Abstract
We evaluated the effectiveness of combining aerobic exercise with a daily dose of Rhizoma Coptidis in lowering cholesterol levels and fat deposits in the liver in Syrian golden hamsters fed a high fat/high cholesterol diet. The experimental diet increased body weight, as well as increasing epididymal, perirenal and liver adipose tissue and overall serum cholesterol levels (82.7%-157.15% increase). The combination of aerobic exercise and Rhizoma Coptidis reduced body weight gain by 11.7%, epididymal adipose tissue by 16.5% (P<0.05), and perirenal and liver adipose tissue by 27.7% and 18.44% (P<0.01). This experimental combination was also effective in lowering serum cholesterol levels by 22.3% to 42.8% (P<0.01), while increasing serum levels of HDL-c level by 11.2%. Histochemical analyses indicated that the combination of aerobic exercise and Rhizoma Coptidis inhibited the accumulation of lipids in the liver, as well as de novo synthesis of cholesterol by down-regulating the expression of 3-hydroxy-3-methylglutaryl-CoA reductase, while accelerating the break-down of cholesterol via an up-regulation of low-density lipoprotein receptor expression. The conversion of cholesterol into bile acids was also promoted via activation of cholesterol 7-alpha-hydroxylase and decreased re-absorption of cholesterol. Future studies are warranted to evaluate the effectiveness of combination of aerobic exercise and Rhizoma Coptidis as a cholesterol-lowering regimen in humans.

Key words: Aerobic Exercise, Rhizoma Coptidis Capsule, High-Fat and High-Cholesterol Diet, Hypercholesterolemia, Cholesterol Metabolism

El Efecto del Ejercicio Aeróbico Combinado con las Cápsulas de Rhizoma Coptidis en la Hipercolesterolemia Inducida por una Dieta Rica en Grasas y en Colesterol en los Hámsters Dorados Sirios--Efectividad del Ejercicio Aeróbico y Rhizoma Coptidis en la Hipercolesterolemia

Resumen
Evaluamos la efectividad de combinar el ejercicio aeróbico con una dosis diaria de Rhizoma Coptidis para reducir los niveles de colesterol y los depósitos de grasa en el hígado en hámsters dorados sirios alimentados con una dieta alta en grasas / colesterol alto. La dieta experimental aumentó el peso corporal y aumentó el epididimo, tejido adiposo perirrenal e hepático y niveles de colesterol en suero en general (aumento del 82,7% -157,15). La combinación de ejercicio aeróbico y Rhizoma Coptidis redujo la ganancia de peso corporal en un 11,7%, el tejido adiposo del epididimo en un 16,5% (P <0,05) y el tejido adiposo perirrenal y hepático en un 27,7% y un 18,44% (P <0,01). Esta combinación experimental también fue efectiva para reducir los niveles de
colesterol sérico en un 22,3% a 42,8% (P <0,01), mientras que aumentó los niveles séricos de HDL-c en un 11,2%. Los análisis histoquímicos indicaron que la combinación de ejercicio aeróbico y Rhizoma Coptidis inhibió la acumulación de lípidos en el hígado, así como la síntesis de novo de colesterol al disminuir la expresión de 3-hidroxi-3-metilglutaril-CoA reductasa, mientras aceleraba la desglosa del colesterol mediante una regulación al alza de la expresión del receptor de lipoproteínas de baja densidad. La conversión de colesterol en ácidos biliares también se promovió a través de la activación de la 7-alfa-hidroxilasa y la disminución de la reabsorción del colesterol. Se justifican estudios futuros para evaluar la efectividad de la combinación de ejercicio aeróbico y Rhizoma Coptidis como un régimen para reducir el colesterol en humanos.

**Palabras clave:** Ejercicio Aeróbico, Rhizoma Coptidis Cápsula, Dieta Alta en Grasas y Colesterol, Hipercolesterolemia, Metabolismo del Colesterol

1. Introduction

Cardiovascular disease is a leading cause of death, with a higher risk than cancer and other diseases combined. As factors associated with cardiovascular disease are increasingly more prevalent, such as obesity and type II diabetes, it is estimated that the prevalence of cardiovascular disease will continue to rise rapidly (World Health Organization, 1993). An elevated serum level of cholesterol is considered to be one of the key factors leading to cardiovascular and other atherosclerotic diseases (Durrington and P, 2003). Therefore, improving serum lipid levels could effectively improve the course of cardiovascular disease.

Aerobic exercise is recognized as a safe and effective means of preventing cardiovascular disease and maintaining cardiovascular health (Zhao, S, Wang, Y, Mu, Y, Yu, B, Ye, P, & Yan, X, et al. 2014). Clinically, statins are commonly used hypolipidemic drugs, which inhibit the capacity of hydroxymethyl glutaryl-CoA reductase to synthesize cholesterol in the body (Topol, E and J. 2004). However, as hypercholesterolemia is a long-term chronic state and prolonged use of statins can lead to liver and muscle injury (Brewer, & Bryan and H 2003), as well as increasing the risk of diabetes (Corrao, G, Ibrahim, B, Nicotra, F, Sorrana, D, Merlino, L, & Catapano, A. L, et al, 2014), an alternative to statins is needed.


Our aim in this study was to induce a state of hypercholesterolemia in golden hamsters using a high fat and higher cholesterol diet (21% fat and 0.1% cholesterol) and use this experimental model to evaluate if the cholesterol-lowering effect of Huang Lian capsules could be potentiated by moderate intensity aerobic exercise. In addition, we used histochemical and DNA analyses to explore potential molecular mechanisms underlying observed effects. Our study was set-up to provide a theoretical basis for the national fitness exercise program that has been established for the prevention of hypercholesterolemia and cardiovascular disease.

2. Materials and Methods

2.1. Ethics Statement

All experimental procedures were in compliance with the Animal Care and Use Committee of Wenzhou University. This study was approved by the Research Ethics Committee of Wenzhou University (11-1029-052).

2.2. Experimental Animal Model

Four-week-old male healthy golden hamsters (SPF grade; body weight, 100±5 g) were purchased from the Chongqing Tengxin Biotechnology Co., Ltd. [SCXK 2012-0001]. All animals were fed in a clean animal room at room temperature of 22±2°C, with a light: dark cycle of 12h:12h, and at a constant humidity of 55±10%. Animals were provided with food and water at libitum. After one week of adaptive feeding, animals were randomly allocated to one of two groups, the normal control group (NC) and the high fat/high cholesterol group (HFFHC), with 10 animals in the NC group and 40 in the HFFHC group. The high fat and high cholesterol diet consisted of 10% lard, 10% egg yolk powder and 1% cholesterol added to the normal diet. After a 6-week exposure to either the normal or high fat diet, animals were fasted for 12 h and blood samples were drawn from the posterior orbital vein and body weight recorded. Blood samples were analyzed for levels of total cholesterol (TC), total triglycerides (TG), LDL-c cholesterol, and HDL-c cholesterol.
2.3. Intervention Program

Animals in the HFHC group were randomly allocated to four experimental groups, with 10 animals in each group, as follows: HFHC only; HFHC with rhizoma coptidis (RC), where rhizoma coptidis is a generic form of berberine; HFHC with aerobic exercise (AE); and HFHC with Huang Lian, combining AE and RC (AE+RC). The intervention for each of the experimental groups was as follows. Animals in the NC group were fed a conventional diet, with daily (PM3) saline by gavage (0.5 ml), for 8 weeks, without exercise or ingestion of Rhizoma Coptidis. Animals in the HFHC group were fed the HFHC diet, with daily (PM3) gavage (batch number, 100601-201002; dose, 0.25 g), without exercise or ingestion of Rhizoma Coptidis. Animals in the RC group received the same HFHC and daily saline gavage as animals in the HFHC group, with no exercise, but did receive a daily Huang Lian capsule per oral (Xianglian Pharmaceutical Co., Ltd., batch number: 100601-201002; dose, 0.7 g per kg of body weight, administered with the physiological saline gavage), for 8 weeks. Animals in the AE group received the same HFHC and daily saline gavage as animals in the HFHC group and, in addition, completed a daily program of moderate intensity treadmill exercise (slope, 0°; speed, 13 m/min; duration, 60 min) for 8 weeks. Animals in the AE+RC group received the same HFHC and daily saline gavage as animals in the HFHC group and performed the same treadmill exercise program as animals in the AE group, and received, in addition, the same daily Huang Lian capsule per oral as animals in the RC group.

2.4. Measurement of Body, Liver, Fecal, and Residual Weight

During the course of the experiment, the amount of food ingested, the change in body weight and the weight of the feces were recorded. Feces were collected, dried and weighted for one week before exposure to the intervention. Subsequently, body weight and the weight of non-ingested food were obtained every 7 days. At the end of the experiment, feces were again collected and dried at 60°C for determination of cholesterol and bile acid levels. Following euthanasia, the liver was removed and weighed, and subsequently frozen at -80°C for sectioning and histochemical analysis.

2.5. Determination of Serum Levels of TC, TG, LDL-c and HDL-c

On the last day of the intervention, following completion of the AE program and administration of the final RC dose, animals were fasted for 12 h and then anesthetized to obtain orbital venous blood samples. Blood samples were maintained at room temperature for 2 h and subsequently centrifuge (5000 r/min) for 10 min. The supernatant was extracted and conserved at -80°C until testing for serum levels of total cholesterol (TC), triglyceride (TG), low-density lipoprotein (LDL-c), and high-density lipoprotein (HDL-c) levels.

2.6. Determination of TC and Total Bile Acid (TBA) In Feces

Feces were collected at three time-points for analysis; after 24 h of adaptive feeding; after the first 24 h of the intervention program; and after 4 weeks of the intervention program. For analysis, feces were dried at 60°C, weighed and preserved at 4°C in a sealed container for subsequent analysis. Determination of the level of cholesterol and bile acid in feces was determined using an automatic biochemical analyzer. For this analysis, 100 mg of feces were soaked in 1 L of distilled water. Subsequently, 8 ml of methanol was added and the solution centrifuged for 10 min at 5000 r/min to extract the supernatant for analysis.

2.7. Histochemical Analysis of Liver and Adipose Tissue Using Oil Red O and Hematoxylin and Eosin (HE) Staining

Immediately after euthanasia, samples of liver and adipose tissue (0.3 cm ~ 0.5 cm cubes), were obtained and fixed in 10% formaldehyde for 12 ~ 24 h, followed by gradient alcohol dehydration. Dehydrated samples were treated with xylene Ⅰ and Ⅱ for 15 ~ 20 min, until transparent, and then embedded in wax and sliced (4 ~ 5 μm thickness). Slices were then dewaxed, using a conventional technique, and stained with oil red O and HE staining. The NIS-elements basic research software was then used to detect the volume of staining in each slice for statistical analysis.

2.8. Total Ribosome Nucleic Acid (RNA) Extraction and Determination of mRNA Expression in Liver Tissue

The total concentration of RNA was extracted from liver tissue samples using the Trizol kit and the absorbance (A) measured using a nucleic acid concentration analyzer (Nanodrop 2000, United States), with a reference A-value of A260 / A280. As well, 1.8 ~ 2.0 samples were subjected to agarose gel electrophoresis, for detection of 28S, 18S and 5S, for cDNA synthesis. cDNA was synthesized using a 25 ul reverse transcription system, with the reaction carried out at 50°C for 50 min, at 85°C for 5 min and, finally, at 4°C for 1 h to
terminate the reaction. The cDNA template was stored at -20°C. Real-time quantitative PCR was performed using the first cDNA as a template, in 20 μL of reaction solution (10 μL of SYBR Green mixture, 3.4 μL of DEPC water, 0.8 μL of 10 pM / μL upstream primer, 0.8 μL of 10 pM / ML downstream primer, 5 μL of 20 ng / μL cDNA). The following reaction conditions were used: 3 min at 94°C pre-denaturation; 10 s at 94°C for denaturation; 30 s at 55°C for annealing; and 30 s at 72°C for extension. Steps 2-4 were repeated for 52 cycles. The relative mRNA content was represented by a 2-ΔΔCT method and GADPH.

2.9. Extraction of Liver Protein for Evaluation of Protein Changes Using Western Blotting

Samples of 100 mg of liver tissue were obtained immediately after euthanasia and ground in liquid nitrogen. RIPA lysate (containing 1 mmol / L of PMSF) was added, with the mixture let to stand for 10 m prior to determination of the protein concentration. According to the measured protein concentration, a 5× buffer loading solution and lysate were added to the protein samples and centrifuged in different proportion until a solution with a protein concentration of 1.5 mg/mL was obtained (in a 1x buffer solution). The mixture was then boiled at 100°C for 10 min to denature the protein, followed by SDS-PAGE electrophoresis and wet transfer to a PVDF membrane for western blotting.

Membranes were soaked in a solution of 10% skim milk at room temperature for 1 h, and subsequently in a diluted solution of 10% skim milk (primary anti-dilution ratio: HMGCR, 1:500; LDL receptor, 1:3000; CYP7A1, 1:500; β-actin, 1:500) at 4°C overnight in a shaking incubator. The membrane was then rinsed with TBST solution 5x, at 5-min intervals, incubated at room temperature for 2 h with secondary antibody HRP (dilution ratio, 1:4000), and then rinsed again with TBST, 5x and 5-min intervals. ECL luminous fluid development was used to evaluate the protein concentration, with the internal parameters of the grey value ratio analyzed using Image J software.

2.10. Statistical Analysis

All data were expressed as a mean±standard deviation. The least squared difference t-test was used to compare measured variables, described above, before and after the intervention and between the groups. Correlation between variables of interest was evaluated using Pearson’s correlation. All analyzes were performed using SPSS 19.0, with a P <0.05 deemed to be statistically significant.

3. Results

3.1. Effects of Huang Lian and aerobic exercise on body weight

As shown in Table 1, there was no significant difference in dietary intake between groups over the duration of the experiment. At the end of the 8-week intervention, animals in the HFHC group had gained significant greater body weight than animals in the NC group (P<0.01). Animals in the RC group also gained weight, but with this weight gain being lower than that of the HFHC group (P<0.05). The most striking effect was for animals in the AE+RC group, whose weight gain was 11.7% less than the weight gain of animals in the HFHC group (P<0.01). The results show that aerobic exercise combined with RC can significantly reduce the weight gain caused by HFHC diet.

3.2. Effects of Huang Lian and aerobic exercise on serum levels of TC, TG, LDL-c and HDL-c

As shown in Table 2, there was no significant difference in dietary intake between groups over the duration of the experiment. At the end of the 8-week intervention, animals in the HFHC group had gained significant greater body weight than animals in the NC group (P<0.01). Animals in the RC group also gained weight, but with this weight gain being lower than that of the HFHC group (P<0.05). The most striking effect was for animals in the AE+RC group, whose weight gain was 11.7% less than the weight gain of animals in the HFHC group (P<0.01). The results show that aerobic exercise combined with RC can significantly reduce the weight gain caused by HFHC diet.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Species</th>
<th>Primer Sequences (5’detec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HMGCR</td>
<td>Mus musculus</td>
<td>R:gcgactatgacgctgaaacaa F:tggagatcgatgtcatgct</td>
</tr>
<tr>
<td>LDLR</td>
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</tr>
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<tr>
<td>ABST</td>
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</tr>
<tr>
<td>GAPDH</td>
<td>Mus musculus</td>
<td>R:acacattggggtggagaac F:aatctgcccattgtgag</td>
</tr>
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RT-PCR, reverse transcription polymerase chain reaction

<table>
<thead>
<tr>
<th>Group</th>
<th>food intake</th>
<th>Body Weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial weight (g)</td>
<td>Final weight (g)</td>
</tr>
<tr>
<td>NC</td>
<td>20.08±1.88</td>
<td>99.93 weig</td>
</tr>
</tbody>
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Table 1. Total RNA Extraction in Liver Tissue and RT-PCR detection of mRNA expressions

Table 2. Food intake, body weight and relative liver weight of hamsters
As shown in Table 2, at the end of the 8-week intervention, serum levels of TC, TG and LDL-c were 82.7%, 157.1% and 33.3% higher, respectively, in the HFHC than NC group (P < 0.01). Compared to the HFHC group, serum levels of TC, TG and LDL-c were 43.8%, 34.6% and 22.3% lower, respectively, in the RC+AE group (P<0.01 versus HFHC), while the level of HDL-c was 11.2% higher (P<0.05 versus HFHC). Therefore, the RC+AE combination produced significant improvements in serum cholesterol levels during exposure to a HFHC diet.

3.3. Effects of Hulian and aerobic exercise on fecal TC and TBA levels

TC and total biliary acid (TBA) levels in feces were examined to compare the effects of RC+AE on cholesterol conversion and excretion. The results in Table 3 show that fecal levels of TC and TBA were significantly higher in the HFHC than NC group at both the 4- and 8-week time points of the intervention (P<0.01). At the 8-week time point, the excretion level of TC and TBA by 21.3% and 39.3%, respectively, with RC alone (P<0.01 versus HFHC). Excretion of TC and TBA further increased to 50.8% and 125%, respectively, with RC+AE (P<0.01 versus HFHC). Therefore, RC+AE can significantly increase cholesterol and bile acid excretion in the intestine, helping to lower serum cholesterol levels.

3.4. Effect of Hulian capsule and aerobic exercise on fatty accumulation in the liver and perirenal and epididymal adipose tissue

![Protein expression of genes involved in cholesterol metabolism pathways](image.png)
A: Western blot; B: relative expression of protein normalized by β-actin

## \*P < 0.01 versus NC; \*\*P < 0.05 versus HFHC, \*\*\*P < 0.01 versus HFHC

The HFHC diet induced increases in epididymal (36.9%) and perirenal (67.6%) adipose tissue and fatty accumulation (49.4%) in the liver, as shown in Figure 1, compared to the NC group (P<0.01). In addition, the HFHC diet increased the weight of the liver (35.49%) compared to the NC group (P<0.01). Both RC and AE independently decreased the weight of epididymal fat (16.5%; P<0.05), perirenal fat (27.7%; P<0.01) and of the liver (18.4%; P<0.01). These independent effects were not significantly improved by combining RC and AE (RC+AE group), with the exception of liver weight (P<0.01 versus RC and AE). Of note, AE was effective in improving body weight. Therefore, RC and AE can reduce the increase in organ fat weight caused by a HFHC diet.

3.5. Effects of Huang Lian and aerobic exercise of fatty accumulation of the liver

![Figure 2. Oil Red O staining of liver sections after one month of exposure to the intervention of the respective experimental groups (bars indicate 100 μm)](image)

Figure 2 shows the significant increase in red lipid droplets in the liver of animals fed a HFHC diet compared to the NC group. Both RC and AE produced a slight decrease in the concentration of lipid droplets within the liver, with this effect being more evident with the RC+AE combination. These findings are consistent with our findings of decreasing liver weight between the HFHC and AE and RC groups, and between the AE and RC groups and the RC+AE group.

3.6. Effects of Huang Lian and aerobic exercise on adipocytes

The HE staining of adipose tissue is shown in Figure 3, with no evidence of fibrous tissue development in any of the groups. The size of adipocytes in NC group was uniform, with a concentration of 1000-5000 μm² and a cell size in the range of 2000-4000 μm². In HFHC fed animals, fat cells increased in volume, with 92.5% of adipocytes having being 2000-9000 μm² in size. With RC administration, 93.7% of adipocytes had a volume of 900-7000μm², with aerobic exercise further decreasing the volume of adipocytes, with 83.1% of cells having a volume in the range of 800-7000 μm². Therefore, both RC and AE exerted a positive effect on adipocytes, with 87.7% of adipocytes across both groups having a volume in the range of 900-6000 μm².
3.7. Effects of Huang Lian and aerobic exercise on the cholesterol metabolism

Possible molecular and genetic pathways of cholesterol's metabolism underlying observed changes were investigated using quantitative reverse transcription polymerase chain reaction (qRT-PCR) and Western blotting (Figures 1 and 3). Hydroxymethylglutaryl-CoA reductase (HMGCR) is a rate-limiting enzyme for in vivo cholesterol synthesis and plays an important regulatory role in the synthesis of cholesterol. The HFHC diet upregulated mRNA and protein expression of HMGCR, likely through a negative feedback loop from the high cholesterol content in the diet. Both RC and AE independently exerted a down-regulation effect on HMGCR expression, which inhibited cholesterol expression, resulting in decreased serum levels of cholesterol. In addition, both RC and AE independently increased the expression of LDLR, compared to the HFHC group (P<0.05), promoting cholesterol metabolism and helping to prevent an excessive accumulation of cholesterol. In addition, in vivo synthesis of cholesterol or food sources of cholesterol can be converted to bile acid under the action of CYP7A1, with this conversion being enhanced by RC and by AE. The combined effects of RC and AE further inhibited the expression of ABST, which improved the absorption of cholesterol by bile acids through the liver and kidneys, resulting in an increased level of cholesterol excretion in feces.

![Figure 4. mRNA expression of genes involved in cholesterol metabolism pathways](image)

**Figure 4.** mRNA expression of genes involved in cholesterol metabolism pathways  
##P< 0.01 vs. NC; *P< 0.05 vs. HFHC, **P< 0.01 vs. HFHC

3.8. Discussion

Our AE+RC intervention was based on previous studies that have reported elevated serum cholesterol level to be a key factor in the development of cardiovascular and atherosclerotic diseases, and that aerobic exercise and the use of statins, which inhibit hydroxymethyl glutaryl-CoA reductase, in lowering cholesterol levels. As well, it is known that long term use of statins can lead to liver injury and muscle damage, as well as increase the risk of new onset of diabetes, as well as other side effects. As such, we elected to select an alternative to statins, namely berberine which is a well-known Chinese medicinal component that is known to be a safe and effective cholesterol-lowering active agent. We hypothesized that combining berberine with aerobic exercise, which accelerates blood flow and improves blood vessel elasticity, could potentiate the cholesterol-lowering effect of berberine, providing an effective long-term strategy for the control (and perhaps prevention) of hypercholesterolemia (He, S. Y., Qian, Z. Y., Wen, N., Tang, F. T., Xu, G. L., & Zhou, C. H., 2007).

We provide evidence of the effectiveness of our combined AE+RC intervention in down-regulating levels of TC (43.8%), TG (34.6%) and LDL-c (22.3%) in animals fed a HFHC diet, while up-regulating levels of HDL-c. Moreover, our intervention decreased gains in epididymal (16.5%) and perirenal (18.4%) fat and fatty accumulation in the liver (27.7%), as well as reducing weight gain (11.7%) produced by the HFHC diet. On further analysis, we identified that the AE+RC combination also increased excretion of intestinal cholesterol and bile acids, which can further lower serum levels of cholesterol.
Cholesterol is an important component of cells, and is necessary for the synthesis of vitamin D3 from raw materials, as well as being a precursor of bile acid. However, an abnormal increase in serum level of cholesterol is a specific risk factor for cardiovascular disease and, therefore, controlling cholesterol levels is clinically important. The multi-enzyme HMGCR pathway is the rate-limiting enzymatic factor of cholesterol synthesis (Zou, Z. Y., Hu, Y. R., Ma, H., Wang, Y. Z., He, K., & Xia, S., et al, 2015) and, therefore, inhibition of HMGCR activity and gene expression of HMGCR will inhibit cholesterol synthesis (Woo, M. N., Bok, S. H., & Choi, M. S., 2009). In our study, we further identified that berberine, in combination with aerobic exercise of moderate intensity, was effective in down-regulating the expression of HMGCR, thereby reducing the level of cholesterol synthesis.

In addition, studies have shown that in vivo synthesis or food sources of cholesterol are largely bound to low-density lipoprotein (LDL) transport, with LDL receptors in the cell membrane (LDLR) mediating the endocytosis into the cells. Once in cells, cholesterol is catabolized, with a resultant decrease in serum levels of cholesterol. However, LDLR production is controlled by the need for cholesterol within cells, and not by the serum level of cholesterol (Yamamoto, T., Hasegawa, K., Onoda, M., & Tanaka and K, 2015). However, up-regulation of the expression of LDLR in the liver can reduce body cholesterol levels, with liver dysfunction generally resulting in hypercholesterolemia (Soutar, & A. and K, 1996). Our findings demonstrate that the AE+RC combination can significantly increase LDLR level, promote cholesterol metabolism and inhibit the accumulation of excessive cholesterol in the body.

Cholesterol conversion to bile acids is the main pathway for cholesterol excretion, with CYP7A1 (7α-hydroxylase) being a key enzyme for the synthesis of bile acids. As such, CYP7A1 is essential to maintaining stable levels of serum cholesterol (Yang, T., Espenshade, P. J., Wright, M. E., Yabe, D., Gong, Y., & Aebersold, R., et al, 2002), with decreases in CYP7A1 levels being associated with an increased risk for hyperlipidemia via a decrease in levels of bile acids (Chiang and J. Y. L, 2004). Functionally, bile acid promotes the absorption of cholesterol in the intestine, with the level of CYP7A1, and hence bile acid, being regulated in negative feedback fashion via an up-regulation in mRNA levels as cholesterol synthesis in the liver increases (Kong, W., Wei, J., Abidi, P., Lin, M., Inaba, S., & Li, C., et al. 2004). Therefore, the activity of CYP7A1 in the liver and the content of bile acids in the intestine are closely related in the transformation and excretion of cholesterol. In our study, we reported on the increased effectiveness of combining berberine with aerobic exercise to significantly up-regulate the expression of CYP7A1 mRNA and protein, which promotes the transformation of cholesterol into bile acid in vivo, resulting in a decrease in serum cholesterol levels.

The ileal NA1/Bile acid cotransporter (IBAT) is a sodium-dependent bile acid-conjugated transporter (Gbagauid, G. F., & Agellon, L. B, 2004) located at the end of the ileum and specifically absorbs bile acids (Shneider, & Benjamin and L, 2001) and, thus, plays a vital role in the control of serum cholesterol. Specifically, suppression of IBAT promotes excretion of bile acids and accelerates the transformation of cholesterol in the liver into bile acids, thereby reducing liver and serum cholesterol levels (Kitayama, K., Nakai, D., Kono, K., Hoop, A. G. V. D., Kurata, H., & Wit, E. C. D., et al. 2006). Changes in the structure and function of the transporter can cause abnormal bile acid absorption, which in turn affects the absorption of cholesterol and lipids. Therefore, the suppression of IBAT is considered as an important target for the control of cholesterol. We identified in our study that an 8-week program of AE+RC inhibited ABST expression, which improved resorption of cholesterol by bile through the liver and kidneys, with a resultant increase in the level of cholesterol excreted into the feces and effectively reducing the accumulation of cholesterol in the body.

4. Conclusions

Our HFHC diet was effective in inducing a state of hypercholesterolemia in our animal model. We provide evidence that both RC and AE can individually improve body weight, cholesterol levels and fatty accumulation in the liver, with these effects being potentiated by combining RC and AE (AE+RC group). We demonstrated that these positive effects on cholesterol levels are mediates principally by a down-regulation in the expression of HMGCR, which inhibits cholesterol synthesis, and inhibition of ASBT, in combination with an increase in LDLR and CYP7A1 expression. Globally, effects of RC and AE accelerated the transfer of peripheral cholesterol to the liver, promoted its decomposition; promoted the transformation of cholesterol into bile acid, with an increased excretion of cholesterol in feces. Future studies will be needed to confirm our findings in humans, which would provide evidence to support the implementation of aerobic exercise programs to improve (and prevent) cardiovascular diseases.

Acknowledgments

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