-CoA carboxylase; AMPK, AMP-activated protein kinase; CGA, Chlorogenic acid; CGAH, Chlorogenic acid high dose; CGAL, Chlorogenic acid low dose; CK, Control Check; CPT, Carnitine palmitoyltransferase; DC, Dendritic cells; FA, Food Allergy; FAO, Fatty Acid Oxidation; IgE, Immunoglobulin E; IgG, Immunoglobulin G; MC, Mast cells; OVA, ovalbumin; P-ACC, Phosphorylation Acetyl-CoA carboxylase; P-AMPK, Phosphorylation AMP-activated protein kinase; SA, Shrimp Allergy.

1 | INTRODUCTION

The global incidence of food allergy (FA) is rising over the years, so research on effective treatments is necessary. FA mainly triggers type I allergic reactions mediated by immunoglobulin E (IgE), which is caused by ingestion of foods such as peanuts, tree nuts, milk, egg, soybean, wheat, shellfish, and fish (Yu et al., 2016). Moreover, type II allergic reactions mediated by immunoglobulin G (IgG) also occur but are not as common as IgE. FA is associated with a series of inflammation and metabolic changes in the immune system as well (Rodriguez-Coira et al., 2021). Some studies have shown that fatty acid metabolism is the core of immune regulation (Arita, 2016), while other studies have found that reprogramming of fatty acid oxidation (FAO) impacts the normal function of immune cells (Sun et al., 2021).

Spleen is the largest immune organ of the human body, which is the concentration region of mature and migration of various immune cells. AMP-activated protein kinase (AMPK) is a highly conserved serine/threonine protein kinase, prevalent in eukaryotic cells, and highly sensitive to changes in energy metabolism (Hardie, 2015). One of its functions is to promote FAO by acetyl-CoA carboxylase (ACC) phosphorylation and enhance carnitine palmitoyltransferase-1 (CPT-1) synthesis (Galic et al., 2018). AMPK plays an essential role in the metabolic regulation of immune cellular function, the silencing of AMPK expression resulted in the development of pro-inflammatory Th cells and enhanced capacity for antigen presentation by dendritic cells, and AMPK phosphorylation also prevents the production of fatty acid-induced inflammatory factors (Peixoto et al., 2017).

Plant polyphenols are a class of compounds with benzene rings and multiple hydroxyl groups, their antioxidant, anti-inflammatory, and metabolic regulation characteristics make them become potential active substances for the treatment of FA. Rutin, quercetin, rat tail, and chlorogenic acid (CGA) are representative polyphenols that can be ingested from food, which are relatively easier to be extracted and utilized by the human body. A phenolic acid compound in plant polyphenols (Lallemant et al., 2012), CGA is easier to be absorbed through the gastrointestinal membrane in the above several polyphenols, and its index of antioxidant biological acceptability is the highest (Xing et al., 2020). CGA has the properties of anti-inflammatory, lipid-lowering, immune regulation, and spleen protection (Naveed et al., 2018), which is a substantial polyphenol component of honeysuckle, green coffee beans, tea, and other plants (Wang & Clifford, 2008). ICR mice models demonstrated that CGA treatment rescued ear swelling and erythema, while reducing the expression of inflammatory cytokines (Luo et al., 2021). Importantly, there are studies have shown that CGA regulates lipid metabolism to inhibit endogenous lipid production by phosphorylating AMPK and enhancing the activity of CPT-1 (Kumar et al., 2020).

Given the relationship between fatty acid metabolism and immune regulation, we hypothesized that AMPK/ACC/CPT-1 fatty acid oxidation pathway is probably a potential therapeutic approach to FA. In addition, the anti-inflammatory and lipid-lowering properties of CGA make it possible to treat FA. However, the anti-allergic mechanism of CGA is poorly investigated, this study evaluated the antiallergic effect of CGA through the establishment of

shrimp food allergy in mice induced by the protein model, explored whether CGA treats FA by influencing the AMPK signaling pathway in the spleen and discussed regulating function of CGA on FAO in the spleen of FA mice. This study aims at providing theoretical bases for the CGA treatment on FA and the development of related functional food.

2 | MATERIALS AND METHODS

2.1 | Materials and reagents

CGA (purity $\geq 98\%$, CAS: 327-97-9) was purchased from Macklin Co (Pudong New Area, Shanghai, China). *Litopenaeus vannamei* were purchased from a local supermarket (Haidian District, Beijing, China). Bovine serum albumin was purchased from Sigma-Aldrich Co (St. Louis, Missouri, USA). Protein quantitative kit was purchased from Beijing Cowin Biotech Co, Ltd (Haidian District, Beijing, China). Ultrapure RNA extraction reagent was purchased from TaKaRa Bio Inc (Kyoto, Japan). Reverse transcription TransScript II Reverse, qPCR kit was purchased from Beijing TransGen Biotech Co, Ltd (Haidian District, Beijing, China). Real-time PCR primers were synthesized by Beijing Liuhe Huada Gene Technology Co, Ltd (Daxing District, Beijing, China). Primary antibodies AMPK α 1, ACC, P-AMPK, and P-ACC were purchased from Cell Signaling Technology (CST) Co (Danvers, Massachusetts, USA). Primary antibody CPT-1 was purchased from Abcam Co (Cambridge, UK).

2.2 | Animals and ethical statement

Female BALB/c mice (SPF), 4–5 weeks old, weighing 18–22 g were procured from Charles River Co (Beijing, China).

All procedures in this study were performed in compliance with relevant laws and institutional guidelines, Animal Ethics Committee at China Agriculture University has approved the mice experimental protocol (animal ethics number: RD-20172041-1).

2.3 | Preparation of shrimp protein

Wash and dry *Litopenaeus vannamei* shrimps that were obtained from a local market in Beijing with sterile water, then shell, and remove the heads, tails, and intestines. Shrimp muscle was cut into small pieces with eye scissors and ground in liquid nitrogen until powdered. Respectively, 1 μ l protease inhibitor, 10 μ l phosphatase inhibitor, and 5 μ l 100mM PMSF were added into the precooled 1 ml Lysis buffer. Leave the mixture on ice for a few minutes. Briefly, 100mg powdered material was mixed thoroughly with the cold mixture in a 1.5 ml precooling centrifuge tube. After 2 h in a 4°C refrigerator, the sample was centrifuged at 10,000rpm for 5 min at 4°C, and the supernatant was taken into the other precooling centrifuge tube as protein extracts. Protein extracts were stored at -80°C . Repeated freezing and thawing has been avoided.

2.4 | Female BALB/c experiment

The 4-week-old female BALB/c mice were fed for 1 week under the freedom of taking food and water. During the experiment, the weight, and growth of the mice were observed weekly. The ambient humidity was controlled at 40%–70%, and the environment temperature was controlled at $23 \pm 3^\circ\text{C}$.

The animals were randomly allocated into four groups according to their body weight, namely the negative control group (CK), chlorogenic acid low-dose group (CGAL), chlorogenic acid high-dose group (CGAH), and shrimp protein allergy group (SA), with six animals in each group.

The sensitization method by J. Sun et al. was used with minor modifications (Sun et al., 2013). On days 0–42, SA, CGAL, and CGAH were orally administered shrimp-allergic protein (1 mg/ml, 0.25 ml/mouse, mixed with aluminum hydroxide in a 3:1 proportion). CK was orally administered PBS (0.25 ml/mouse). The mice in CK and SA were taken blood on the 35th day to measure IgE and IgG1 levels to determine whether the model was established successfully. The mice in CK and SA were given a challenge with a high dose of shrimp-allergic protein (2 mg/ml, 0.25 ml/mouse) to stimulate the allergic reaction on day 42. Rectal temperature was measured, and the allergic symptoms are shown in Table 1.

After 30 min of antigen stimulation, the blood of mice in each group was drawn from the inner canthus venous plexus and collected in the centrifuge tube with the anticoagulant. After standing for 2 h, the blood samples were centrifuged at 5000 rpm for 10 min at 4°C . Serums were separated and stored at -20°C . The mice were sacrificed after anesthesia. The spleens were removed from the mice and stored at -80°C after liquid nitrogen quick freezing.

From days 42 to 56, CGAL was given 50 mg/kg, CGA and CGAH were given 200 mg/kg CGA daily, and both received allergen challenge with shrimp allergic protein (1 mg/ml, 0.25 ml/mouse) 30 min after the treatment (Dong et al., 2020). All mice in the CGA groups were given a high dose of shrimp allergy protein (2 mg/ml, 0.25 ml/mouse) to stimulate the allergic reaction on day 56, and the subsequent process was the same as in SA and CK.

2.5 | Enzyme-linked immunosorbent assay

The specific IgG1/IgE levels were detected by ELISA, as an earlier study described (Lv et al., 2021). The extracts of shrimp protein

were enveloped in 96-well microtiter plates (100 μl /well). After being coated overnight (4°C) and washed (5 times) with 300 μl TBST, the plates were blocked (37°C , 2.5 h) with a TBST solution of 5% skim milk. Following the blocking step, the plates were rinsed (5 times with 300 μl TBST) and further added the mouse serum diluted well in 30 μl of 1% skim milk (IgE, 1:10; IgG1, 1:800), then incubated at 37°C for 2 h. After the rinsing step (5 times with 300 μl TBST), the plate was incubated at 37°C for 1.5 h with antimouse IgE HRP-labeled antibody (1:5000, 100 μl /well) and antimouse IgG1 HRP-labeled antibody (1:8000, 100 μl /well) both diluted in 1% skim milk. Following the final rinsing step, the plates were incubated at 37°C for 20 min after adding TMB (100 μl /well). The reaction was stopped with 2 M sulfuric acid. The absorbance data of each well was measured at 450 nm wavelengths. Each sample was tested in a triple.

2.6 | RNA isolation and real-time PCR

Total RNA was obtained from 20 to 30 mg of the spleen tissue according to the kit procedure, as the previous study described (Gong et al., 2020). The quality and concentration of total RNA were detected by a nucleic acid protein analyzer. The integrity of RNA was detected by 5 μl RNA electrophoresis with 1% agarose gel. The A260/A280 ratio was about 2.0, and the RNA content was calculated. According to the procedure of the reverse transcription kit, genomic DNA was removed from 0.5 μg total RNA, and cDNA was synthesized by reverse transcription. Based on the sequences provided by GeneBank, primers (ACC: NM_022193, AMPK α 1: NM_019142, CPT-1: NM_013200) (Table 2) were designed to be synthesized by Beijing Liuhe Huada Gene Technology CO, Ltd. AMPK α 1, ACC, and CPT1 genes were detected by real-time PCR. The reaction system was set to 20 μl , and the PCR cycling conditions were 1 cycle (95°C 30 s) and 40 cycles (95°C 5 s $\rightarrow$ 60°C 30 s). After the reaction, the relative expression of mRNA was analyzed by the $2^{-\Delta\Delta\text{CT}}$ approach, which was standardized with β -actin as an internal reference.

2.7 | Western blot analysis

Western blot analysis was followed as the previous method with slight modifications (Gao et al., 2018). Spleen tissue was routinely crushed

TABLE 1 Standard of food allergy assessment

Score	Symptom
0	no symptoms
1	scratching and rubbing around the nose and head
2	puffiness around the eyes and mouth, pilar erection, reduced activity, and/or decreased activity with increased respiratory rate
3	wheezing, labored respiration, and cyanosis around the mouth and the tail
4	no activity after prodding or tremor and convulsion
5	death

(RIPA + PMSF + phosphatase inhibitors). The protein concentration was assigned by the BCA method. Proteins in 8% SDS-PAGE gel were moved onto the PVDF membrane. TBST with 5% skim milk was used to preserve membranes for 2 h, and then each membrane was incubated overnight at 4°C with anti-pAMPK (1:1000), anti-AMPK (1:1000), anti-pACC (1:1000), anti-ACC (1:1000), and anti-CPT-1 (1:1000). The membranes were rinsed six times (5 min each time) with TBST after overnight. HRP-labeled antirabbit IgG antibody (1:8000) was added to membranes and incubated for 2 h. Then, membranes were washed six times with TBST (5 min each time). Consequently,

TABLE 2 Sequences of primers used for real-time PCR

Gene	Primer sequences (5'-3')
β -Actin	Forward: CAC TAT CGG CAA TGA GCG Reverse: AGG AGC CAG GGC AGT AAT C
ACC	Forward: CGC CTC TTC CTG ACA AAC G Reverse: TGA GCG ACT GCC GAA ACA TC
AMPK α 1	Forward: CCC CTC AGT ATT TGA ATC CCT C Reverse: GGT CCT GGT GGT TTC TGT TGT AG
CPT-1	Forward: GTG CTG GAG GTG GCT TTG G Reverse: CAG TGC TTG ACG GAT GTG G

membranes were added HRP-DAB-enhanced color solution for coloration in a dark environment. Finally, the protein bands were detected with the ECL imaging system. Image analysis software (ImageJ) was used to quantify the area and gray level of protein bands. Protein expression was expressed in relative grayscale.

2.8 | Statistical analysis

All results are expressed as the mean $\pm$ SE. Data were analyzed by one-way ANOVA or *t* test by using GraphPad Prism 8.0 software. *p* < .05 was considered statistically significant.

3 | RESULTS

3.1 | Successful modeling of food allergy

We established the SA model of mice by intragastric administration of shrimp protein solution. The mice with an allergic constitution produce a large amount of IgE after allergen stimulation, which is an important biological indicator in type I hypersensitivity, and the

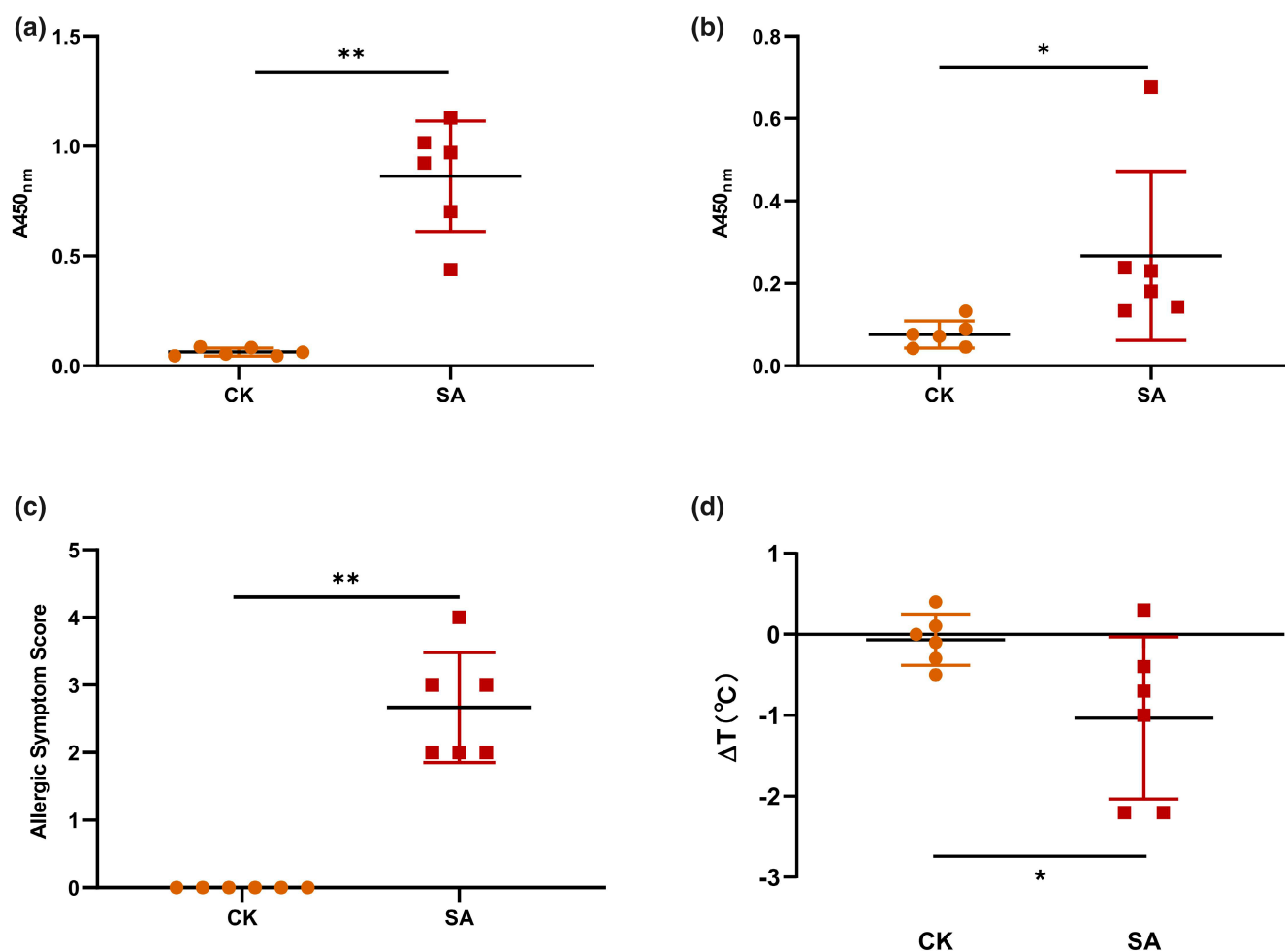


FIGURE 1 (a) Effect of FA on immunoglobulin E in serum of SA mice. (b) Effect of FA on immunoglobulin G in serum of SA mice. (c) Food allergy symptom score of BALB/c. (d) rectal temperature. **p* < .05 vs SA and ***p* < .01 vs SA

increase in its concentration promotes the generation of allergic symptoms. IgG is a crucial antibody that causes cell dissolution and tissue damage in type II hypersensitivity.

We collected blood on the 35th day to determine the mass concentrations of IgE and IgG1 in the CK group and SA group, and the results were analyzed by absorbance. As shown in Figure 1, the levels of IgE and IgG1 in the SA group were meaningfully higher than those in the CK group ($p < .05$), and allergic symptom increase and temperature decline also indicated that the food-allergic mice sensitized by shrimp protein were successfully established.

3.2 | Therapeutic effect of CGA on SA mice

In order to evaluate the therapeutic effect of CGA on food-allergic mice, we measured the temperature change of each group after the antigen challenge and scored the FA symptoms. As shown in

Figure 2c,d, the allergic symptom and temperature decline in the CGA group were considerably decreased compared with the SA group.

The blood samples were collected to determine the mass concentrations of IgE and IgG1, and the results were analyzed by absorbance. The mice that received two different doses of CGA significantly decreased the concentrations of IgE and IgG1 Compared with the SA group, and the inhibitory effect of CGA on the two immunoglobulins was dose dependent (Figure 2).

3.3 | Effects of food allergy on mRNA transcription levels of AMPK α 1, ACC, and CPT-1 in the spleen

Variations in the mRNA levels of AMPK/ACC/CPT-1 were investigated by qRT-PCR. In the spleen tissue, FA significantly inhibited FAO compared with the CK group, as shown by the decline in AMPK α 1 and CPT-1 levels and the increase in ACC level ($p < .05$) (Figure 3).

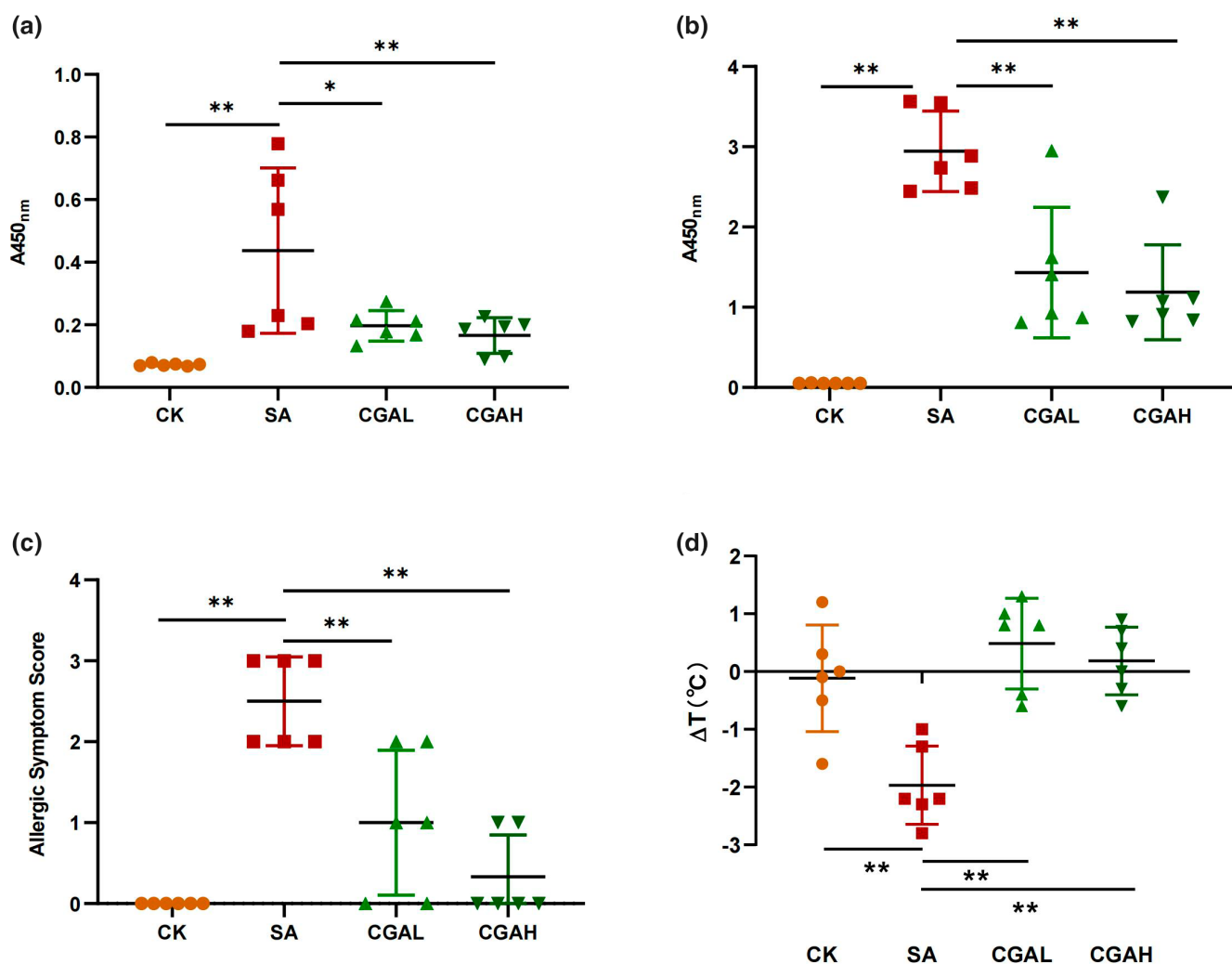


FIGURE 2 (a) Effect of CGA on immunoglobulin E in serum of SA mice, (b) effect of CGA on immunoglobulin G in serum of SA mice, (c) food allergy symptom score of BALB/c, (d) rectal temperature. * $p < .05$ vs SA and ** $p < .01$ vs SA

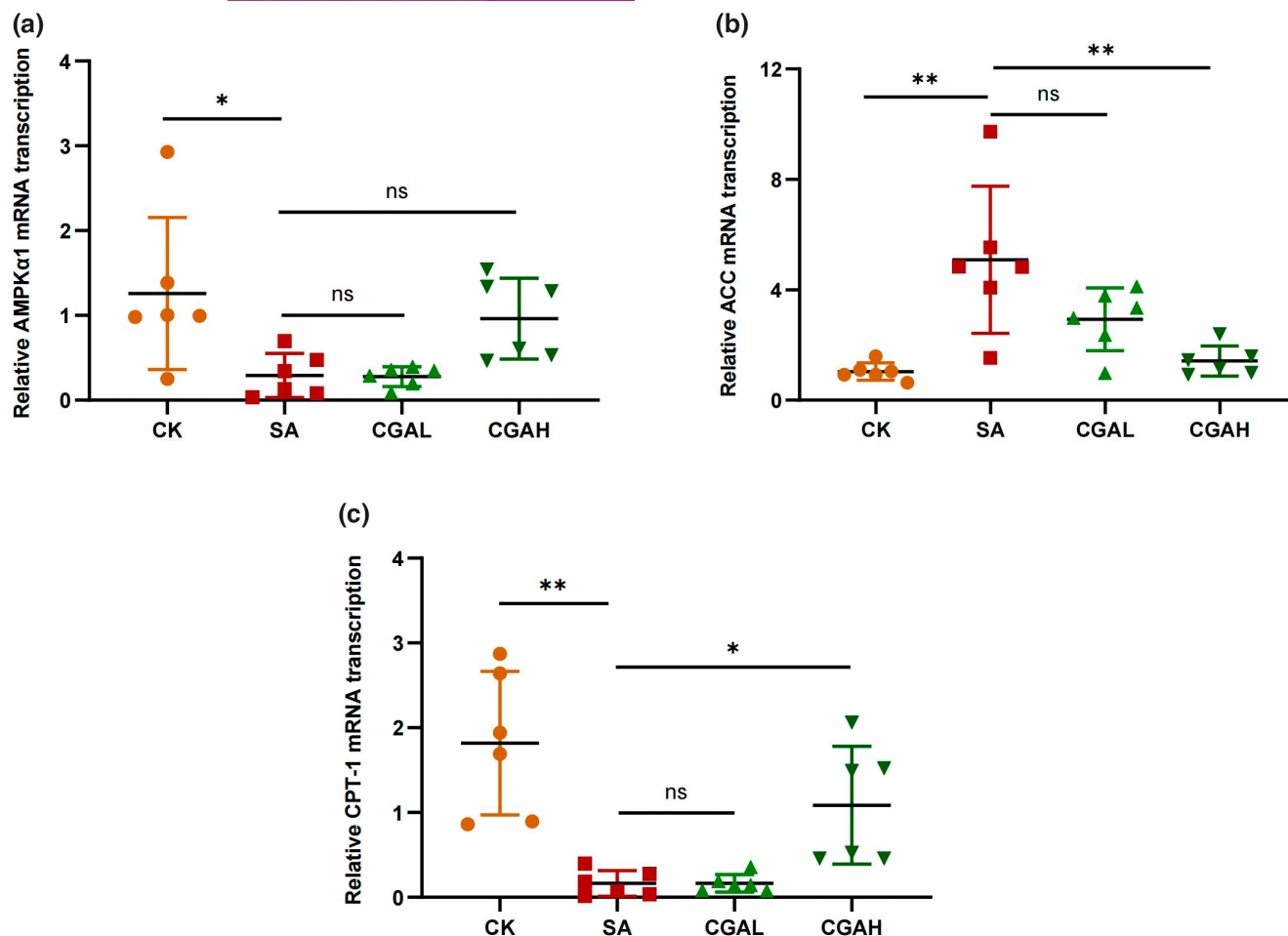


FIGURE 3 (a) Effect of CGA and FA on AMPK α 1 mRNA level in the spleen of SA mice, (b) effect of CGA and FA on ACC mRNA level in the spleen of SA mice, (c) effect of CGA and FA on CPT-1 mRNA level in the spleen of SA mice. * $p < .05$ vs SA and ** $p < .01$ vs SA

3.4 | Effect of food allergy on AMPK α 1/ACC/CPT-1 pathway protein expression in the spleen

In the spleen tissue, the protein expressions of P-AMPK, P-ACC, and CPT-1 in the SA group were significantly inhibited ($p < .01$) compared with those in the CK group (Figure 4). The current results demonstrated that FA led to the metabolic disorder of the AMPK-guided FAO pathway in the spleen.

3.5 | Effects of CGA on mRNA levels of AMPK α 1, ACC, and CPT-1 in the spleen of SA mice

The mRNA levels of AMPK α 1 and CPT-1 in the CGAL group were not significantly different from the SA group. The mRNA level of ACC in the CGAL group was lower than that in the SA group, with no difference from food-allergic mice ($p \geq .05$).

As shown in Figure 3, although there was no significant upsurge in the AMPK α 1 mRNA level of the CGAH group compared with the SA group ($p \geq .05$), an increasing trend was still found. In contrast, the CPT-1 mRNA level in the CGAH group was significantly higher than that in the SA group, and ACC mRNA levels also significantly decline in the CGAH group ($p < .05$).

3.6 | Effect of CGA on the AMPK α 1/ACC/CPT-1 pathway protein expression in the spleen

The protein expression of P-AMPK, P-ACC, and CPT-1 showed a noticeable effect after CGA treatment (Figure 5). CGA increased the phosphorylation level of ACC by increasing the phosphorylation level of AMPK and promoted the expression of CPT-1 to regulate the FAO in the spleen.

In the spleen tissue, the CGAH group significantly increased the protein expression of P-AMPK and CPT-1 ($p < .01$), whereas the CGA group significantly increased the protein expression of P-ACC compared with the SA group ($p < .05$).

4 | DISCUSSION

In this study, we established a mice food-allergic model with an allergen of shrimp protein. We mainly explored the therapeutic effect of CGA on food-allergic mice and its intervention effect on the AMPK/ACC/CPT-1 pathway in the mouse spleen. On the one hand, food-induced allergies are mainly manifested in the immune-mediated allergic reactions that occur after the body receives the corresponding food allergen stimulation. The reaction mainly shows

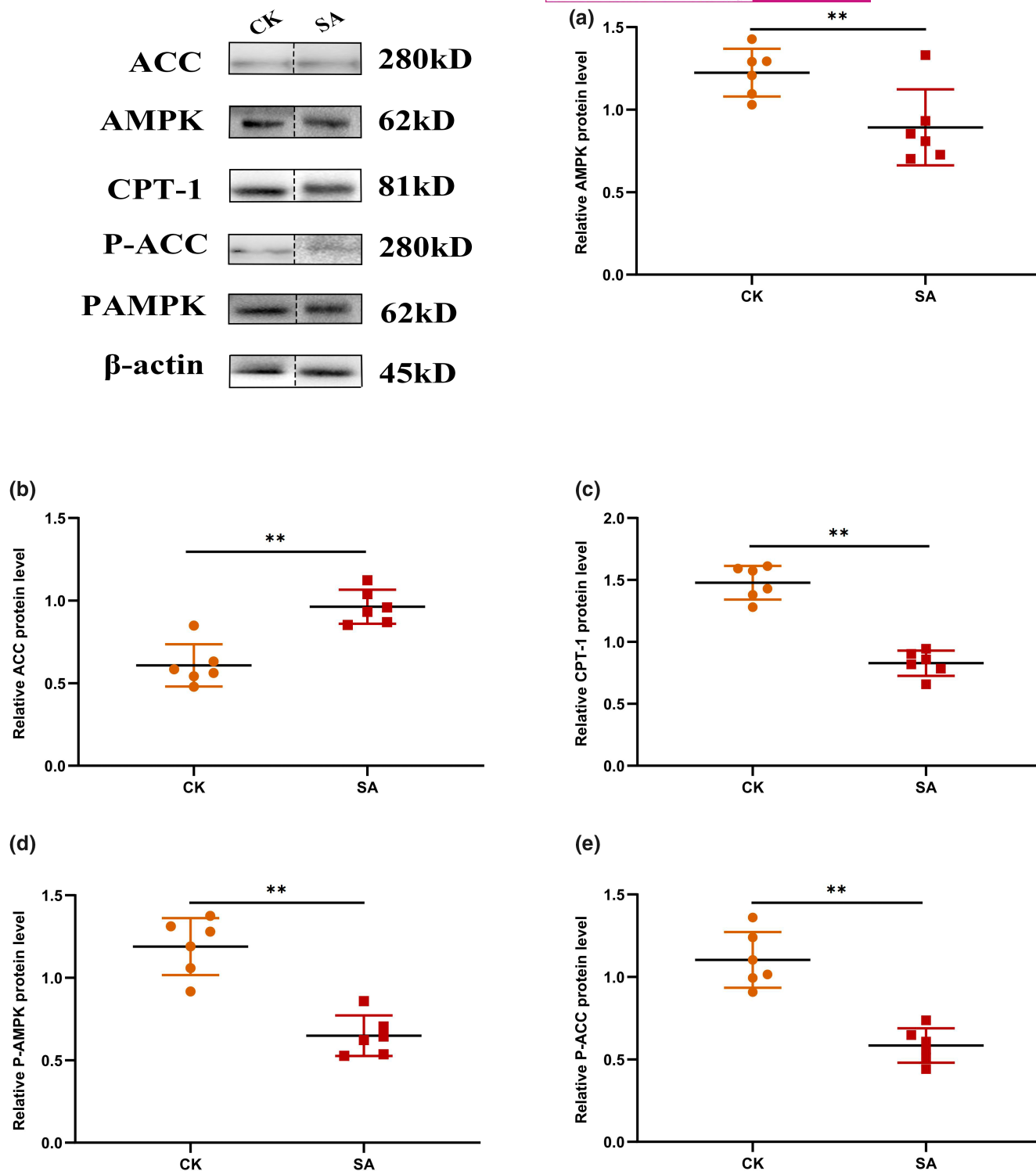


FIGURE 4 Effect of FA on the AMPK, ACC, CPT-1, P-AMPK, and P-ACC levels in the spleen of SA mice. The quantified results of AMPK (a), ACC (b), CPT-1 (c), P-AMPK (d), and P-ACC (e) expression were measured by western blot. * $p < .05$ vs SA and ** $p < .01$ vs SA. Dotted demarcations in blots represent bands spliced from different original blots. Declare: All blots in Figure 4 are consistent with CK and SA groups in Figure 5

an increase in the content of immunoglobulins (such as IgE and IgG), which promotes a large number of pro-inflammatory factors (TNF- α , IL-1 β , IL-6, etc.) and histamine from mast cells (MC). In this study, the relative expression levels of IgE and IgG1 in the CGA group and FA group can prove that CGA has a therapeutic effect on FA, which is

consistent with the research results by others (Lu et al., 2018; Zhang et al., 2021). We verified the improvement of CGA on immunity, especially in FA. On the other hand, the rate-limiting enzyme involved in FAO is CPT-1, which is inhibited by malonyl-CoA formed by ACC catalyzes acetyl-CoA. It catalyzes fatty acid to be transported to

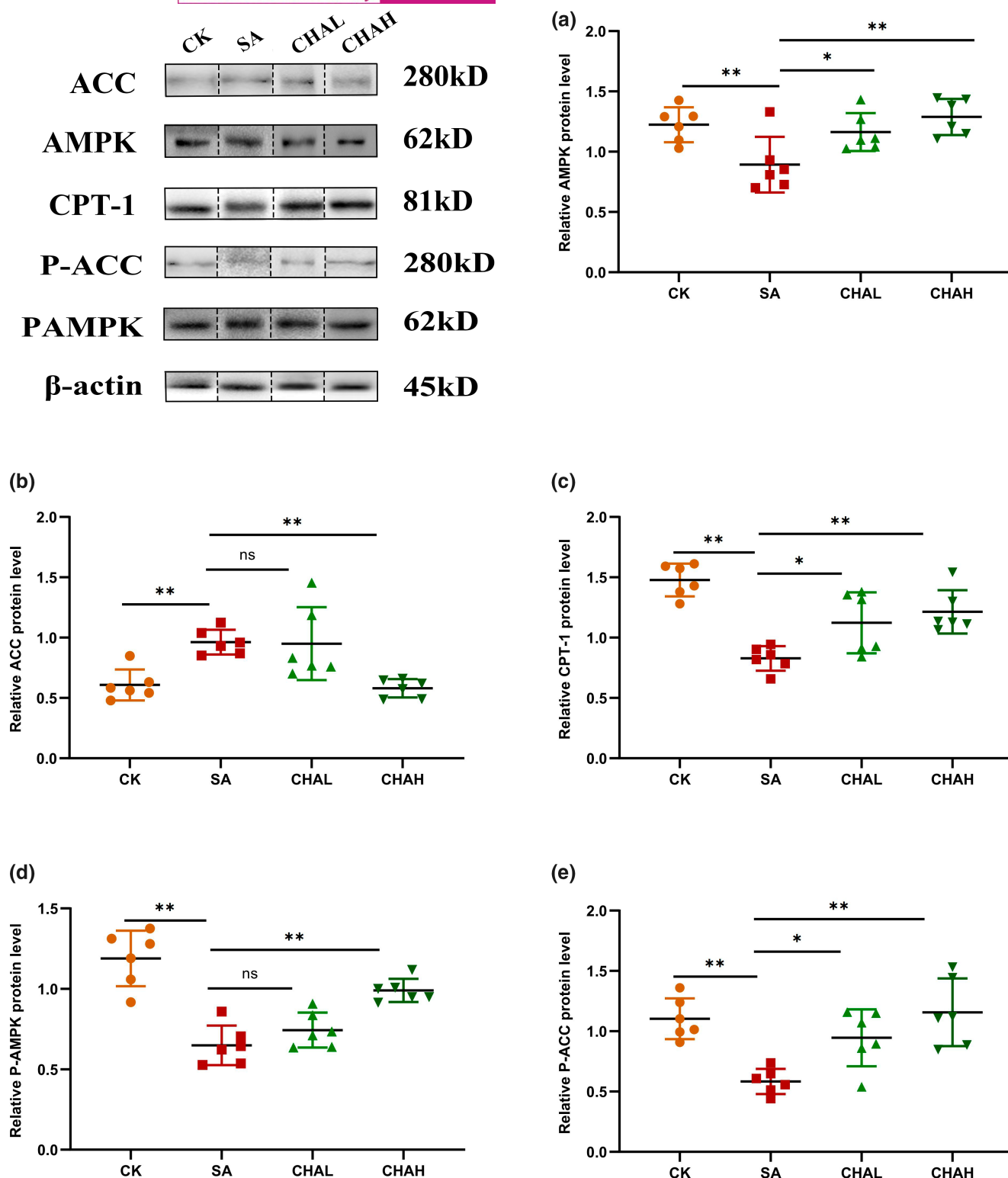


FIGURE 5 Effect of CGA on the AMPK, ACC, CPT-1, P-AMPK, and P-ACC level in the spleen of SA mice. The quantified results of AMPK (a), ACC (b), CPT-1 (c), P-AMPK (d), P-ACC, and (e) expression measured by western blot. * $p < .05$ vs SA and ** $p < .01$ vs SA. Dotted demarcations in blots represent bands spliced from different original blots

mitochondria. ACC phosphorylation can reduce the formation of malonyl-CoA by upregulating the phosphorylation of AMPK, thereby relieving the ACC inhibitory effect on CPT-1 and promoting the transport of fatty acids and its oxidation. This study found that

CGA can inactivate ACC by phosphorylating AMPK and enhance the expression of CPT-1 while promoting FAO, especially at high doses.

FA affects 6%–8% of children and 2%–5% of adults currently with increasing incidence, which has caused various troubles in people's

quality of life and nutritional intake (Iweala et al., 2018). There are some problems with the current food-allergic immunotherapy, such as unsatisfactory desensitization persistence, great individual differences, high cost, and so on (Long et al., 2020). Some research have shown that metabolism is of great significance to the immune homeostasis of allergic diseases, such as intestinal flora metabolism (Luu et al., 2020), fatty acid metabolism (Trinchese et al., 2015), and glucose metabolism (Vanherwegen et al., 2019). Similarly, it was reported that cellular fatty acid in mast cells increased after IgE-mediated stimulation, while lack of fatty acid turnover from peroxisomes functions at rest and during IgE-mediated activation (Meghnam et al., 2022). Metabolic reconstitution is expected to become a new way to treat FA, while better meeting the demands of different allergic patients.

CGA, which is a polyphenolic acid extensively exists in natural diets, not only has the effect of relieving inflammation, but also hypoglycemic and cholesterol-lowering effects. In the influence of CGA on tissues, CGA inhibited the NF- κ B signaling pathway by downregulating CD14 so that it decreased the secretion of pro-inflammatory cytokines and maintains the integrity of the intestinal barrier (Yu et al., 2021), but the effect of chlorogenic acid on the AMPK pathway in intestinal related lymphoid tissue has not been found so far. Additionally, a recent report of CGA anti-inflammation on the liver showed that CGA reduced the hepatic mRNA expression of some pro-inflammatory molecules in mice and alleviated acetaminophen-induced liver inflammatory injury (Hu et al., 2020). Similar to the present study, symptoms of allergic rhinitis mice induced by ovalbumin (OVA) were ameliorated by CGA regulation on Th17 cells, with a decline in the serum levels of IgE and nasal mucosa of histopathological abnormalities (Shi et al., 2020).

Moreover, CGA could regulate fatty acid metabolism by phosphorylating AMPK and ACC in the liver (Lukitasari et al., 2018). In addition, previous studies also demonstrated that polyhydroxy phenolic acid (curcumin, sagerphenol, astragalus polysaccharide) is able to treat metabolic diseases by activating AMPK (Campbell et al., 2019; Liu et al., 2013). Thus, studying the regulation of CGA on AMPK-related pathways is greatly significant to explore the mechanism of dietary active compounds in the treatment of FA.

AMPK is a "cellular energy regulator" that is widely found in active tissues, and it will become a new drug target for metabolic diseases (Trefts & Shaw, 2021). ACC is the downstream target protein of AMPK to regulate fatty acid metabolism. AMPK can promote the synthesis of CPT-1 by inhibiting the activity of ACC, thus induce FAO and eliminate lipid accumulation, which has been confirmed in liver, skeletal muscle, and adipose tissue (Zhang et al., 2019). The spleen is the critical organ to regulate the immune system, AMPK could regulate the proportion of CD4+CD44+T cells in the spleen and participate in anti-inflammatory immunity (Peng et al., 2019). Moreover, the role of AMPK in anti-inflammation and regulation of the innate immune response has also been demonstrated by several studies (Silwal et al., 2018), specifically, an OVA-induced asthma mice model for ligustrazine antiallergy evaluation indicated that an increase of p-AMPK expression level resulted in a decrease of NF-kappaBp65

expression and alleviated airway inflammation (Xu et al., 2021). An in vitro experiment showed that FAO was stimulated by AMPK on ACC phosphorylation, which changed the mitochondrial dysfunction and cell metabolic function of dendritic cells, thereby regulating innate and pathological Th2 and Th17 immune response (Elesela et al., 2020). Significantly, this suggests that fatty acid oxidation regulated by AMPK in relevant immune tissues can regulate the inflammatory response of dendritic cells (DC) caused by FA.

The development of CGA-related products is based on a clear molecular mechanism of CGA function and target validation, but they are insufficient for current CGA research (Wang et al., 2020). In this research, we did not block and activate the signaling pathway, and there is a potential limitation in the mice model regarding allergy response in humans. We still need to continue to explore the effect of CGA on the AMPK/ACC/CPT-1 signaling pathway in immune-related tissues and cells, such as small intestine, DC, MC, macrophages, etc., for providing human allergic treatments with more useful experimental data.

5 | CONCLUSION

In conclusion, FA is associated with immune metabolic disorders, and the main task for related research is to explore FA treatment. In this study, we clarified the therapeutic effect and mechanism of CGA intervention on food allergy. The results show that CGA could effectively treat FA and inhibit the occurrence of FA by regulating the metabolism of FAO in the spleen. This regulatory mechanism may be achieved by the AMPK/ACC/CPT-1 signaling pathway. This study provides a theoretical basis for the further development of CGA-related products and encourages us to find more dietary polyphenols that can improve FA and explore their mechanism of them.

AUTHOR CONTRIBUTIONS

Ze Yun: Conceptualization; methodology; visualization; writing – original draft; investigation. **Zhiying Zou:** Conceptualization; writing – original draft; visualization. **Shanfeng Sun:** Investigation. **Huilian Che:** Resources; project administration; funding acquisition; supervision.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

Research data are not shared.

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