Combination of citrus polymethoxyflavones, green tea polyphenols and Lychee extracts suppresses obesity and hepatic steatosis in high-fat diet induced obese mice

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Abstract

Scope: SlimTrym® is a formulated product composed of citrus polymethoxyflavones (PMFs), green tea extract and lychee extract. We investigated the effect of dietary SlimTrym® on diet-induced obesity and associated non-alcoholic fatty liver disease (NAFLD) in mice.

Methods and results: Male C57BL/6 mice were fed a normal diet (ND), high fat diet (HFD) or HFD containing 0.1% or 0.5% SlimTrym® for sixteen weeks. Dietary SlimTrym® significantly reduced
weight gain and relative perigonadal, retroperitoneal, mesenteric fat weight as well as the size of adipocyte in HFD-fed mice. SlimTrym® supplementation also effectively diminished hepatic steatosis and the serum levels of glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT), triacylglycerol (TG) and total cholesterol (TCHO). Down-regulation of peroxisome proliferator-activated receptor (PPAR)γ, sterol regulatory element-binding protein (SREBP)-1, and the activation of AMP-activated protein kinase (AMPK) signaling by SlimTrym® in both adipose tissue and liver may be responsible for the observed anti-obesity effects.

**Conclusion:** SlimTrym® supplementation potentially diminished diet-induced obesity and hepatic steatosis via regulating AMPK signaling and molecules involved in lipid metabolism.

1. **Introduction**

The prevalence of overweight and obesity has increased substantially worldwide over the past decade. It is simply defined as a condition by abnormal or excessive fat accumulation in adipose tissue resulted from imbalance between energy intake and expenditure [1]. Nowadays, obesity has been recognized with huge impact on human health because it per se is not only regarded as a simple disease but also highly contributes to the development of the metabolic diseases and cardiovascular diseases [2]. The effective obesity prevention and treatment is known as dietary control- and physical exercise-based behavioral therapies and/or in combination of pharmacologic therapies, however, it is difficult for most people because of their lifestyle [3]. Although many pharmacological agents are current available for treatment of obesity but with limited efficacy and concerned side effects [4]. Thus, research in identification of natural products from diet and herbs with anti-obesity properties is increasing popular recently.
Flavonoids are naturally polyphenolic compounds that widely occurring almost in fruits, vegetables, herbs, other plants and also known as essential nutrients for human because they cannot be biosynthesized in animals [5]. It was suggested that flavonols, flavones and isoflavones are the most abundant in human diet, such as tea, fruits and soybean [6,7]. The physiological, biological properties and beneficial effects for human health of flavonoids are extensively investigated [8]. Citrus peel has been originally used as traditional Chinese medicine for numerous purposes since ancient times. Polymethoxyflavones (PMFs) are a unique class of flavonoids that with two or more methoxy groups and almost exclusively in citrus peel [9]. They are reported to display a broad spectrum of biological and pharmacological properties including anti-obesity [10]. Several in vitro and in vivo studies show that PMFs or hydroxylated PMFs (characterized by the presence of one or two hydroxyl groups instead of methoxy groups) inhibit adipogenesis in adipocytes and reduce adipose mass in diet-induced obese mice [11-14]. Green tea (Camellia sinensis) is regarded as nutraceuticals of modern life because of its health-promoting and disease prevention effect [15]. The beneficial effect of green tea against obesity has been recognized and associated with the rich in polyphenols, in particular catechins such as epigallocatechin-3-gallate (EGCG). Consumption of green tea polyphenols reduces body weight and fat mass in animal models as well as several clinical trials through multiple mechanisms including increased energy expenditure, fat oxidation, suppression of adipocyte differentiation and proliferation and inhibition of fat absorption [16,17]. Lychee (Litchi chinensis Sonnerat) is a subtropical fruit that contains abundant phenolic compounds, such as flavanol, and exhibits biological activities [18,19]. Previous studies indicate that lychee fruit extract contains oligomerized flavanols [20,21]. Various in vitro and in vivo studies also demonstrated numerous biological activities of lychee fruit extract with oligomerized flavanols, including
antioxidant, anticancer, antiviral [20], anti-inflammatory [22], and improving insulin resistance [23].

Dietary lychee fruit extract with oligomerized flavanols reduced adipose mass, adipocyte size and adipokines secretion as well as hepatic steatosis in high fat diet-fed mice through modulating multiple molecules such as PPARγ, mammalian target of rapamycin (mTOR)/SREBP-1 and AMPK signaling [23-25].

The dietary consumption of a mixture of bioactive compounds via targeting different mechanisms or enhance their efficacy through synergistic interactions has become an innovative approach for prevention and treatment of obesity. SlimTrym® is a botanical formula with a 1:1:1 herbal blend consisting of citrus PMFs, green tea extract and lychee polyphenols based on their multiple targeted mechanisms of actions in anti-obesity. The citrus peel extract in the SimTrym® formula is standardized for 40% of total PMFs and the major PMFs is tangeretin with 33.0%. The green tea extract and lychee extract contain 114.37 mg/g of total catechins in SlimTrym® (Supplementary Table 1). Because there is no scientific reports are available on the weight-loss effect of this botanical formula, and therefore, this study was aimed to investigate the effect of SlimTrym® on HFD-induced obesity and nonalcoholic fatty liver diseases (NAFLD) in C57BL6 mice and explore the underlying mechanisms.

2. Materials and Methods

2.1 Preparation of SlimTrym®

The individual ingredients of SlimTrym® were obtained utilizing green technology without application of environmentally harmful solvents, with ethyl alcohol and water, and with the CO₂ superstitial extraction process. The CO₂ supercritical extract of Citrus sp. dry peels standardized to a
minimum 40% PMFs was provided by the India Glycols Ltd. (Noida, UP, India), the green tea hydroalcoholic extract standardized for 60% catechins and water extract of Lychee fruits standardized to flavan-3-ol monomers and dimers was provided by Hunan Sunshine Bio-Tech Co., Ltd. (Changsha, China).

The three individual ingredients of SlimTrym® were blended in 1:1:1 ratio to obtain the final product in the GMP facilities, India Glycols Ltd.

**2.2 Experimental animals and treatment**

Male, 4-week-old C57BL/6J mice were purchased from the BioLASCO Experimental Animal Center (Taiwan Co., Ltd., Taipei, Taiwan). The mice were housed in a room maintained at 25 ± 1 °C with 50% relative humidity and 12 h of light/dark cycle and were with free access to water and the Purina 5001 diet (LabDiet, PMI Nutrition International) for 1 week. All animal experimental protocol used in this study was approved by the Institutional Animal Care and Use Committee of the National Kaohsiung Marine University (IACUC, NKMU). Mice were randomly distributed into four dietary groups (n = 8 per each group): normal diet (ND, 15% energy as fat), HFD (45% energy as fat), and HFD supplemented with 0.1 or 0.5% SlimTrym®, respectively, for 16 weeks. The composition of the experimental diet was based on the Purina 5001 diet as described previously [26]. Food consumption and the body weight were recorded daily and weekly, respectively. At the end of the experiments, all animals were fasted overnight and sacrificed by CO₂ asphyxiation. Blood, liver, spleen, kidney, and adipose tissues (perigonadal, retroperitoneal and mesenteric adipose tissue) were immediately collected, weighed, and photographed.
2.3 Biochemical Analysis

Plasma samples were separated by centrifugation at 3500 ×g for 10 min. Plasma levels of GOT, GPT, TG, and T-chol were measured using a commercial assay kit. Briefly, serum was spotted onto respective Fujifilm Dri-Chem slides (Fujifilm, Kanagawa, Japan), and each biochemical indicator was determined using a blood biochemistry analyzer (Fujifilm Dri-Chem 3500s; Fujifilm, Tokyo, Japan) according to the manufacturer’s instructions.

2.4 Histopathological examinations

A portion of the median lobe of liver and the perigonadal adipose tissue was dissected and fixed in 10% buffered formalin for at least 24 h, dehydrated with a sequence of ethanol solutions, and processed for embedding in paraffin. Sections of 2-3 μm in thickness were cut, deparaffinized, rehydrated, stained with hematoxylin and eosin (H&E), and subjected to photomicroscopic observation. Adipocyte size was measured at 200× magnification according to previous study [27].

2.5 Western blot analysis

Liver tissue, adipose tissues and cell proteins were extracted and prepared as described previously [26]. The total proteins were measured by Bio-Rad Protein Assay (Bio-Rad Laboratories, Munich, Germany). The samples (50 μg of protein) were mixed with 5× sample buffer containing 0.3 M Tris-HCl (pH 6.8), 25% 2-mercaptoethanol, 12% sodium dodecyl sulfate (SDS), 25 mM EDTA, 20% glycerol, and 0.1% bromophenol blue. The mixtures were boiled at 100 °C for 10 min and were subjected to 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) at a constant current of 20 mA. After 3 h, the SDS-PAGE was transferred on the PVDF membrane.
(Millipore Corp., Bedford, MA, USA) with transfer buffer containing 25 mM Tris-HCl (pH 8.9), 192 mM glycine, and 20% methanol. The membranes were put in the blocking solution containing 20 mM Tris-HCl buffer with 1% of bovine serum albumin at room temperature for 1 h, and then primary antibodies. The blots were rinsed three times with TPBS buffer (0.2% Tween 20 in 1× PBS buffer) for 10 min each. Then blots were incubated with 1:5000 dilution of the horseradish peroxidase (HRP)-conjugated secondary antibody (Zymed Laboratories, San Francisco, CA, USA) and then washed again three times with TPBS buffer. The transferred proteins were visualized with an enhanced chemiluminescence detection kit (ECL; Amersham Pharmacia Biotech, Buckinghamshire, UK).

2.6 Statistical analyses

Data are presented as means ± SE for the indicated number of independently performed experiments. Comparisons of statistical significance between groups were made by one-way analysis of variance (ANOVA). A P value <0.05 was considered statistically significant.
3. Results

3.1 Effect of SlimTrym® on body weight and relative organ weight in C57BL/6 Mice Fed HFD

Fig. 1 and Table 1 show the effect of SlimTrym® on body weight and relative organ weight of the mice fed with HFD for 16 weeks. Mice fed HFD increased their body weight than the mice fed a ND. A significant decrease in both final body weight and body weight gain was observed in both 0.1 and 0.5%SlimTrym® group compared to the HFD group. The body weight gain in the 0.5% SlimTrym®-fed group was less than in the HFD-fed group by ~21% (Table 1). Meanwhile, no significant differences in food intake and the relative organ weights of kidney or spleen among any of the groups in the experimental period were observed.

3.2 Effect of SlimTrym® on fat mass and serum lipid parameters

The relative weight of fat mass in the 0.1% SlimTrym® group was significantly decreased compared to those of the HFD group (Fig. 2B). Particularly, 0.5% SlimTrym® treatment significantly decreased relative weight of perigonadal, retroperitoneal and mesenteric fat compared with the HFD group by about 25, 52 and 43%, respectively (Fig. 2A-C). The H&E staining of epididymal fat tissue revealed an increased adipocyte size in mice fed the HFD compared to those of the ND mice. SlimTrym® supplementation at 0.1% and 0.5% both significantly and dramatically decreased the size of adipocytes in HFD-fed mice (Fig. 2D), indicating the reduction in body weight by dietary SlimTrym® might associate with a result of reduced adipose mass. We also analyzed the serum lipid profiles among four groups and were shown in Table 1. The mice in HFD group had higher serum levels of TG and TC than the ND group whereas they were significantly ameliorated by both 0.1%
and 0.5% SlimTrym® consumption. These results revealed that SlimTrym® was effective in preventing HFD-induced adiposity and improving systemic lipid homeostasis.

### 3.3 Effect of SlimTrym® on the expression of adipogenic markers in adipose tissue

Western Blot analysis was performed to investigate the protein expression of PPARγ, SREBP-1c and its downstream target FAS in epididymal adipose tissue. Mice fed HFD showed markedly increased protein expression of PPARγ, SREBP-1c and FAS (Fig. 2E). SlimTrym® supplementation significantly reduced above protein levels compared to the HFD group, providing evidence of the anti-adipogenic effect of SlimTrym®. We also found the increased protein level of Dlk1/Pref-1, a marker for preadipocytes, in epididymal adipose tissue in SlimTrym® treated mice. Moreover, the increased in phospho-AMPK that in accompany with inactivation of ACC, was occurred in SlimTrym® group than those in the HFD-fed group. These results indicate that SlimTrym® supplementation attenuates adiposity through the regulation of lipogenesis.

### 3.4 Effect of SlimTrym® on hepatic steatosis in HFD-induced obese mice

Obesity is often associated with hepatic steatosis following by other biological changes such as hepatic injury and inflammation [28]. We found HFD treatment caused an increase of both serum GOT and GTP levels than in the ND group, accompanied with the increased liver weight (Table 1) and severe hepatic steatosis by both gross morphological examination and histological examination (Figure 3A). A significant hepatocyte ballooning, hepatic vacuoles and lipid droplets were observed in HFD-fed mice. Dietary SlimTrym® exhibited a reduced liver weight followed by markedly decreasing ballooning degeneration and smaller lipid droplets. SlimTrym® treatment also significantly lowered the increase in GOT and GTP levels found in the HFD-fed mice. These results suggested SlimTrym®
supplementation not only effectively attenuated hepatic steatosis but also improved hepatic injury induced by HFD. To further explore the effect of SlimTrym® on hepatic steatosis, the protein levels involved in hepatic lipogenesis were evaluated. Mice fed with HFD showed the increase in protein expression of C/EBPβ, PPARγ and SREBP-1; and their downstream target FAS was significantly reduced by SlimTrym® (Figure 3B). SlimTrym® also upregulated the protein levels of phospho-AMPKα and PPARα compared with the HFD group. These results indicated that SlimTrym supplementation attenuated HFD-induced hepatic steatosis through the regulation of lipogenesis.

4. Discussion

Growing evidence of numerous epidemiological studies have suggested the beneficial effect of consumption of flavonoids on human health [8]. In the present study, we showed that dietary consumption of SlimTrym® formulation that consists of different flavonoids and green tea polyphenols, significantly reduced obesity and hepatic steatosis in HFD-fed mice. Mice administered 0.1% and 0.5% SlimTrym® (the dose of approximately 0.73 g/60 kg and 3.65 g/60kg for human, respectively) for 16 weeks showed significant differences in body weight gain. In particular, adipose fat mass, the size of adipocytes and serum lipid levels were reduced significantly by SlimTrym® consumption compared with HDF control. There is no significant difference on daily caloric intake of mice among the HFD and SlimTrym® groups, suggesting SlimTrym® attenuated HFD-induced obesity through metabolic regulation.

The potential mechanism by which dietary SlimTrym® against HFD-induced obesity was involvement of reducing both adipocyte hyperplasia (adipocyte number increase) and hypertrophy (adipocyte size increase). C/EBPβ, PPARγ and SREBP-1c are critical transcription factors involved in
adipocyte differentiation and adipogenesis through modulating expression of downstream marker molecules, such as FAS that provides nonesterified fatty acid substrate for triacylglycerol synthesis [29]. Here we found the protein levels of three transcription factors and FAS were decreased in adipose tissue by SlimTrym® treatment. Dlk-1 is also known as preadipocyte factor 1 (Pref-1) which exists in preadipocytes but disappears during they differentiate into adipocytes following by up-regulation of adipocyte-specific factor, such as PPARγ [30]. In this study, SlimTrym® supplementation was found to up-regulation of Dlk1/Pref-1, indicating the role of SlimTrym® in the maintenance of the preadipose state in epididymal adipose tissue. The decreased adipogenic transcription factors and increased Dlk1/Pref-1 by SlimTrym® may contribute to reduce the adipocyte hyperplasia during HFD treatment. The anti-obesity effect of SlimTrym was also associated with attenuated adipocyte hypertrophy that evidenced by a significant reduced size of adipocyte in epididymal adipose tissue. AMPK is an important metabolic regulator implicated in lipid and glucose metabolism [31]. Phosphorylation of ACC at Ser79 by AMPK leads to inhibition of ACC activity that leading to reduce levels of malonyl-CoA product [32]. Thus, the decreased size of adipocytes caused by SlimTrym® might derive from the increase in activation of AMPK and down-regulated ACC, resulting in reduce TG accumulation in adipocytes.

Accumulating studies reveled that HFD-induced obesity is associated with abnormal hepatic lipid metabolism [33]. HFD intake causes an increased serum lipid levels and lipid influx into the liver, which further precipitating TG synthesis and ultimately hepatic steatosis [28,33]. We found that SlimTrym® suppressed serum TG and TC levels from C57BL/6 mice fed HFD with a significant decrease in liver weight, indicating SlimTrym® reduced HFD-induced hepatic steatosis most likely by decreasing lipid influx into the liver. Another important mechanism by which dietary SlimTrym®
abolished HFD-induced hepatic steatosis was proposed by down-regulation of lipogenesis. This finding was supported by the reduced hepatic lipogenic protein levels of C/EBPβ, PPARγ, SREBP-1c and FAS in SlimTrym® treated mice. Moreover, the cleavage, nuclear translocation and transcriptional activity of SREBP-1c is negatively regulated by AMPK [34]. Here we showed that SlimTrym® elevated the phosphorylation of AMPK and down-regulated protein level of SREBP-1c, which then attenuated fatty acid synthesis in the liver from HFD-fed mice. PPARα is abundant in hepatocytes and is involved in hepatic lipid metabolism, gluconeogenesis and inflammation [35]. It has been reported that mice lack of PPARα enhances hepatic steatosis and increases susceptibility to high fat-induced NASH [36]. In our study, SlimTrym® supplementation caused an increase of hepatic PPARα speculating that SlimTrym® may stimulate fatty acid oxidation, but further investigation is necessary. Taken together, our findings suggest that SlimTrym® reduced HFD-induced hepatic steatosis by activation of AMPK and regulating gene expression involved in lipid metabolism.

The result of chemical composition of SlimTrym® showed citrus peel extract in this formula is 40% of total PMFs and the major PMFs is tangeretin with 33.0%. The total catechins with 11% that included catechin, epicatechin, EGC, EGC and EGCG were contributed by green tea extract. Here we showed two anthocyanidins, Malvidin and Peonidin, were identified in lychee pulp extract, although the content was relatively low (Supplementary Table 1). Nine compounds were identified in SlimTrym® as showed in Supplementary Figure 1. Studies have shown that dietary citrus tangeretin and nobiletin reduced weight gain and improved insulin resistance in HFD-fed mice [12,14]. Citrus PMFs also have been reported to inhibit adipogenesis in adipocytes and HFD-induced weight gain as well as fatty liver in mice through down-regulating multiple adipogeneic molecules and activating AMPK signaling [13,26]. PMFs with multiple lipophilic methoxyl groups are considered to enhance
bioavailability because of their greater permeability and membrane transport ability than other flavonoids [37], including green tea catechins and flavanols in lychee fruit extract, despite the anti-obesity effect and molecular mechanism has been widely investigated of later two. We also identified two minor anthocyanidins, Malvidin and Peonidin in the lychee pulp extract but not in the peel which presented as anthocyanins [19]. Thus we assumed combination of three individual ingredients in SlimTrym® might enhance its anti-obesity efficacy, and PMFs appears to have more importance with others on exhibiting potential efficacy on mitigating diet-induced obesity and hepatic steatosis. Furthermore, the potential action of SlimTrym supplementation on attenuation of obesity and associated NAFLD might via a synergistic effect among three natural components, but further investigation is necessary. Collectively, our study provides evidence that SlimTrym® supplementation exerts an anti-obesity effect and inhibited fatty liver by regulating AMPK signaling and molecules related to lipid metabolism.

Acknowledgments

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Conflict of interest

The authors declare that there are no conflicts of interest.
5. References


Ref Type: Journal (Full)


Ref Type: Journal (Full)


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Figure captions

Figure 1. Effect of supplement with SlimTrym on body weight in HFD-fed mice. Mice were fed HFD supplement with or without SlimTrym (0.1% and 0.5%) for 16 weeks. ND group was administered control diet as vehicle. At the end of experiment, all mice of each group were killed by CO₂ asphyxiation. (A) Representative photographs of each group are shown at the end of week 16. (B) Body weight was monitored weekly. Values are the mean ± SEM (n = 8 per group). Mean values within each column with different labels (a-c) are significantly different (p < 0.05) according to one way ANOVA analysis of variance and Duncan’s multiple range test.

(A)
Figure 1.
Figure 2. Effect of SlimTrym supplement on relative adipose tissue weights in HFD-fed mice.
The relative (A) perigonadal, (B) retroperitoneal, and (C) mesenteric fat weight was expressed as a percentage of body weight. (Adipose tissue weight/body weight × 100). (D) Histology of epididymal adipose tissues by H&E staining (200× magnification) and the adipocyte size was quantified under microscope quantified from representative sections. Data were presented as the mean ± SE (n = 8 per group). Mean values within each column with different labels (a–c) are significantly different (p < 0.05) according to one way ANOVA analysis of variance and Duncan’s multiple range test. (E) The protein levels of SREBP-1c, PPARγ, FAS, Dlk-1(Pref-1) and AMPK signaling were evaluated by Western blot analysis. The relative protein expression is expressed as the fold increased in comparison to ND group after normalized to β-actin. The values are presented as mean ± S.E.M.
(D)

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<td>HFD</td>
<td>b</td>
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<td>HFD + 0.1% SlimTrym</td>
<td>c</td>
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<tr>
<td>HFD + 0.5% SlimTrym</td>
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(E)

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<td>SREBP-1c</td>
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<td>FAS</td>
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<td>DLK-1(PreF-1)</td>
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<td>β-actin</td>
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Figure 2.
**Figure 3. Effect of SlimTrym on hepatic steatosis in HFD-fed mice.** (A) The gross morphology of livers (upper) and representative histological images of H&E-stained sections (lower) (400 × magnification). (B) The hepatic protein levels of SREBP-1c, PPARγ, PPARα, FAS and AMPK signaling were evaluated by Western blot analysis. The relative protein expression is expressed as the fold increased in comparison to ND group after normalized to β-actin. The values are presented as mean ± S.E.M.
Figure 3.
Table 1. Body weight gain, relative organ weights, food intake and serum parameters in C57BL6 mice

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<th>HFD + 0.5% SlimTrym</th>
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<td>Initial wt (g)</td>
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<td>20.22 ± 0.28&lt;sup&gt;a&lt;/sup&gt;</td>
<td>20.04 ± 0.39&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>Wt gain (g)</td>
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<td>18.60 ± 096.&lt;sup&gt;a&lt;/sup&gt;</td>
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<td><strong>Relative organ weights&lt;sup&gt;b&lt;/sup&gt;</strong></td>
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<td>Liver (%)</td>
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<td>3.50 ± 0.16&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>Kidney (%)</td>
<td>1.20 ± 0.10&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>Spleen (%)</td>
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<td>0.22 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>TG (mg/dl)</td>
<td>98.50 ± 23.09&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td>134.88 ± 6.97&lt;sup&gt;a&lt;/sup&gt;</td>
<td>110.25 ± 8.79&lt;sup&gt;b&lt;/sup&gt;</td>
<td>89.75 ± 12.41&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>TCHO (mg/dl)</td>
<td>88.38 ± 13.02&lt;sup&gt;d&lt;/sup&gt;</td>
<td>203.88 ± 15.98&lt;sup&gt;a&lt;/sup&gt;</td>
<td>164.50 ± 6.60&lt;sup&gt;b&lt;/sup&gt;</td>
<td>150.88 ± 11.73&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>GOT (U/l)</td>
<td>60.13 ± 24.20&lt;sup&gt;b&lt;/sup&gt;</td>
<td>197.38 ± 73.40&lt;sup&gt;a&lt;/sup&gt;</td>
<td>98.75 ± 32.98&lt;sup&gt;b&lt;/sup&gt;</td>
<td>109.13 ± 38.60&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>GPT (U/l)</td>
<td>15.50 ± 6.98&lt;sup&gt;c&lt;/sup&gt;</td>
<td>70.38 ± 10.92&lt;sup&gt;a&lt;/sup&gt;</td>
<td>35.38 ± 10.16&lt;sup&gt;b&lt;/sup&gt;</td>
<td>29.00 ± 8.66&lt;sup&gt;b&lt;/sup&gt;</td>
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</tbody>
</table>

<sup>a</sup> The relative organ weight was expressed as a percentage of body weight (liver weight/body weight × 100). Data were presented as the mean ± SE (n = 8 per group). Mean values within each column with different labels (a–d) are significantly different (p < 0.05) according to one way ANOVA analysis of variance and Duncan’s multiple range test. GOT, glutamate oxaloacetate transaminase; GPT, glutamate pyruvate transaminase; TG, triglycerides; TCHO, total cholesterol.
Dietary SlimTrym reduced hepatic lipogenic protein levels of C/EBPβ, PPARγ, SREBP-1c and FAS via increasing the activation of AMPK and down-regulated ACC, resulting in reduce TG accumulation in adipocytes.