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ORIGINAL CONTRIBUTION

Theabrownin from Pu-erh tea together with swinging exercise synergistically ameliorates obesity and insulin resistance in rats

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Abstract

Purpose Theabrownin (TB)-containing Pu-erh tea has been shown to be hypolipidemic in rats fed a high-fat diet. Physical exercise such as swinging is also known to reduce obesity. We hypothesized that TB in combination with swinging can synergistically ameliorate obesity and insulin resistance in rats with metabolic syndrome.

Methods TB, rosiglitazone, or lovastatin (controls) was administered by gavage to rats fed a diet high in fat, sugar, and salt. A subgroup of the rats was subjected to a 30-min daily swinging exercise regimen, whereas the other rats did not exercise.

Results Theabrownin in combination with swinging was found to significantly improve serum lipid status and prevent development of obesity and insulin resistance in rats. Liver transcriptomics data suggested that theabrownin activated circadian rhythm, protein kinase A, the adenosine monophosphate-activated protein kinase, and insulin signaling pathways by enhancing cyclic adenosine monophosphate levels and, hence, accelerating nutrient metabolism and the consumption of sugar and fat. The serum dopamine levels in rats increased significantly after exercise. In parallel work, intraperitoneal dopamine injections were shown to significantly reduce weight gain and prevent the elevation in triglyceride levels that would otherwise be induced by the high fat-sugar–salt diet. Theabrownin prevented obesity and insulin resistance mainly by affecting the circadian rhythm, while swinging exercise stimulated the overproduction of dopamine to accelerate metabolism of glucose and lipid.

Conclusions Theabrownin and exercise synergistically ameliorated metabolic syndrome in rats and effectively prevented obesity.

Keywords Dopamine · Insulin resistance · Metabolic syndrome · Exercise · Theabrownin · Pu-erh tea

Introduction

Pu-erh tea produced in Yunnan Province, China, by a solid-state microbial fermentation, is being increasingly consumed worldwide. Pu-erh tea is characterized by its distinctive aroma, dark red color, and mild taste [1]. Pu-erh tea has been shown to regulate blood lipid concentration, blood glucose, fat levels in the liver, and weight loss [1–6]. These bioactive properties of Pu-erh tea are attributed mainly to theabrownin, a complex water soluble polyphenolic substance that is insoluble in non-polar organic solvents. The theabrownin content of Pu-erh tea can exceed 18% by weight, but is only about 9% in the well-known black tea [7]. Theabrownin is a brown pigment with multiple aromatic rings and attached residues of polysaccharides and proteins. The major functional groups in theabrownin are carboxyl, hydroxyl, amino, and methyl. Theabrownin has a significant hypolipidemic effect in rats fed a high-fat diet [8, 9] and may have a preventive effect on metabolic syndrome.

Enkai Wu and Tingting Zhang have contributed equally to this work.

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Consumption of theabrownin, or Pu-erh tea, in combination with exercise using a swing, for example, has the potential to synergistically reduce obesity and improve other biochemical health indices, relative to exercise alone. This hypothesis drove the present research. Swings are readily accessible worldwide and are easily used by all age groups without any specific training. Swings have been used since ancient times. For example, since Han dynasty (202–220 BC), swinging has been popular at the traditional Chinese festivals [10, 11]. Regular swinging has been claimed to benefit the nervous system, the respiratory system, the cardiovascular system, skeleton, and muscles [10]. The leg and arm swinging during human walking and running have been shown to improve physical and mental health [12, 13], but it is unclear how swinging alone improves metabolic health in humans.

Metabolic syndrome is an important health issue of the current era. The main features of metabolic syndrome include insulin resistance, hypertension (high blood pressure), abnormal levels of blood cholesterol, and an increased risk of blood clotting. People diagnosed with this syndrome are generally overweight or obese [14].

Although the etiology of metabolic syndrome remains unclear, it is believed to include a variety of genetic, immunological, and environmental factors. Insulin resistance is recognized as a leading cause of metabolic syndrome and an increased physical activity is the primary means of its prevention and treatment [15].

In the present study, we hypothesized that consumption of theabrownin can prevent metabolic syndrome, and further hypothesized that theabrownin consumption in combination with exercise (swinging, in the present work) can synergistically ameliorate obesity and insulin resistance in rats with metabolic syndrome.

To test these hypotheses, we fed rats a diet high in fat, sugar, and salt, and administered a gavage of theabrownin. A swinging motion was applied to the rats' cages. Although this was different from the use of an actual swing by an animal, during swinging of the cages, the rats needed to continually shift their body weight to retain balance and, therefore, consumed more metabolic energy compared to sedentary rats in a static cage. The continual cyclic weight shifting response in effect simulated the use of a swing, although with a reduced physical activity. The various relevant biochemical factors were measured to characterize the metabolic response under various experimental conditions.

Materials and methods

Preparation of theabrownin from Pu-erh tea

Theabrownin from Pu-erh tea was prepared as described previously [16]. Briefly, 20 kg of tea leaves were infused for 30 min with 200 L of distilled water at 70 °C, with intermittent agitation. The suspended solids were removed by filtration. The recovered solids were extracted a second time exactly as above. The clear filtrates were combined and concentrated at a reduced pressure (0.07 MPa, 65 °C). The concentrate was cooled to room temperature and mixed with absolute ethanol (0.25 L ethanol/L concentrate) to precipitate TB. The solids were recovered by centrifugation (3500*g*, 10 min). The recovered solids were lyophilized as the TB extract (125 g/kg Pu-erh tea). TB comprised 75.3% by weight of the lyophilized solids, as measured by a previously published colorimetric method [17].

Experimental diets, animals, and dosages

One-hundred-and-sixty-eight 8-week-old male Sprague–Dawley rats (specific-pathogen-free grade) were sourced from the Experimental Animal Center of Kunming Medical University (experimental animal license: SCXK (Dian) K2015-0002). In the first week, all rats were fed a basal diet [8] with freely accessible food and water to allow them to adapt to the environment and the feeding method. At the beginning of the second week, the rats were randomly divided into 14 groups (12 rats per group) based on their serum cholesterol (TC), triglyceride (TG), and glucose levels. The following groups were not subjected to the swinging regimen: normal group 1 (NG1), model group 1 (MG1), rosiglitazone hydrochloride group 1 (RHG1), lovastatin group 1 (LG1), low-dose theabrownin group 1 (LTB1), medium-dose theabrownin group 1 (MTB1), and high-dose theabrownin group 1 (HTB1). The following groups were subjected to the swinging regimen: normal group 2 (NG2), model group 2 (MG2), rosiglitazone hydrochloride group 2 (RHG2), lovastatin group 2 (LG2), low-dose theabrownin group 2 (LTB2), medium-dose theabrownin group 2 (MTB2), and high-dose theabrownin group 2 (HTB2). The repartition of the animals in different groups is further clarified in Fig. S1.

The intervention trial started after the 1-week adaptation period and lasted for 9 weeks. Apart from the normal control group, all groups were fed a high-fat-salt–sugar diet (Kunming Medical University) (620.0 g/kg basal diet, 100.0 g/kg lard oil, 100.0 g/kg yolk powder, 15.0 g/kg cholesterol, 5.0 g/kg bile salt, 10.0 g/kg table salt, and 150.0 g/kg white granulated sugar), with free access to 15% (*w*/*v*) fructose water daily. The groups subjected to swinging experienced 30 min of swinging each day. For the swinging, the cages were suspended 20 cm above the ground level with a 70 cm-long rope, and swung through an angle of 40° at a frequency of 34–36/min (Fig. S2).

Gavage dosage: an oral gavage of theabrownin was administered just before the daily swinging activity. The recommended daily intake level of theabrownin for humans

is 2.7 g/60 kg body weight [8]. In compliance with the requirements of Health Food Evaluation Procedures and Inspection Methods in China, we trialed low, medium, and high doses of theabrownin. The low dose was defined as the recommended dose for humans, which converted to a rat equivalent dose by multiplying by a conversion factor of 6.25 as recommended by the United States Food and Drug Administration [18]. Thus, the low dose was 0.2812 g/kg body weight (i.e. 2.7 × 6.25/60). The medium dose was defined as 2 × the low dose, or 0.5625 g/kg body weight, and the high dose was defined as 4 × the low dose, i.e., 1.125 g/kg body weight.

The RHG groups of animals were given the insulin-sensitizing drug rosiglitazone hydrochloride (0.13 mg/kg body weight, i.e., the upper limit specified in dosage instructions for this drug) and the LG groups were given the cholesterol lowering drug lovastatin (1.33 mg/kg body weight; i.e., the upper limit specified in the dosage instructions for this drug). Both drugs were administered by daily oral gavage. The other groups received an equal volume of distilled water daily by oral gavage.

Effect of dopamine on body weight

Sixty 8-week-old male Sprague–Dawley rats (specific-pathogen-free grade) were sourced as noted in the previous section. In the first week adaptation period, all rats were fed a basal diet as explained in the previous section and given free access to food and water. At the beginning of the second week, the rats were randomly divided into five groups (12 rats per group) based on their serum TC, TG, and glucose levels. The groups were a normal group 3 (NG3), the model group 3 (MG3), and three dopamine hydrochloride groups [low-dose dopamine, 0.2 mg/kg body weight (LDH); medium-dose dopamine, 0.4 mg/kg body weight (MDH); and high-dose dopamine, 0.8 mg/kg body weight (HDH), administered daily by intraperitoneal injection]. The dopamine hydrochloride intervention trial started after the 1-week adaptation period and lasted 9 weeks. Apart from the NG3 group, all groups were fed a high fat-sugar-salt diet as specified in the previous section. Figure S1 further clarifies the repartition of the animals into various groups.

Preparation of serum and liver specimens

Serum and liver specimens were prepared as previously described [4, 8]. Briefly, the rats were fasted for 12 h and blood samples were collected from their orbits. The samples were held at 37°C for 1 h, and then, the serum was recovered by centrifuging at 5635*g* for 15 min. The recovered serum was kept at 4°C for subsequent analyses. The liver tissue samples were collected after sacrificing the animals by cervical dislocation. The collected tissue was rapidly frozen in liquid nitrogen for subsequent analyses.

Analyses and measurements

Activity and fur status were observed daily and animal deaths were recorded. Body weight and body length were measured weekly, and gavage dose was adjusted weekly according to the body weight. Food and water intake were recorded daily. Serum lipid and fasting serum glucose levels were measured using an automatic biochemical analyzer (model 5421; Olympus, Japan) using the appropriate assay kits specific for each metabolite (Shanghai Kehua Bio-Engineering Co., Ltd.). Fasting serum insulin, stearoyl-coenzyme A desaturase (SCD2), hormone-sensitive triglyceride lipase (HSL), acetyl-CoA carboxylase (ACC), interleukin-1 (IL-1β), interleukin-6 (IL-6), and dopamine were analyzed by enzyme-linked immunosorbent assay on a microplate reader (Mk3; Thermo Fisher Scientific, Waltham, MA, USA) using the relevant assay kits purchased from Shanghai Enzyme-Linked Biotechnology Co., Ltd. Homeostatic model assessments of insulin resistance (HOMA-IR), β-cell function (HOMA-β), and insulin sensitivity (HOMA-IS) were computed as specified by Matthews et al. [19].

Transcriptomic analysis of liver

Extraction of RNA from rat liver tissue was performed using the Trizol method in accordance with the protocol provided the manufacturer (TRIzol®; Thermo Fisher Scientific, Waltham, MA, USA). The quality of total RNA was tested using an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). Samples for sequencing met the following requirements: concentration > 200 ng/μL, total quantity > 20 μg, OD260/OD280 ≥ 1.8, and *28S*/*18S* ≥ 1.0. After total RNA extraction, mRNA was enriched using magnetic oligo(dT) beads (Thermo Fisher Scientific, USA). The resulting mRNA was cut into short fragments by adding a fragmentation buffer. These fragments of mRNA were used as a template for the first-strand cDNA synthesis with a six-base random primer (Random Primer 6; Thermo Fisher Scientific, USA). Subsequently, the buffer, dNTPs, RNase H, and DNA Polymerase I were added for second-strand cDNA synthesis. The products were purified using a QiaQuick PCR kit (Beijing, China) and eluted with the EB buffer, followed by end repair, poly(A) addition, and sequencing adaptor ligation. Fragments of the target size were recovered by agarose gel electrophoresis and amplified by PCR to complete the cDNA library. Sequencing was performed on an Illumina HiSeq 2000 platform (Illumina, San Diego, CA, USA).

Real-time RT-PCR verification of transcriptome sequencing data

The real-time RT-PCR reactions (20 μL) were performed with 50 ng–2 μg of liver total RNA as the template using a FastQuant RT Kit (with gDNase) (KR106, Tiangen; Beijing, China) according to the manufacturer's instructions. Melting curve analysis was conducted in the temperature range of 65–95 °C.

Quantitative RT-PCR primers were designed using Premier 5.0 (Premier Biosoft International, Palo Alto, CA, USA). Rat β-actin (GenBank accession number: NM_031144.3) served as an internal control.

To reduce experimental errors, each sample was amplified three times. Data were analyzed using the $2^{-\Delta\Delta Ct}$ method.

Statistical analysis

Data are presented as the mean ± standard deviation. All data were first subjected to Grubbs' test for outliers, and then analyzed by Duncan's test using SPSS 19.0 (IBM, USA). A p value < 0.05 was considered statistically significant.

Results

Theabrownin and swinging exercise prevented excess body weight gain

There were no significant group differences in body weights at the beginning of the experiment. After 1 week of the high fat-sugar-salt diet, MG1 rats had a significantly higher body weight than NG1 rats ($p < 0.05$) (Table 1). After 3–9 weeks of intervention with theabrownin, MTB1 and HTB1 rats had significantly lower body weights than MG1 rats ($p < 0.05$) and did not significantly differ from NG1 rats, indicating that the medium and high theabrownin doses played a significant role in preventing excess body weight gain in rats. After 9 weeks of intervention, rosiglitazone and lovastatin had no significant effects on body weight in rats fed the high fat-sugar-salt diet ($p > 0.05$; Table 1).

With swinging, no significant differences in body weight were observed between the NG2, MG2, RHG2, LG2, LTB2, MTB2, and HTB2 groups. Apart from the NG group, the average body weight was significantly lower in the groups subjected to swinging ($p < 0.05$; Table 1). This indicates that swinging effectively prevented excess weight gain in rats.

Theabrownin and swinging exercise improved serum lipid indices

Theabrownin alone effectively prevented the elevation of triglyceride (TG) in rats fed the high fat-sugar-salt diet after only 3 weeks at medium and high dose (Fig. 1). However, theabrownin alone showed little effect on cholesterol (CHO) and low-density lipoprotein cholesterol (LDL-C) levels. Surprisingly, CHO and LDL-C were higher in the HTB1 group (without swinging) than in the model MG1 group at week 9. In our previous study, theabrownin could prevent the elevation of CHO and LDL-C in rats fed with a high-fat diet [8]. In another parallel work, theabrownin showed insignificant effect on preventing the elevation of CHO in rats fed with a high sugar diet [20]. Therefore, we suspect that the high fat-sugar-salt diet resulted in a more complicated metabolic syndrome in rats than a high-fat diet alone. This was further reinforced by the fact that either rosiglitazone hydrochloride or lovastatin did not prevent increases in CHO and LDL-C levels in RHG1 and LG1 groups, repetitively (Fig. 1).

Interestingly, swinging prevented the elevation in serum TG, TC, and LDL-C levels in rats fed the high fat-sugar-salt diet after only 3 weeks (Fig. 1). The combination of long-term swinging activity (9 weeks) and a high dose of theabrownin prevented the elevation in TG, TC, and LDL-C levels in rats fed the high-fat-sugar-salt diet ($p < 0.05$).

Theabrownin and swinging exercise synergistically ameliorated insulin resistance

By 9 weeks, swinging had significantly reduced insulin resistance (HOMA-IR) and fasting serum insulin (FINS) level, and increased insulin sensitivity (HOMA-IS) in all groups ($p < 0.05$) except the RHG group (Fig. 2c, d, and f). With or without swinging, HOMA-IR values were significantly higher in the MG1, LG1, LTB1, and MTB1 groups than the NG1 group ($p < 0.05$), showing that the high fat-sugar-salt diet induced insulin resistance in rats. The high dose of theabrownin alone also significantly reduced insulin resistance (HTB1) ($p < 0.05$), but achieved a better effect when combined with swinging (HTB2). With swinging, HOMA-IR values were significantly lower in the HTB2 group than the MG2 group and HTB1 group with no swinging ($p < 0.05$). This suggests that swinging and theabrownin synergistically ameliorated insulin resistance in rats.

With no swinging, theabrownin, rosiglitazone, and lovastatin had no significant effects on serum IL-6 or IL-1β levels, but a high dose of theabrownin alone had a significant effect on serum IL-6. After 9 weeks of swinging, serum IL-6 and IL-1β levels were significantly lower than those of the non-swinging groups ($p < 0.05$), except for IL-6 in the HTB group (Fig. 3). This indicates that swinging effectively regulated serum levels of some inflammatory markers. Thus, our findings indicate that swinging can affect the health of rats by modulating serum levels of inflammatory markers.

Table 1 Effect of theabrownin from Pu-erh tea and swinging on the body weight of rats

Group	Body weight at adaptive time (g)	Body weight (g) at intervention time								
		Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9
NG1	199.0 ± 13.5^{AB}	225.3 ± 24.8^{CD}	235.8 ± 22.4^{AB}	248.0 ± 23.2^{ABC}	270.0 ± 23.7^{ABCD}	285.7 ± 21.3^{ABCD}	305.1 ± 18.2^{BCDE}	315.7 ± 22.3^{BCD}	335.4 ± 22.9^{CD}	346.8 ± 26.0^{CD}
MG1	207.9 ± 13.2^{B}	251.9 ± 14.9^{E}	274.9 ± 22.1^{D}	291.8 ± 19.4^{E}	313.6 ± 23.5^{E}	331.5 ± 27.8^{E}	353.6 ± 29.1^{F}	361.0 ± 30.1^{E}	388.2 ± 31.3^{F}	393.9 ± 34.5^{E}
RHG1	208.6 ± 16.4^{B}	231.6 ± 20.8^{DE}	244.5 ± 19.2^{AB}	264.2 ± 17.4^{BCD}	287.2 ± 20.7^{D}	310.3 ± 22.0^{DE}	328.1 ± 25.6^{EF}	337.1 ± 26.5^{DE}	366.6 ± 28.0^{EF}	374.9 ± 31.6^{DE}
LG1	203.7 ± 13.9^{AB}	235.0 ± 12.6^{DE}	248.6 ± 11.6^{AB}	263.7 ± 15.3^{BCD}	278.5 ± 22.7^{CD}	301.0 ± 22.1^{CD}	322.1 ± 22.9^{CDE}	334.3 ± 19.6^{CD}	360.7 ± 22.2^{DE}	371.3 ± 21.1^{DE}
LTB1	204.2 ± 14.7^{AB}	232.9 ± 17.9^{DE}	259.7 ± 19.3^{BCD}	279.7 ± 22.1^{DE}	292.6 ± 24.9^{DE}	308.7 ± 22.4^{DE}	324.6 ± 21.2^{DE}	336.9 ± 23.4^{DE}	357.8 ± 29.6^{DE}	367.8 ± 28.6^{DE}
MTB1	203.8 ± 12.7^{AB}	222.3 ± 19.9^{BCD}	247.1 ± 16.4^{AB}	262.7 ± 15.1^{BCD}	274.9 ± 22.7^{BCD}	291.7 ± 20.3^{BCD}	300.7 ± 22.9^{ABCD}	315.4 ± 23.0^{BCD}	334.8 ± 28.0^{CD}	346.2 ± 30.5^{CD}
HTB1	208.3 ± 16.3^{B}	227.4 ± 12.9^{CD}	254.2 ± 12.7^{ABC}	267.9 ± 11.8^{CD}	276.00 ± 12.74^{BCD}	290.6 ± 21.1^{BCD}	300.4 ± 26.2^{ABCD}	305.6 ± 22.6^{ABC}	329.2 ± 24.8^{BC}	326.9 ± 25.7^{BC}
NG2	195.1 ± 22.0^{AB}	215.4 ± 25.8^{ABCD}	222.0 ± 30.8^{AB}	236.5 ± 28.3^{A}	258.2 ± 24.2^{ABC}	275.7 ± 27.3^{ABC}	300.4 ± 25.3^{ABCD}	309.1 ± 29.8^{ABC}	328.3 ± 28.7^{BC}	325.2 ± 29.8^{ABC}
MG2	187.7 ± 16.7^{A}	201.2 ± 18.9^{AB}	215.8 ± 22.1^{AB}	242.6 ± 20.5^{AB}	262.7 ± 18.5^{ABC}	276.9 ± 17.1^{ABC}	294.1 ± 18.2^{AB}	303.1 ± 18.5^{ABC}	324.1 ± 20.5^{ABC}	314.2 ± 19.8^{AB}
RHG2	190.5 ± 27.5^{AB}	208.0 ± 33.1^{ABC}	217.5 ± 17.1^{AB}	239.5 ± 20.9^{A}	256.3 ± 19.0^{ABC}	274.7 ± 21.8^{AB}	286.9 ± 24.6^{AB}	292.7 ± 22.8^{ABC}	314.0 ± 22.1^{ABC}	311.2 ± 25.7^{AB}
LG2	188.3 ± 19.0^{A}	198.8 ± 23.9^{A}	206.8 ± 24.2^{A}	230.5 ± 22.6^{A}	250.9 ± 24.6^{AB}	268.3 ± 27.5^{AB}	280.0 ± 30.2^{AB}	290.8 ± 28.0^{AB}	309.7 ± 32.7^{ABC}	308.5 ± 28.5^{AB}
LTB2	206.1 ± 13.7^{AB}	213.8 ± 19.1^{ABCD}	218.9 ± 21.8^{AB}	233.4 ± 23.6^{A}	249.0 ± 32.8^{A}	280.9 ± 28.4^{ABC}	296.2 ± 26.7^{ABC}	306.0 ± 30.5^{ABC}	319.7 ± 27.7^{ABC}	317.6 ± 29.5^{AB}
MTB2	203.0 ± 18.6^{AB}	217.2 ± 20.3^{ABCD}	218.23 ± 25.1^{AB}	236.3 ± 28.1^{A}	255.0 ± 32.0^{ABC}	271.4 ± 32.3^{AB}	283.4 ± 37.1^{AB}	290.9 ± 36.6^{AB}	305.2 ± 34.0^{AB}	303.9 ± 37.3^{AB}
HTB2	203.3 ± 16.39^{AB}	214.3 ± 18.9^{ABCD}	214.28 ± 24.6^{AB}	231.9 ± 26.5^{A}	247.3 ± 22.2^{A}	262.5 ± 27.1^{A}	274.8 ± 27.9^{A}	285.3 ± 28.1^{A}	298.2 ± 28.5^{A}	295.2 ± 30.6^{A}

Data are presented as mean ± standard deviation

There is no significant difference between the data marked by the same capital superscript letters in the a given column ($p > 0.05$); the difference between the data marked by different capital superscript letters is significant ($p < 0.05$)

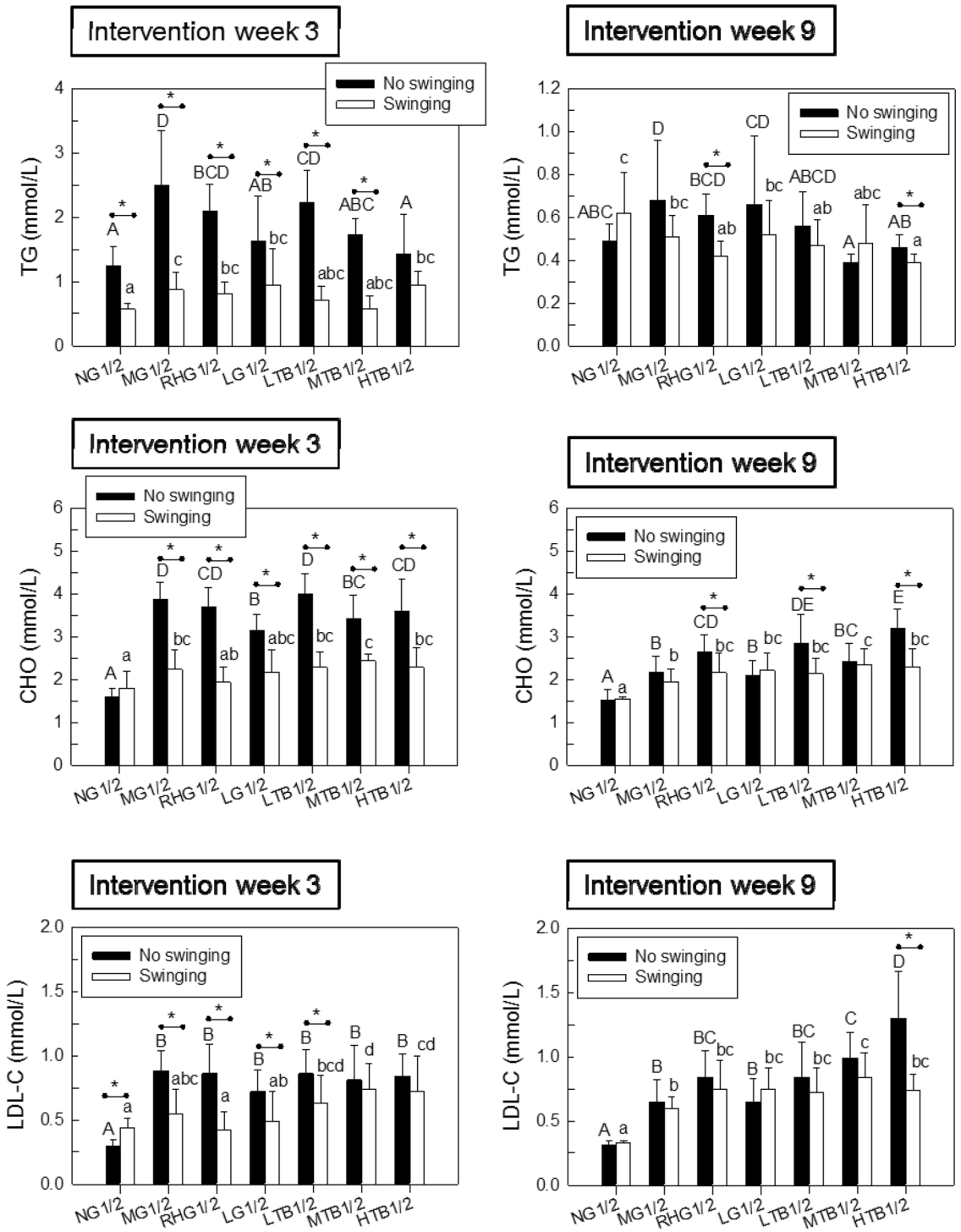

Fig. 1 Synergistic effects of theabrownin and swinging on the serum lipid level. $^{A-E}p < 0.05$ between no swinging groups; $^{a-d}p < 0.05$ between swinging groups; $^{*}p < 0.05$ between no swinging and swinging groups

Theabrownin and swinging exercise synergistically stimulated the expression of genes related to glucolipid metabolism and energy expenditure

We analyzed liver gene expression by transcriptomic analysis and verified the transcriptome sequencing data by quantitative RT-PCR. The difference in the relative expression level was consistent with the trend in the $\log_2$ (FC) value of

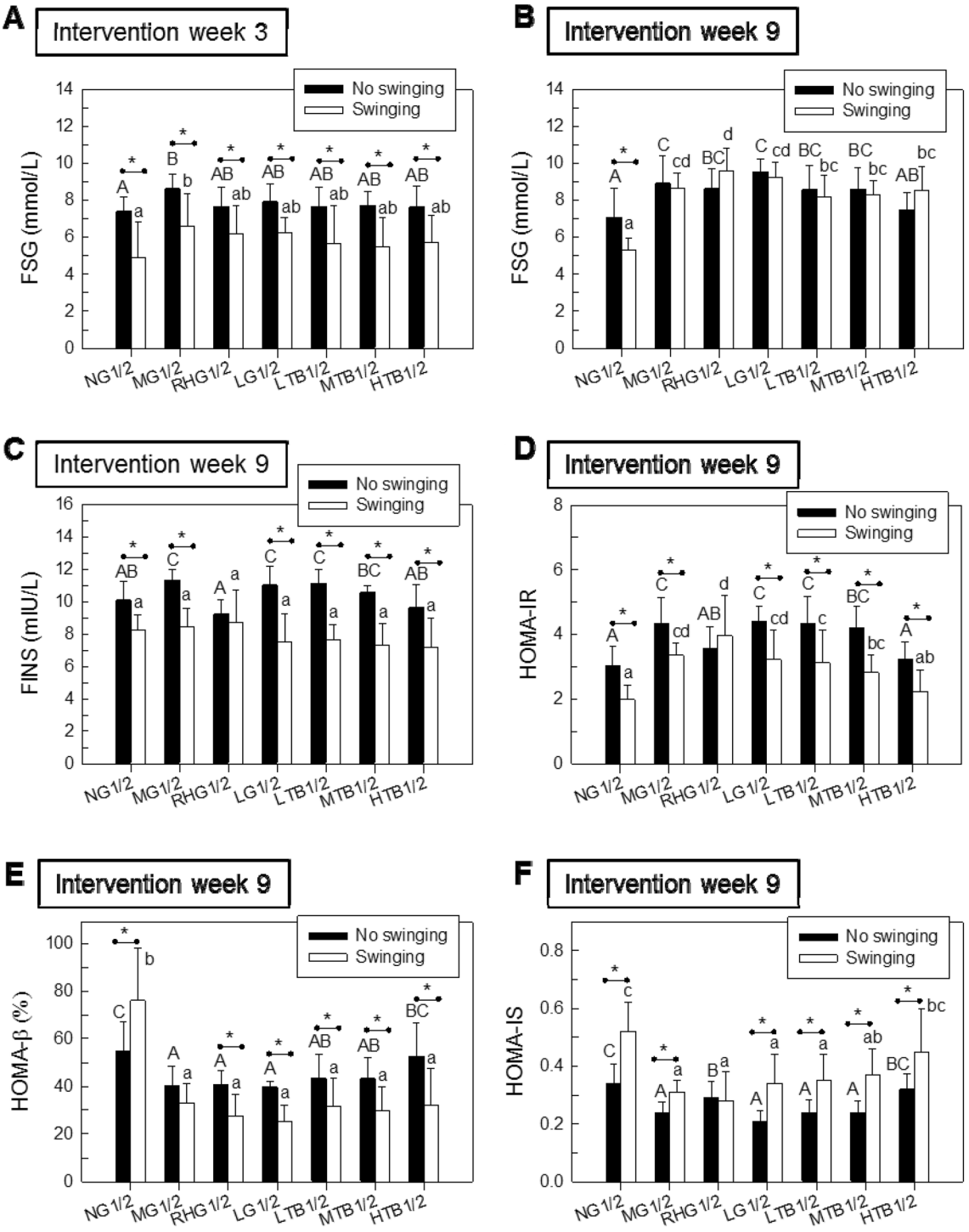

Fig. 2 Synergistic effects of theabrownin and swinging on fasting serum glucose (**a**, **b**), fasting insulin (**c**), HOMA-IR (**d**), HOMA-β (**e**), and HOMA- IS (**f**) obtained by homeostasis model assessment. $^{A-C}p < 0.05$ between no swinging groups; $^{a-d}p < 0.05$ between swinging groups; $^{*}p < 0.05$ between no swinging and swinging groups

the transcriptome (Fig. S4). Swinging mainly affected the immune system, environmental adaptation, signal transduction, transport and catabolism, and development pathways in rat liver (Fig. S5). The high fat-sugar-salt diet had no effect on circadian rhythm in rats under swinging or non-swinging conditions (Fig. 4a, c), and mainly affected the immune system, ribosome, transport and catabolism, signaling molecules and interaction, development, and signal transduction

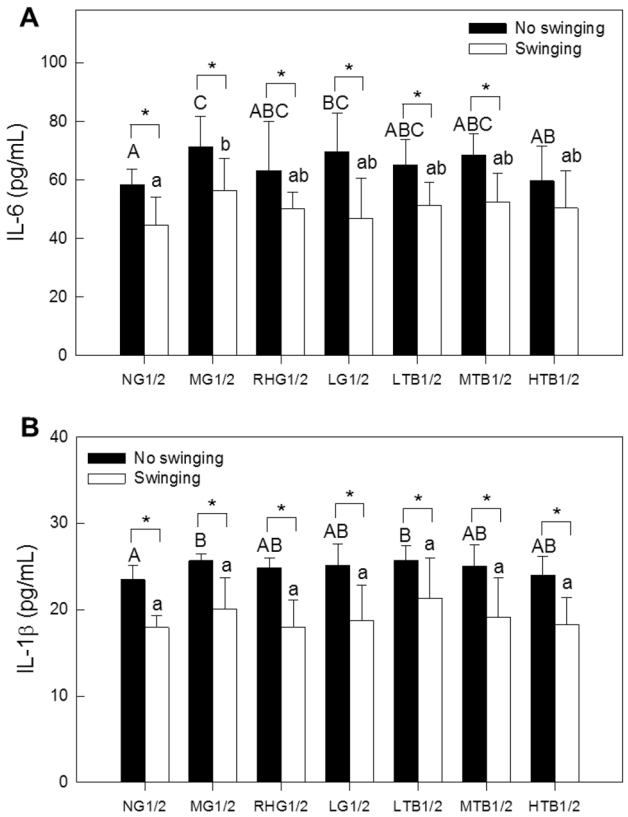

Fig. 3 Synergistic effects of theabrownin and swinging on inflammatory markers IL-6 (**a**) and IL-1β (**b**) in rats. $^{A–C}p < 0.05$ between no swinging groups; $^{a, b}p < 0.05$ between swinging groups; $^{*}p < 0.05$ between no swinging and swinging groups

pathways. In contrast, theabrownin had a significant effect on circadian rhythm in rats fed the high fat-sugar-salt diet under swinging and non-swinging conditions (Fig. 4b, d), and significantly affected glycerolipid metabolism, the insulin signaling pathway, peroxisome proliferators-activated receptors (PPAR) signaling pathway, antigen processing and presentation, and the AMPK signaling pathway.

Under non-swinging conditions, in the MG1 group, *Adcy10* (− 2.591, *Cpt1a* (− 1.108), *Per2* (− 1.334), and *Fos* (− 1.089) were significantly downregulated, and *Pparδ* (+ 1.201), *Slc2a4* (+ 3.585), *Srebf1* (+ 2.399), *Scd* (+ 4.818), *Acc* (+ 1.878), *Fas* (+ 2.525), and *Gck* (+ 2.567) were significantly upregulated, compared with the NG1 group (Table S1). These changes may play a role in the development of obesity, hyperlipidemia and insulin resistance in MG1 rats. In the HTB1 group, *Pparγ* (− 1.072), *Slc2a4* (− 2.932), *Cry1* (− 1.18), *Fas* (− 2.021), *Gck* (− 3.044), and *Bmal1* (*Arntl*) (− 4.767) were significantly downregulated, and *Adcy10* (+ 2.077), *Cpt1a* (+ 1.123), *Per2* (+ 2.393), *Fos* (+ 1.083), *Per3* (+ 3.247), *Nr1d1* (+ 2.837), *Pgc1α* (+ 1.591), *Pparδ* (+ 1.711), *Cyp7a1* (+ 2.079), and *Fads2* (+ 1.347) were significantly upregulated, compared with the MG1 group. These changes may play a role in the regulation of obesity, hyperlipidemia, and insulin resistance by theabrownin in rats.

Under swinging conditions, in the MG2 group, *Adcy10* (− 1.525), *Fads2* (− 1.492), *Per2* (− 1.377), and *Per3* (− 2.665) were significantly downregulated, and *Pgc1α* (+ 1.048), *Slc2a4* (+ 9.861), *Srebf1* (+ 2.951), *Scd* (+ 6.236), *Acc* (+ 2.493), *Fas* (+2.654), *Gck* (+ 1.681), and *Fos* (+ 1.293) were significantly upregulated, compared with the NG2 group (Table 2). In the HTB2 group, *Pparδ* (− 1.118), *Slc2a4* (− 1.954), *Cry1* (− 1.124), *Fas* (− 1.508), *Gck* (− 3.207), *Bmal1* (*Arntl*) (− 3.752), *Clock* (− 1.326), and *Fos* (− 1.692) were significantly downregulated, and *Pgc1α* (+ 1.253), *Cyp7a1* (+ 1.669), *Fads2* (+ 1.033), *Per2* (+ 1.782), *Per3* (+ 4.789), and *Nr1d1* (+ 2.773) were significantly upregulated, compared with the MG2 group. Theabrownin significantly upregulated *Cyp7a1* expression, indicating that it may accelerate cholesterol catabolism in rats.

The transcriptomics results indicate that theabrownin may increase energy expenditure in rats by activating *Adcy10* and *Pgc1α* and regulating circadian rhythm. In addition, theabrownin may reduce fat storage and thus body weight gain by downregulating *Srebf1* and *Scd*.

Swinging exercise prevented weight gain by increasing serum dopamine levels

Swinging significantly increased serum dopamine levels ($p < 0.05$; Fig. 5a), leading us to speculate that the swinging-induced amelioration of metabolic syndrome may be associated with elevated dopamine levels. To investigate this, we administered different doses of dopamine by intraperitoneal injection to rats fed the high fat-sugar-salt diet under resting conditions. After 8 consecutive weeks of intervention, dopamine significantly prevented excess weight gain ($p < 0.05$), compared with the MG3 group, so that there was no significant difference compared with the NG3 group (Fig. 5b). This indicates that dopamine had an obesity-preventive effect. In addition, after 8 weeks of intervention, different doses of dopamine significantly decreased serum TG levels ($p < 0.05$), compared with the MG3 group, so that there was no significant difference compared with the NG3 group (Fig. 5b). However, dopamine had little effect on serum LDL-C, TC, or high-density lipoprotein cholesterol levels. This indicates that dopamine played a role in regulating TG metabolism. The NG3 and HDH groups had significantly higher hormone-sensitive triglyceride lipase activity than the MG3 group ($p < 0.05$; Fig. 5c–f). Medium and high doses of dopamine significantly inhibited ACC and SCD2 activities ($p < 0.05$), and significantly reduced insulin resistance ($p < 0.05$).

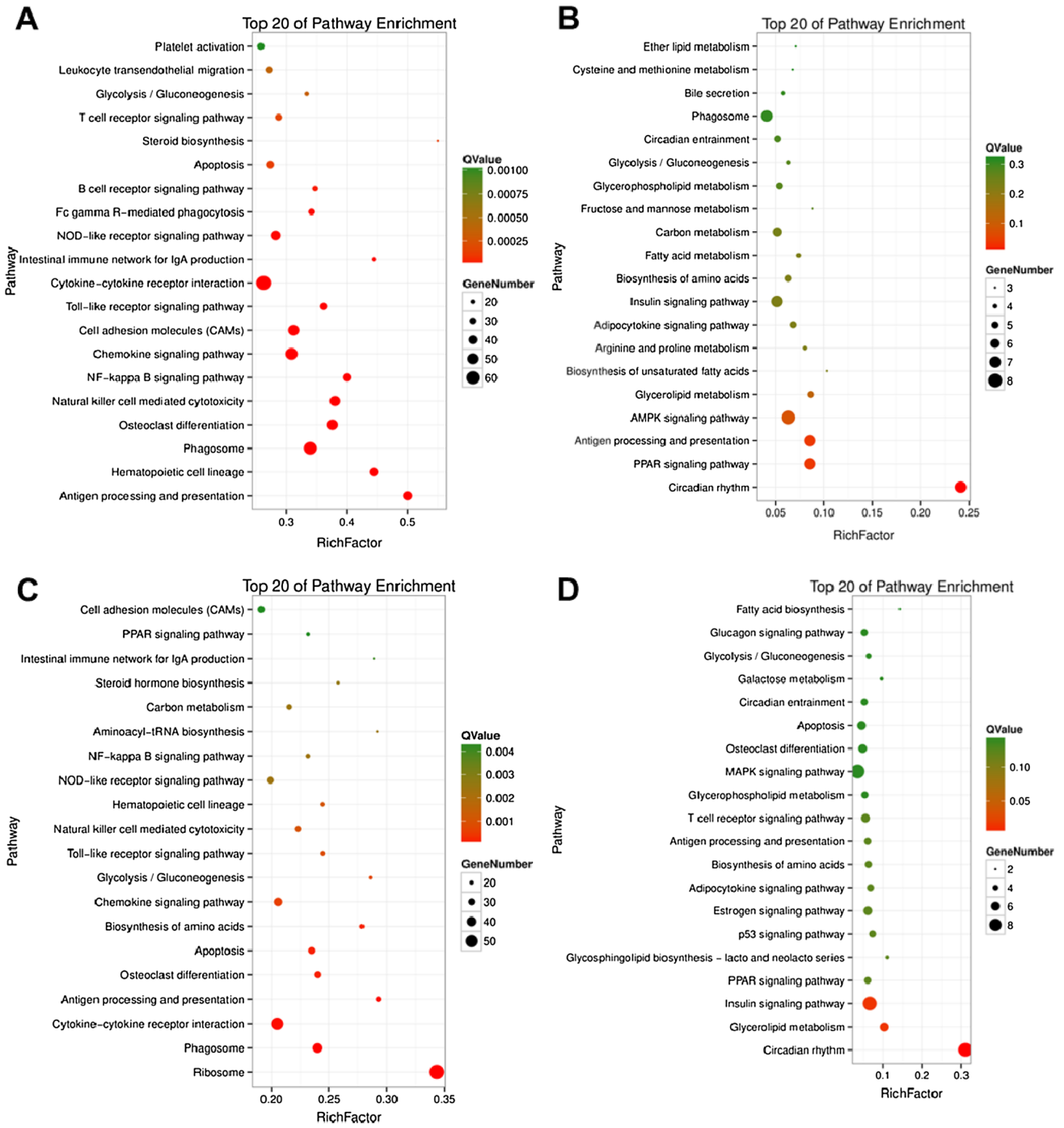

Fig. 4 Effects of swinging and its absence on the pathway enrichment in rat liver: **a** NG1 versus MG1; **b** MG1 versus HTB1; **c** NG2 versus MG2; and **d** MG2 versus HTB2

Discussion

Under non-swinging conditions, prolonged feeding with a high fat-sugar-salt diet induced obesity, insulin resistance, and elevated serum TG, TC, and LDL-C levels in rats. Intervention with rosiglitazone or lovastatin achieved some beneficial effects, but was less effective than intervention with a medium or high doses of theabrownin (Table 1, Figs. 1, 2). Statistically significant differences were found between the model group (MG1) and control group (NG1) in body weight and serum TG, TC, LDL-C, and HOMA-IR levels after 9 weeks of intervention. Thus, the response of the animal model was consistent with the diagnostic criteria for metabolic syndrome [15]. On the basis of these results, the

Table 2 Effect of swinging on expression of the genes related to lipid metabolism, glucose metabolism, and energy metabolism in rats' liver

Gene	NG1-vs-NG2 [$\log_2$ (FC)]	MG1-vs-MG2 [$\log_2$ (FC)]	RHG1-vs-RHG2 [$\log_2$ (FC)]	LG1-vs-LG2 [$\log_2$ (FC)]	LTB1-vs-LTB2 [$\log_2$ (FC)]	MTB1-vs-MTB2 [$\log_2$ (FC)]	HTB1-vs-HTB2 ($\log_2$ (FC))
Adcy10	0.776	1.842[a]	1.209[a]	1.133[a]	1.208[a]	0.589	0.347
Per1	2.010[a]	− 0.517	− 1.797[a]	0.485	− 0.611	0.482	0.026
Per3	1.216[a]	− 1.509[a]	− 0.717	2.602[a]	0.183	0.809	0.032
Noct	0.523	− 0.948	− 0.367	1.268[a]	− 0.363	0.772	− 0.437
Fasn	− 0.443	− 0.323	− 1.147[a]	0.816	− 1.070[a]	0.041	0.189
Cpt1b	0.722	1.495[a]	0.238	0.032	0.603	0.204	1.020[a]
Cpt1a	0.336	0.890	0.669	0.469	0.494	0.541	0.380
Cpt2	0.127	0.489	0.292	0.068	− 0.034	0.341	0.632
Scd	− 0.859	0.559	0.065	0.735	− 0.588	− 0.174	0.482
Scd2	− 0.409	− 1.096[a]	− 0.467	1.253[a]	− 0.730	− 0.103	− 0.602
Fads2	0.243	0.371	0.381	0.745	0.852	0.044	0.057
Fads1	0.181	0.117	0.648	0.654	0.926	− 0.101	− 0.081
Cyp7a1	0.977	− 0.146	0.133	0.374	0.492	0.409	− 0.556
Srebf1	− 0.328	0.223	0.180	− 0.117	0.223	0.135	0.093
Srebf2	− 0.073	− 0.192	− 0.142	0.093	− 0.155	− 0.142	0.141
Irs3	− 0.702	− 1.378[a]	0.000	− 0.580	0.893	0.630	0.585
Irs2	− 0.162	0.247	0.200	0.396	0.229	0.666	0.224
Irs1	− 0.063	0.367	0.226	− 0.012	0.105	0.257	0.264
Slc2a1	− 0.088	− 1.165[a]	− 0.203	0.820	− 0.313	− 0.222	− 0.848
Slc2a4	− 6.129[a]	0.147	− 0.771	0.811	− 0.273	0.193	1.125[a]
Slc2a5	− 0.933	− 0.887	− 1.368[a]	− 0.165	0.339	0.216	− 0.043
Gck	1.525[a]	0.630	0.231	− 1.465[a]	− 0.227	− 0.871	0.468

$\log_2$ (FC) is the pair value of the difference multiple of Sample 1 and Sample 2 FPKM (Fragments Per Kilobase of transcript per Million mapped reads); at the base of 2, we used FDR (false discovery rate) and $\log_2$ (FC) to screen the differential genes with the screening conditions FDR < 0.05 and $|\log_2 (FC)| > 1.0$)

Per1 Period circadian clock 1, *Noct* nocturnin, *Cpt1b* carnitine palmitoyltransferase 1B, *Cpt2* carnitine palmitoyltransferase 2, *Scd* stearoyl-CoA desaturase, *Scd2* stearoyl-Coenzyme A desaturase 2, *Fads1* fatty acid desaturase 1, *Srebf2* sterol regulatory element-binding transcription factor 2, *Irs1* insulin receptor substrate 1, *Irs2* insulin receptor substrate 2, *Irs3* insulin receptor substrate 3, *Slc2a1* solute carrier family 2 member 1 (also known as glucose transporter type 1, GLUT-1), *Slc2a5* solute carrier family 2 member 5 (also known as glucose transporter type 5, GLUT-5)

[a]Significant

transcriptomics data, and the relevant indices (Table S1, Fig. 4 and Figures S4–S5), we propose the following mechanism to explain how theabrownin ameliorates metabolic syndrome in rats in the absence of swinging activity: theabrownin enhances adenylate cyclase (*Adcy10*) activity and elevates the cyclic adenosine monophosphate (cAMP) level, which, in turn, activates protein kinase A (PKA), the AMP-activated protein kinase (AMPK), the circadian rhythm, and the other signaling pathways, and accelerates nutrient metabolism and consumption of sugar and fat, thereby effectively ameliorating the high fat-sugar-salt diet-induced metabolic disease (Fig. S6A). In view of the complex chemical structure of theabrownin, the question of how its metabolic products enhance *Adcy*10 activity in rats needs to be explored in future studies.

Our results show that when rats were subjected to 30 min of a swinging regimen daily, there were no significant differences in body weight between the NG2, MG2, RHG2, LG2, LTB2, MTB2, and HTB2 groups. Swinging also inhibited the release of inflammatory cytokines IL-6 and IL-1β and reduced insulin resistance (Fig. 3). An elevation in IL-6 is an important pathological change in obesity type II diabetes. A short-term IL-6 treatment in skeletal muscle has been shown to improve insulin sensitivity, whereas prolonged IL-6 exposure induced resistance [21]. IL-1β is known to regulate inflammation and is an important inflammatory cytokine in atherosclerotic plaque. An elevation in IL-1β is a pathological mechanism known to trigger insulin resistance [22]. Moreover, a high dose of theabrownin (HTB) combined with swinging significantly reduced insulin resistance ($p < 0.05$; Fig. 2). Further analysis showed that serum dopamine levels significantly increased in rats under swinging conditions ($p < 0.05$; Fig. 5a). When rats fed the high fat-sugar-salt diet were injected intraperitoneally with

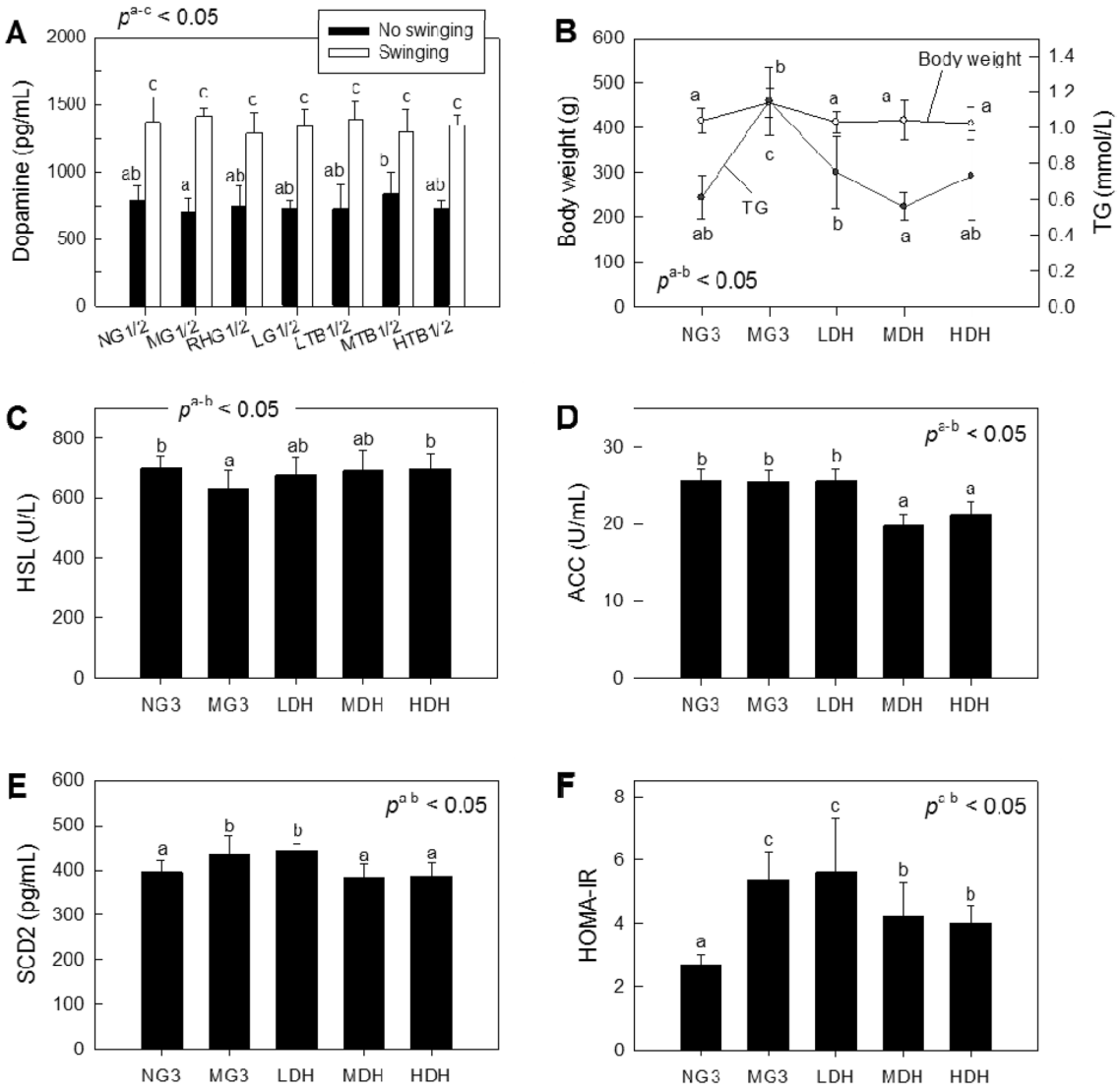

Fig. 5 Effect of swinging and different doses of dopamine (given by intraperitoneal injection) on the rats fed the high fat-sugar-salt diet after 9-week intervention. Effects of swinging on dopamine level in rat serum (**a**). Effects of the injected dopamine dose on: **b** the body weight and TG content in rat serum; **c** the HSL activity; **d** the ACC activity; **e** the SCD2 activity; and **f** the HOMA-IR activity; $^{a-c}p < 0.05$

dopamine, we found that dopamine significantly prevented excess weight gain and serum TG levels, and, thus, induced hypolipidemic and body weight-reducing effects (Fig. 5b). Dopamine is a key neurotransmitter in the hypothalamus and pituitary, a neurotransmitter for happiness signals, and one of the most important catecholamine neurotransmitters in the central nervous system. Dopamine plays a major role in regulating various functions of the brain such as motion, cognition, emotion, positive reinforcement, feeding, and incretion [23, 24]. Adenylate cyclase activity is enhanced and cAMP levels are elevated after activation of the D1 dopaminergic receptor [25, 26].

Under swinging conditions, in the HTB2 group, *Pgc1α*, *Cyp7a1*, *Fads2*, *Per2*, *Per3*, and *Nr1d1* were significantly upregulated, and *Pparδ*, *Slc2a4*, *Cry1*, *Fas*, *Gck*, *Bmal1* (*Arntl*), *Clock*, and *Fos* were significantly downregulated compared with the MG2 group (Table 1). BMAL1 is also known as aryl hydrocarbon receptor nuclear translocator-like protein 1 and the *Bmal1* (*Arntl*) has been identified as a candidate gene for susceptibility to hypertension, diabetes, and obesity [27, 28]. Mutations in BMAL1 have been linked to infertility, gluconeogenesis and lipogenesis problems, and altered sleep patterns. BMAL1, according to genome-wide profiling, is estimated to target more than 150 sites in the human genome, including all of the clock genes and genes encoding for proteins that regulate metabolism [29]. Recent phenotype data suggest that *Arntl* gene [30] and its partner *Clock* play a role in the regulation

of glucose homeostasis and metabolism [31], which can lead to hypoinsulinemia, or diabetes, when disrupted [32]. Another study has shown that the CLOCK/BMAL1 complex upregulates human LDLR promoter activity, suggesting that the *Arntl* gene also plays a role in cholesterol homeostasis [33]. *Pgc-1α* is a co-activator of numerous transcription factors in energy metabolism pathways and plays a crucial role in energy metabolism regulation. In addition to regulating adaptive thermogenesis, *Pgc-1α* is also closely related to glucose metabolism and fatty acid oxidation [34]. *Pgc-1α* has also been shown to be a potent co-activator of *Pparδ*. Overexpression of active *Pparδ* in white and brown adipose tissues can upregulate gene expression associated with fatty acid oxidation and energy expenditure, preventing high-fat diet-induced obesity and fatty liver, and alleviating hyperlipidemia [35]. Cyp7a1 is a rate-limiting enzyme in the bile acid synthesis pathway, catalyzing the breakdown of cholesterol into bile acids in the liver [36].

Swinging also activated *Adcy10* gene transcription in rat liver (Table 2) in the MG2, RHG2, LG2, and LTB2 groups, probably as a result of the swinging-induced increase in dopamine secretion. This would help to accelerate energy metabolism and increase calorie expenditure, thereby affecting body weight. Moreover, swinging affected the transcription of glucolipid metabolism-related enzyme genes such as *Cpt1b*, *Cpt1a*, *Cpt2*, *Slc2a1*, *Scd*, *Slc2a4*, *Gck*, and *Irs* (Table 2). Both swinging and theabrownin affected circadian rhythm in rats, particularly *Per1*, *Per2*, *Per3*, and *Noct* (nocturnin) (Table 2).

Existing research shows that high-fat diets can increase the body weight of mice by affecting the oscillation of clock genes and their target genes [37]. This weight-increasing effect has been found to be inhibited by short-term energy constraints [38]. Paschos et al. [39] reported that adipocyte-specific deletion of *Arntl* (also known as *Bmal1*), a gene encoding a core molecular clock component, results in obesity in mice with a shift in the diurnal rhythm of food intake.

High-fat diets can induce changes in the expression cycles of many core clock genes and clock-controlled genes, while nighttime feeding and insulin resistance can cause significant changes in the amplitude of certain genes [40].

In the present study, both theabrownin and swinging affected the circadian rhythm and modulated the expression of clock-controlled genes (e.g., *Per1*, *Per2*, *Per3*, *Noct*, and *Cry*), thereby influencing body weight and insulin resistance in rats. This may be an important mechanism by which theabrownin and swinging ameliorate metabolic disease in rats. On the basis of our findings, we speculate that swinging promotes dopamine production in rats, and that dopamine binds to its receptor and enhances *Adcy*10 activity, thereby increasing cAMP levels and activating PKA, AMPK, circadian rhythm, and insulin signaling pathways.

The externally generated swinging of the cages physically exercised the rats as their whole body muscles had to respond to the shifting position of a cage to keep the body in balance. Swinging-induced activity mainly affected the immune system and the environmental adaptation pathways, and stimulated the production of dopamine which, in turn, regulated metabolism and feeding behavior. Theabrownin had a significant effect on circadian rhythm, insulin signaling pathway, and peroxisome proliferators-activated receptors (PPAR) in the liver, and may have activated pathways of glucose and lipid metabolism by accelerating energy metabolism and depletion, thus preventing metabolic syndrome. Swinging and theabrownin were synergistically effective in preventing metabolic syndrome in rats (Fig. S6 B).

Concluding remarks

Theabrownin consumption can effectively prevent obesity and insulin resistance in rats. Theabrownin consumption combined with swinging-induced exercise synergistically prevented obesity and ameliorated metabolic syndrome in rats. A regular consumption of theabrownin-rich Pu-erh tea alone and sustained physical activity alone are both known to independently ameliorate metabolic syndrome in humans. Based on this and the results in the rat model, the dietary consumption of theabrownin combined with readily accessible and mild physical activity may synergistically ameliorate metabolic syndrome in humans.

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Author contributions JSG obtained financial support, and designed and oversaw this study. EKW performed the swinging research and analyzed data. TTZ performed the non-swinging research and analyzed data. CT designed the experimental swing. QPW, CXP, and YC prepared, reviewed, and edited the manuscript. All authors contributed to the discussion, read the final manuscript and approved it. JSG is the guarantor of this work.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

Animal ethics statement Experiments on animals were conducted in full compliance with the Yunnan Agricultural University institutional and Chinese national guidelines for care and use of laboratory animals.

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