Effects of Phytol on Metabolic Enzyme Activity and Muscle Fiber Type in Mouse Skeletal Muscle Postprint

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Abstract

This study aimed to investigate the effects of dietary phytol supplementation on metabolic enzyme activities and muscle fiber types in mouse skeletal muscle. Forty 3-week-old male Kunming mice were randomly assigned to 4 groups (n=10 per group) and individually housed. The experimental diets were supplemented with 0 (control group), 0.05%, and 0.50% phytol, with 0.48% stearic acid supplementation serving as a positive control. The experimental period lasted 28 days. Measurements included average body weight, relative gastrocnemius muscle weight, activities of succinate dehydrogenase (SDH), hexokinase (HK), and lactate dehydrogenase (LDH), as well as triglyceride (TG) content in the gastrocnemius muscle; muscle fiber types were analyzed via ATPase staining. The results showed: 1) Compared with the control group, dietary supplementation with 0.50% phytol significantly reduced average body weight during the first 2 weeks of the experiment ($P<0.05$) and significantly increased relative gastrocnemius muscle weight ($P<0.05$). 2) Compared with the control group, 0.50% phytol supplementation significantly decreased TG content ($P<0.05$) and significantly increased HK activity ($P<0.05$) in the gastrocnemius muscle; 0.05% phytol supplementation significantly increased SDH activity ($P<0.05$) in the gastrocnemius muscle. 3) Compared with the control group, 0.50% phytol significantly increased the proportion of type I muscle fibers in the gastrocnemius muscle ($P<0.05$). In summary, dietary phytol supplementation can increase the relative weight of mouse gastrocnemius muscle, enhance SDH and HK activities in the gastrocnemius muscle, and elevate the proportion of type I muscle fibers.

Full Text

Effects of Phytol on Metabolic Enzyme Activity and Muscle Fiber Type in Mouse Skeletal Muscle

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Abstract: This study investigated the effects of dietary phytol supplementation on metabolic enzyme activity and muscle fiber type in mouse skeletal muscle. Forty 3-week-old male Kunming mice were randomly assigned to four groups (n=10 per group) and housed individually. The experimental diets were supplemented with 0 (control), 0.05%, and 0.50% phytol, with 0.48% stearic acid serving as a positive control. Parameters measured included average body weight, relative gastrocnemius weight, activities of succinate dehydrogenase (SDH), hexokinase (HK), and lactate dehydrogenase (LDH), triglyceride (TG) content in the gastrocnemius, and muscle fiber types via ATPase staining. The 28-day trial demonstrated that: (1) Compared with the control group, 0.50% phytol supplementation significantly reduced average body weight during the first two weeks ($P<0.05$) and significantly increased relative gastrocnemius weight ($P<0.05$). (2) Dietary supplementation with 0.50% phytol significantly decreased TG content ($P<0.05$) and increased HK activity ($P<0.05$) in the gastrocnemius, while 0.05% phytol significantly elevated SDH activity ($P<0.05$). (3) The 0.50% phytol group showed a significantly higher proportion of type I muscle fibers in the gastrocnemius compared with the control group ($P<0.05$). These results indicate that dietary phytol supplementation can increase relative gastrocnemius weight, enhance SDH and HK activities, and elevate the proportion of type I muscle fibers in mouse gastrocnemius.

Keywords: phytol; gastrocnemius; muscle fiber type; ATPase staining

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Recent studies have demonstrated that muscle fiber type directly determines numerous meat quality indicators including color, tenderness, and intramuscular fat content, representing a core factor influencing meat quality [1-3]. Consequently, investigating the nutritional regulation of muscle fiber types holds significant theoretical and practical value for meat quality improvement. Research on pork color scoring revealed a positive correlation between the proportion of oxidative (type I) muscle fibers and a* values in meat color assessment [4]. Leseigneur et al. [5] found that oxidative muscle fibers contain higher phospholipid content than glycolytic fibers, and muscles with high type I fiber content exhibit greater intermuscular fat, superior flavor, and enhanced juiciness, whereas muscles rich in type IIb fibers demonstrate poorer meat quality and may even develop PSE (pale, soft, exudative) meat. De Souza et al. [6] confirmed that peroxisome proliferator-activated receptor (PPAR) agonists increase PPAR expression specifically in slow-twitch (type I) muscles without affecting fast-twitch (type II) muscles. Russell et al. [7] discovered that elevating PPAR expression and mitochondrial fatty acid oxidation enzyme activity in skeletal muscle can induce fiber type transition from type II to type I. Recent findings have established a close relationship between phytol and PPAR activation, with phytol acting as a direct ligand to activate PPAR . Luciferase reporter gene assays demonstrated that phytol significantly activates PPAR , with an activation effect four times greater than phytanic acid [8]. An et al. [9] reported that dietary phytol supplementation in mice activates PPAR , thereby ameliorating obesity-induced metabolic disorders. Based on these findings, this study aims to investigate the effects of dietary phytol supplementation on muscle metabolism-related enzyme activities and muscle fiber type transitions in mice.

1.1 Experimental Animals and Diets

Three-week-old male Kunming mice were purchased from the Guangdong Provincial Medical Laboratory Animal Center. The basal diet was provided by the same center, with composition and nutrient levels shown in Table 1 . The basal diet was ground and supplemented with 0, 0.05%, or 0.50% phytol, and 0.48% stearic acid for the positive control group. After thorough mixing, the diets were re-pelleted and dried at 37°C.

Analytical-grade phytol was purchased from Ruiding Chemical (Shanghai) Co., Ltd. Detection kits for triglycerides (TG), hexokinase (HK), lactate dehydrogenase (LDH), and succinate dehydrogenase (SDH) were obtained from Nanjing Jiancheng Bioengineering Institute.

1.3 Experimental Design and Husbandry

Following an acclimation period, forty 3-week-old male Kunming mice (13±1 g) were divided into four groups and housed individually. The control group received the basal diet, the positive control group received the basal diet supplemented with 0.48% stearic acid, and the two experimental groups received diets containing 0.05% and 0.50% phytol, respectively. Mice were maintained under natural lighting conditions with ad libitum access to feed and water throughout the 28-day experimental period.

1.4 Sample Collection

At the conclusion of the experiment, the gastrocnemius and extensor digitorum longus muscles were dissected, blotted dry with filter paper, weighed, placed in centrifuge tubes, and immediately immersed in liquid nitrogen. Samples were subsequently transferred to -80°C for storage. Prior to analysis, frozen gastrocnemius samples were thawed and gently rinsed with ice-cold 0.86% saline, blotted dry, and weighed. Tissue was transferred to 5 mL centrifuge tubes with nine volumes of ice-cold 0.86% saline (w/v), minced on ice, and homogenized. The resulting 10% tissue homogenate was centrifuged at 3,000 rpm for 15 minutes at low temperature. The supernatant was aliquoted and stored at -20°C until analysis.

1.5.1 Growth Performance Measurement

Mice were weighed individually on a weekly basis to calculate average body weight for each group.

1.5.2 Determination of Relative Gastrocnemius and Extensor Digitorum Longus Weight

Prior to slaughter at the end of the experiment, body weight was recorded. Following slaughter, the gastrocnemius and extensor digitorum longus muscles were dissected, blotted dry, and weighed to calculate relative muscle weights using the following formulas:

Relative gastrocnemius weight (%) = (gastrocnemius weight / body weight) × 100
Relative extensor digitorum longus weight (%) = (extensor digitorum longus weight / body weight) × 100

1.5.3 Detection of Metabolic Biochemical Indicators in Gastrocnemius

Colorimetric assays were employed to determine TG content and activities of HK, LDH, and SDH in the gastrocnemius. All measurements were performed according to kit instructions using a microplate reader (Gene5 Multi-Mode Microplate Reader, BioTek, USA).

1.5.4 Histological Determination of Muscle Fiber Type

Muscle fiber types were determined following the enzyme histochemistry method of Ashmore et al. [10]. For section preparation, gastrocnemius samples for histochemical analysis were gradually warmed from liquid nitrogen to -80°C, then to -30°C before cryosectioning perpendicular to muscle fiber orientation (freezing microtome, Leica, Germany). The cryostat head temperature was set at -20°C, chamber temperature at -25°C, and section thickness at 10 m, with five sections prepared per sample.

Using the five-point method on each section, type I fibers stained dark blue, type IIa light blue, and type IIb white. Fibers were observed and photographed under a microscope (×100 magnification), and the proportions of each fiber type were analyzed using Motic Images Advanced 3.2 software.

1.6 Statistical Analysis

Data were analyzed using one-way ANOVA in SPSS 18.0 software, with Duncan' s multiple range test applied for post-hoc comparisons. Results are expressed as mean ± standard error (S.E.), with $P<0.05$ considered statistically significant.

2.1 Effects of Phytol on Average Body Weight of Mice

As shown in Table 2 , supplementation with 0.50% phytol significantly reduced average body weight during the first two weeks compared with the control group ($P<0.05$), after which body weight gradually normalized.

2.2 Effects of Phytol on Relative Weight of Gastrocnemius and Extensor Digitorum Longus

Table 3 demonstrates that dietary supplementation with both 0.05% and 0.50% phytol significantly increased relative gastrocnemius weight compared with the control group ($P<0.05$), while no significant effects were observed on relative extensor digitorum longus weight ($P>0.05$).

2.3 Effects of Phytol on TG Content and Metabolic Enzyme Activities in Gastrocnemius

According to Table 4 , dietary supplementation with 0.50% phytol significantly decreased TG content ($P<0.05$) and increased HK activity ($P<0.05$) in the gastrocnemius compared with the control group. Supplementation with 0.05% phytol significantly elevated SDH activity ($P<0.05$). No significant effects of phytol supplementation were observed on LDH activity ($P>0.05$), and the 0.48% stearic acid positive control showed no significant influence on any of these metabolic parameters ($P>0.05$).

2.4 Effects of Phytol on Muscle Fiber Type in Gastrocnemius

Figure 1 [Figure 1: see original paper] and Table 5 reveal that dietary supplementation with 0.50% phytol significantly increased the proportion of type I muscle fibers in the gastrocnemius compared with the control group ($P<0.05$), while showing no significant effects on the proportions of type IIa and IIb fibers ($P>0.05$).

3.1 Effects of Phytol on Average Body Weight of Mice

Current literature on phytol' s effects on animal growth and development is limited and inconsistent. Mackie et al. [11] reported that dietary supplementation with 0.5% and 0.1% phytol for three weeks significantly reduced mouse body weight. Hashimoto et al. [12] similarly found that 0.5% dietary phytol decreased feed intake and slowed weight gain in mice. The present results align with these previous findings, showing that 0.50% phytol supplementation significantly reduced body weight after two weeks compared with controls, though the underlying mechanism requires further investigation.

3.2 Effects of Phytol on Metabolic Enzyme Activities and TG Content in Gastrocnemius

Muscle metabolism can be broadly classified as oxidative, glycolytic, or intermediate. Type I muscle fibers are primarily oxidative, while type II fibers are mainly glycolytic [13]. Under similar conditions, the metabolic profile of muscle depends on its intrinsic characteristics, with LDH, SDH, and HK activities serving as indicators of metabolic status [14]. SDH links oxidative phosphorylation with electron transport, providing electrons to the respiratory chain in eukaryotic mitochondria and various prokaryotic cells. Its activity generally reflects tricarboxylic acid cycle function, and uniquely among TCA cycle enzymes, SDH is embedded in the mitochondrial inner membrane as an integral component [15]. Research has shown that athletes engaged in speed and power training exhibit selective hypertrophy of fast-twitch fibers, whereas endurance athletes show selective hypertrophy of slow-twitch fibers with elevated SDH activity [16]. Sieck et al. [17] measured SDH activity across fiber types in rat diaphragm muscle, finding highest activities in type I and IIa fibers. He et al. [18] similarly reported that human skeletal muscle SDH activity was highest in type I fibers, intermediate in type IIa, and lowest in type IIb. HK catalyzes the first step of glycolysis, converting glucose to glucose-6-phosphate, with glucokinase representing an HK isoform. LDH is a marker protein reflecting anaerobic glycolytic activity [19]. Comparative studies in guinea pigs have shown that the soleus muscle (type I-rich) exhibits the lowest LDH activity and highest HK activity, while the white vastus lateralis (type IIb-rich) shows the opposite pattern [20-21], with Peter et al. [22] reporting consistent results. These findings indicate that type I fibers possess higher SDH and HK activities but lower LDH activity compared with type II fibers. The present results demonstrate that phytol supplementation increased HK and SDH activities in mouse gastrocnemius, suggesting that phytol enhances oxidative metabolic enzyme activity and thus improves muscle oxidative metabolism. Additionally, the 0.50% phytol group showed significantly lower TG content in gastrocnemius compared with controls. Although oxidative muscles are traditionally considered to have higher intramuscular fat content [23], this view remains controversial, with evidence showing no correlation between intramuscular fat and muscle metabolic type [24]. While oxidative muscle fibers contain more lipids than glycolytic fibers, the absolute lipid content in muscle fibers is substantially lower than in adipocytes, making intramuscular fat content primarily determined by adipocyte content. Previous studies have demonstrated that feeding mice diets containing 0.2% and 0.5% phytol for four weeks reduced liver TG content by 3-fold and 4-fold, respectively [25], consistent with the present findings showing TG-reducing effects of phytol in tissue.

3.3 Effects of Phytol on Muscle Fiber Type in Gastrocnemius

Muscle fibers constitute the fundamental units of muscle, and variations in fiber type composition represent an important factor underlying meat quality differences [26], showing significant relationships with tenderness, pH, color, and water-holding capacity [27-31]. Fiber type transition may thus be key to meat quality improvement. Muscles with high type I fiber proportions exhibit elevated pH at 24 hours postmortem, increased oxidative enzyme activity, reduced glycolytic enzyme activity, and lower drip loss [32]. Studies have shown that increasing type II fiber proportion in pigs accelerates postmortem pH decline, increases meat lightness and paleness, elevates cooking loss and protein denaturation, and reduces water-holding capacity [33-36]. The present findings that 0.50% phytol supplementation increased type I fiber proportion suggest that phytol may have beneficial effects on meat quality.

4 Conclusion

In summary, dietary phytol supplementation can increase the relative weight of mouse gastrocnemius, enhance HK and SDH activities, and elevate the proportion of type I muscle fibers in the gastrocnemius.

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Note: Figure translations are in progress. See original paper for figures.