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Original Article

Effect of diet and physical exercise on endocannabinoid system and energy homeostasis in obese mice

Fangyuan Ju[1], Xiaofeng Zhu[2], Guanggao Zhao[3], Qin Yang[4]

[1]Department of Physical Education, College of Sports and Recreation, National Taiwan Normal University, Taipei, China; [2]Child Development Research Center of Jiaxing University, Jiaxing University Pinghu Campus, Jiaxing, China; [3]Department of Physical Education, Nanchang University, Nanchang, China; [4]International College of Football Tongji University, Shanghai, China

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Abstract: This study investigated whether a combination of diet and exercise could improve endocannabinoid system (ECS) and related-energy homeostasis during weight loss in obese mice compared to standard diet intervention. High-fat diet (HFD)-induced obese mice were randomly divided into three groups: an HFD group (OB), a standard diet (SD) group (D) and a diet plus exercise group (D+E) fed with SD and trained on treadmill for 840 m/d, 6 d/w at 14 m/min and lasted for 6 weeks. More significant effect of weight loss (25.47 ± 0.35 vs. 28.99 ± 1.75 g; P<0.01) and reduction of body fat rate (2.30 ± 0.57 vs. 2.89 ± 0.57%, P<0.05) were observed in the D+E group compared to the D group. Both interventions demonstrated comparable changes in improving cannabinoid levels (decreased AEA (10.31 ± 8.14 vs. 25.02 ± 10.45 pg/ml, P<0.05) in D group and decreased 2-AG (77.84 ± 47.97 vs. 180.81 ± 57.49 pg/ml, P<0.05) and AEA (9.69 ± 6.64 vs. 25.02 ± 10.45 pg/ml, P<0.01) in D+E group compared to OB group in serum), cannabinoid receptor CB2 and transcriptional expression of metabolic enzymes except for CB1 receptor. Besides, total energy expenditure, respiratory exchange rate, and daily energy intake showed no significant difference between the two interventions. Fasting blood glucose levels was positively correlated with brain AEA levels (r=0.522, P=0.026) and negatively correlated with brain CB1 mRNA levels (r=-0.735, P=0.003), indicating an inherent regulatory relationship between central ECS signal and physiological blood glucose stability after weight loss. Taken together, these findings provide a new perspective on the effects of diet alone and a combination of diet-exercise-induced weight loss on lipid metabolism regulation pathway-ECS signal.

Keywords: Diet, exercise, training, weight loss, endocannabinoid system, energy metabolism

Introduction

Obesity-related metabolic disorders are closely related to the over-activation of endocannabinoid system (ECS). The ECS is a complex signaling cascade consisting of endocannabinoid receptor 1 and 2 (CB1 and CB2), enzymes for the synthesis (diacylglycerol lipase α/β (DAGLα/β) and N-acyl-phosphatidyl-ethanolamine-selective phospholipase D (NAPE-PLD)) and degradation (monoacylglycerol lipase (MAGL), and fatty acid amide hydrolase (FAAH)) of endogenous ligands (mainly 2-AG and AEA) (**Figure 1**) [1, 2]. Previous studies have found an elevated ECS tone in the liver, muscles, adipose tissue (AT), and hypothalamus during obese status [3, 4]. Over the last decade, second and third-generation of CB1 receptor antagonists have been used for the treatment of obesity [5]. CB1 antagonist has been shown to reduce weight and enhance improvements in cardiometabolic risk parameters in obese populations [6, 7]; and their effect has been compared to diet intervention and physical exercise [8-10]. Nevertheless, the effects of the diet on ECS tend to be sporadic and may vary during weight loss [11, 12]. Englei *et al.* found that CB1 and FAAH mRNA levels were decreased in adipose tissue of obese individuals but were unaffected after a 5% weight loss through calorie restriction [13]. Bennetzen *et al.* found increased levels of 2-AG in subcutaneous AT, increased expression of CB1 in abdominal AT, and decreased expression of CB1 in gluteal AT after diet-induced weight loss in obese individuals [14]. A similar study using dietary intervention reported the persistence of some lipid metabolites, including the EC components dur-

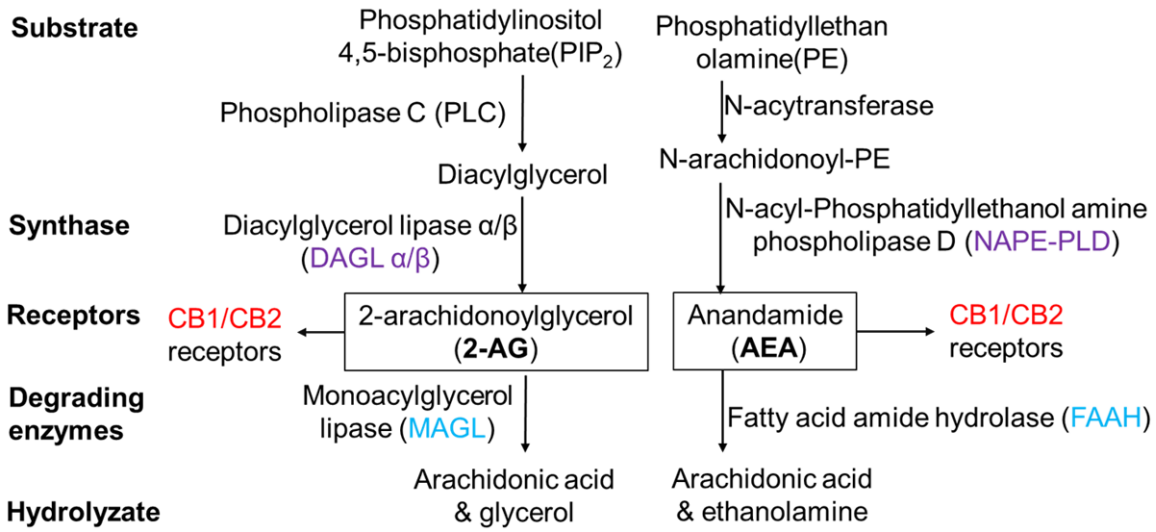

Figure 1. Primary metabolic pathways regulating 2-AG (left) and AEA (right) synthesis and degradation.

ing weight loss, which may not be conducive to metabolic improvement and weight maintenance [15]. In contrast, the effect of exercise on ECS appears to be distinctly dual [16]. Acute moderate-intensity exercises can increase plasma AEA, but not 2-AG levels [17]. Moreover, long-term aerobic exercise reduces the CB1 expression in the striatum and hippocampus of adolescent rats, and adipose tissue of rats treated with a high fat diet (HFD) [9, 18]. Additionally, a combined effect of diet and physical exercise on ECS showed a superior effect on weight loss and metabolism [19]. The long term effect of diet and exercise intervention may lead to a significant decrease in plasma 2-AG and AEA levels in obese subjects [13, 20]. Basic circulating EC levels have been reported to be related to the degree of metabolic improvement [21]. Yet, so far, only a few studies have explored whether the combination of diet and physical exercise may improve the ECS in metabolic organs compared to diet intervention only. Also, it is well known that weight loss methods can affect energy balance; while the relationship between changes in energy balance and ECS in metabolic organs after weight loss have not yet been explored.

In this study, we investigated the effects of diet and physical exercise on ECS in central and peripheral metabolic organs during weight loss and analyzed whether changes in ECS components are associated with changes in blood lipids and energy homeostasis after weight loss. We hypothesized that a combination of diet and physical exercise might improve ECS components compared to diet intervention only. This study elucidated the possible role of ECS components during a diet with or without exercise-induced weight loss and provided a basis for understanding the abnormal presence of lipid metabolites after weight loss.

Materials and methods

Animal grouping

C57Bl/6J male mice, 4-week-old, were purchased from Experimental Animal Center of Second Military Medical University, Shanghai, China. All the animals were housed in an environment with a temperature of 22 ± 1°C, relative humidity of 50 ± 1%, and a light/dark cycle of 12/12 hr. All animal studies (including the mice euthanasia procedure) were conducted in accordance with the ethical guidelines of Shanghai University of Sport institutional animal care and were in complete compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Every effort was made to minimize any pain or discomfort, and the minimum number of animals was used.

After a week of adaptive feeding, mice were fed with a high-fat diet (D12492, Research Diets, containing 60% fat, 20% protein, and 20% carbohydrate of total calories) or standard diet (SD, D12450J, Research Diets, containing 10% fat, 20% protein, and 70% carbohydrate of total calories) for 12 weeks to establish obese mice

Table 1. Primer sequences used for RT-PCR

Gene ID	Sense primer (5' to 3')	Anti-sense primer (5' to 3')
CB1	TGCTGTTGCTGTTCATTGTG	TTGCCATCTTCTGAGGTGTG
CB2	CGACTCCAACGCTATCTTCC	GGTAGGCGGGTAACACAGAC
DAGLα	GCCTGGCCATCTTGGTGATT	CAGTGATGCAGACGCTGAGA
DAGLβ	CTCCACCAGCAACAAGACAA	CAAACGGCATGTAATGATGG
NAPE-PLD	GCCGCACACTTCATACCTTT	TCTCCCGCCATGTCTCTATT
MAGL	AACATCTCAACCACTAAGCCC	GAGAAAGGGAAGTGTGAGGTG
FAAH	GTATCGCCAGTCCGTCATTG	GCCTATACCCTTTTTCATGCCC
GAPDH	GGTTGTCTCCTGCGACTTCA	TAGGGCCTCTCTTGCTCAGT

Note: CB1/2, endocannabinoid receptor 1/2; DAGLα/β, diacylglycerol lipase α/β; NAPE-PLD, N-acyl-phosphatidyl-ethanolamine-selective phospholipase D; MAGL, monoacylglycerol lipase; FAAH, fatty acid amide hydrolase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

model. Obese mice were then randomly divided into three groups (n=9 for each group): obese group (OB group) fed with HFD; diet group (D group) fed with SD; Diet+Exercise group (D+E group) fed with SD and trained on the treadmill for 14 m/min, 840/d, 6 d/w. The experiment lasted for 6 weeks.

Body weight (BW) was measured every week. Body fat percentage (BFP) was measured according to the following formula: *BFP = bilateral perirenal fat weight plus epididymal fat weight)/BW*100%*. Blood samples were allowed to stand for 2 hours at 4°C, after which they were centrifuged at 2500 rpm for 10 min to collect serum.

All mice were sacrificed two days after the final intervention session with a 12 h fasting period. Consequently, their brain, liver, eWAT, and gastrocnemius were collected, weighed, and snap-frozen in liquid nitrogen before being stored at -80°C until analyses.

Blood metabolic indicators assays

Fasting blood glucose (FBG, Catalog: CK-E00592M, R&D systems), triglycerides (TG, Catalog: K622-100, BioVision) and high/low-density lipoprotein cholesterol (H/LDL-C, Catalog: MAK045, Sigma) were measured by enzyme-linked immunosorbent assay (ELISA) kits using a microplate spectrophotometer (BioTek Instruments).

RNA extraction and real-time PCR (RT-PCR)

After collection, samples (~50 mg of tissue) were treated with 0.5 ml TRIzol (Lot: 117206, Invitrogen) according to the manufacturer's instructions. The final preparation of RNA was considered DNA- and protein-free if the ratio between readings at 260/280 nm was ≥1.8 and ≤2.1. Total RNA was then reverse-transcribed to cDNA using a Revert Aid First Strand cDNA Synthesis Kit (catalog: K1622, Thermo Scientific). The transcripts were stored at -80°C until analysis. For RT-PCR, SYBR Green Real-Time PCR Master Mix (Lot: 16385700, Roche) was used in a total volume of 20 μL. The primers sequences (synthesized by Sangon Biotech Co. Ltd. Shanghai, China) are listed in **Table 1**. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was selected as an internal reference gene. The samples were evaluated in duplicate using an equal load of 10 ng of cDNA/well, and data were collected using the ABI StepOne Plus system (Applied Biosystems). The relative gene expression levels were determined according to the $2^{-\Delta\Delta Ct}$ method.

Endocannabinoid assays

2-AG and AEA contents in the whole brain and serum were detected by LC-MS/MS methods using Agilent 1290 systems (Agilent Technologies, Wilmington, Delaware, USA) as previously described [22].

Energy metabolic measurements

Metabolic chambers (PhenoMaster; TSE Systems, Germany) were used to measure oxygen consumption (VO_2), CO_2 production (VCO_2), total energy expenditure (TEE), voluntary activity, and food intake according to previous methods [23]. The respiratory exchange ratio (RER) was calculated using the following equation: $RER = VCO_2/VO_2$. Metabolic chambers were debugged according to the manufacturer's instructions. All parameters were monitored every 33 min and were normalized to body weight. Before the measurement, six mice were randomly selected from each group and individually placed in metabolic cages for two days acclimatization.

Statistical analyses

Data are expressed as mean ± SE. Kolmogorov-Smirnov testing was used to ensure that data were normally distributed. A two-way ANOVA with repeated measures, followed by a Tukey

Table 2. Changes of morphological and blood indicators in two groups

	OB (n=8~9)	D (n=8~9)	D+E (n=7~8)
BW (g)	39.21 ± 1.10	28.99 ± 1.75**	25.47 ± 0.35**,##
BFP (%)	7.72 ± 1.5	2.89 ± 0.57**	2.30 ± 0.57**,#
FBG (mmol/L)	6.08 ± 0.45	5.75 ± 0.50	5.88 ± 0.53
TG (mmol/L)	3.30 ± 0.56	1.34 ± 0.59**	1.31 ± 0.28**
HDL-C (μg/μL)	1.43 ± 0.12	1.96 ± 0.36**	2.09 ± 0.16**
LDL-C (μg /μL)	1.29 ± 0.03	0.80 ± 0.07**	0.84 ± 0.27**

Note: OB: obese group; D: standard diet group; D+E: diet+exercise group. BW, body weight; BFP, body fat percentage; FBG, fasting blood glucose; TG, triglycerides; H/LDL-C: high/low-density lipoprotein cholesterol. *vs. OB group, **P<0.01; #vs. diet group, #P<0.05, ##P<0.01.

post-hoc test was used to compare the differences among OB, D and D+E groups using SPSS25.0 (Armonk, NY). Two-tailed independent t-test was conducted to compare the continuous variables between D and D+E groups. Pearson's correlation was used to analyze the relationship between the changes in morphology, blood indicators, energy homeostasis levels, and ECS components under the condition of taking group D and group D+E as a whole. A 2-tailed P value <0.05 was considered to statistically significant.

Results

changes in morphological and blood indicators after two interventions (diet vs. diet+exercise)

Compared to the OB group, reduced BW, BFP, TG, and LDL-C and increased HDL-C levels were observed in the D group and D+E group (P<0.05 for all comparisons), while there was no difference in FBG levels (P=0.58 and P=0.76, respectively) (**Table 2**). Moreover, BW (28.99 ± 1.75 *vs.* 25.47 ± 0.35 g, P<0.01) and BFP (2.89 ± 0.57 *vs.* 2.30 ± 0.57%, P<0.05) were significantly lower in the D+E group compared to the D group (**Table 2**).

changes of ecs components in central and peripheral metabolic organs after two interventions (diet vs. diet+exercise)

Compared to the OB group (25.02 ± 10.45 pg/ml), the AEA levels in the serum, but not in the brain (P=0.141 and P=0.085, respectively) were significantly reduced in the D+E group (9.69 ± 6.64 pg/ml, P<0.01) and D group (10.31 ± 8.14 pg/ml, P<0.01). Moreover, the 2-AG levels in the serum were decreased in the D+E group (77.84 ± 47.97 pg/ml) compared to the OB group (180.81 ± 57.49 pg/ml, P<0.05).

As **Figure 2** shows, in the brain, both interventions reduced CB1 (P<0.01 for both comparisons), DAGLα/β (P<0.05 for each comparison), NAPE-PLD, MAGL and FAAH (P<0.01 for all comparisons) transcriptional expression.

Compared to the OB group, increased transcriptional expression of CB1 was found in the liver, eWAT, and gastrocnemius (P<0.01 for all comparisons) of the D group. Moreover, the transcriptional expression of CB2 decreased in eWAT (P<0.01 and P<0.01) and gastrocnemius (P<0.05 and P<0.01); the DAGLα decreased in liver (P<0.01 and P<0.01) and eWAT (P<0.01 and P<0.01), and increased in gastrocnemius (P<0.05 and P<0.01); the DAGLβ decreased in liver (P<0.01 and P<0.01) and eWAT (P<0.01 and P<0.01); the NAPE-PLD decreased in liver (P<0.01 and P<0.01) and eWAT (P<0.05 and P<0.01); the FAAH increased in liver (P<0.01 and P<0.01) and gastrocnemius (P<0.01 and P<0.01) in both D and D+E group. In addition, diet+exercise intervention reduced MAGL transcriptional expression in eWAT (P<0.01), while diet intervention reduced MAGL transcriptional expression in gastrocnemius (P<0.01), which suggests an organ-specific expression trend following different interventions.

comparison of energy homeostasis between groups (diet vs. diet+exercise)

Next, we compared the energy homeostasis levels between the D and D+E groups. Although there were differences in RER and TEE between groups within some individual periods, no significant difference was found in the overall average RER (Light: 0.81 ± 0.05 *vs.* 0.80 ± 0.05, P=0.73; Dark: 0.88 ± 0.05 *vs.* 0.90 ± 0.02, P=0.35) and TEE (Light: 19.78 ± 1.01 *vs.* 21.39 ± 1.64 kcal/kg/hr, P=0.07; Dark: 24.14 ± 2.18 *vs.* 26.10 ± 1.40 kcal/kg/hr, P=0.09) levels between groups. Also, there was no significant difference in the amount of daily food intake (Light: 1.05 ± 0.16 *vs.* 0.91 ± 0.44 g, P=0.45; Dark: 3.10 ± 0.68 *vs.* 2.74 ± 0.63 kcal/kg/hr, P=0.09) throughout the day and the levels of voluntary activity (Light: 1.50 ± 0.32 *vs.* 0.93 ± 0.59 × 10^3 counts, P=0.06) during the daytime, while the levels of voluntary activity during the nighttime greatly decreased (Dark: 4.86 ± 2.37 *vs.* 2.31 ± 0.91 × 10^3 counts, P<0.05) (**Figure 3**), which may be due to a long

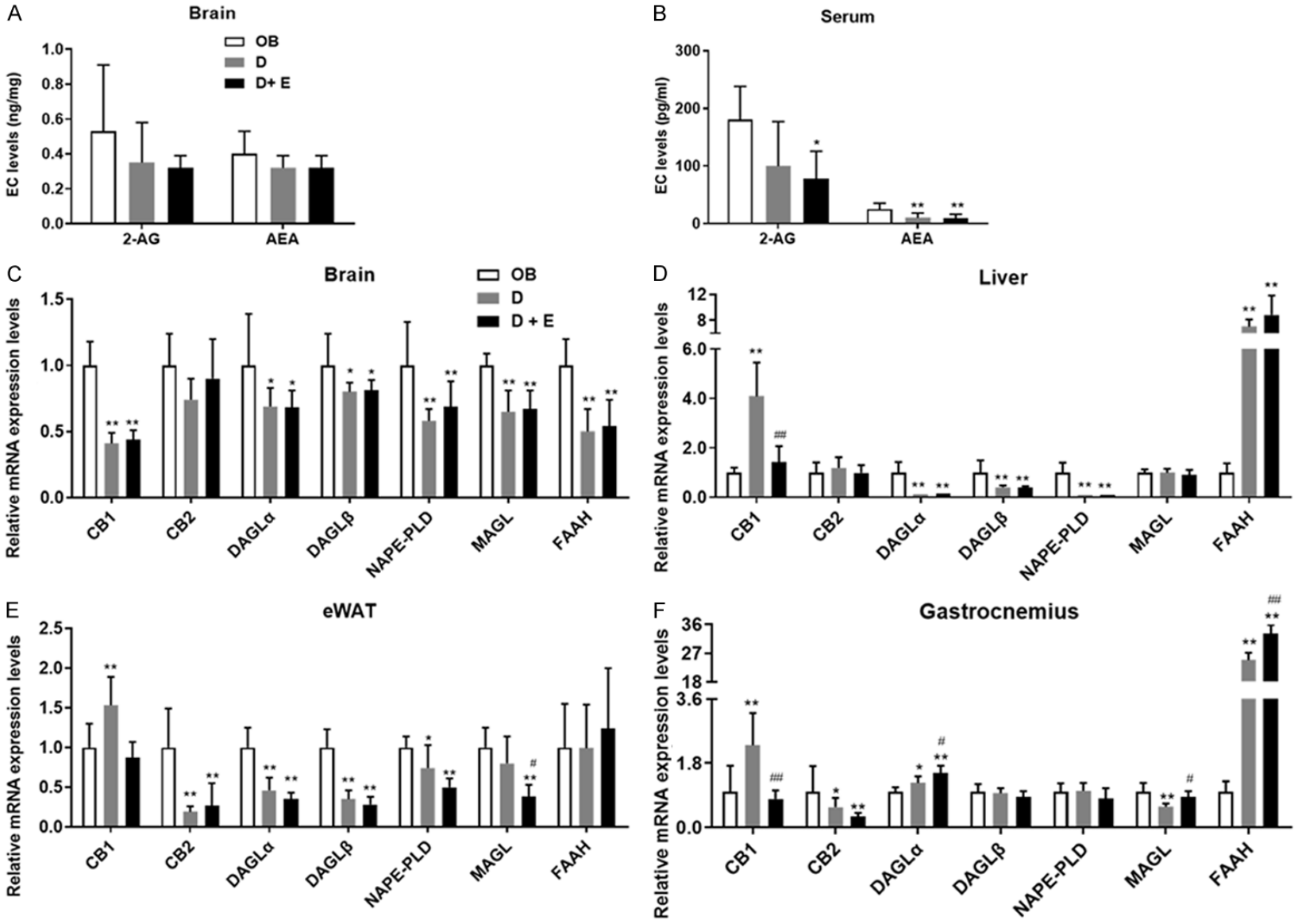

Figure 2. 2-AG/AEA levels in the brain (A) and serum (B), and the transcriptional expression of cannabinoid receptors and metabolic enzymes in the brain (C), liver (D), epididymal fat (E) and gastrocnemius (F) between the D group and D+E group. (n=9 in each group). Note: *vs.* OB group, $^{*}P<0.05$; $^{**}P<0.01$; $^{\#}$ *vs.* D group, $^{\#}P<0.05$, $^{\#\#}P<0.01$. OB: obese group; D: standard diet group; D+E: diet+exercise group. CB1/2, endocannabinoid receptor 1/2; DAGLα/β, diacylglycerol lipase α/β; NAPE-PLD, N-acyl-phosphatidyl-ethanolamine-selective phospholipase D; MAGL, monoacylglycerol lipase; FAAH, fatty acid amide hydrolase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

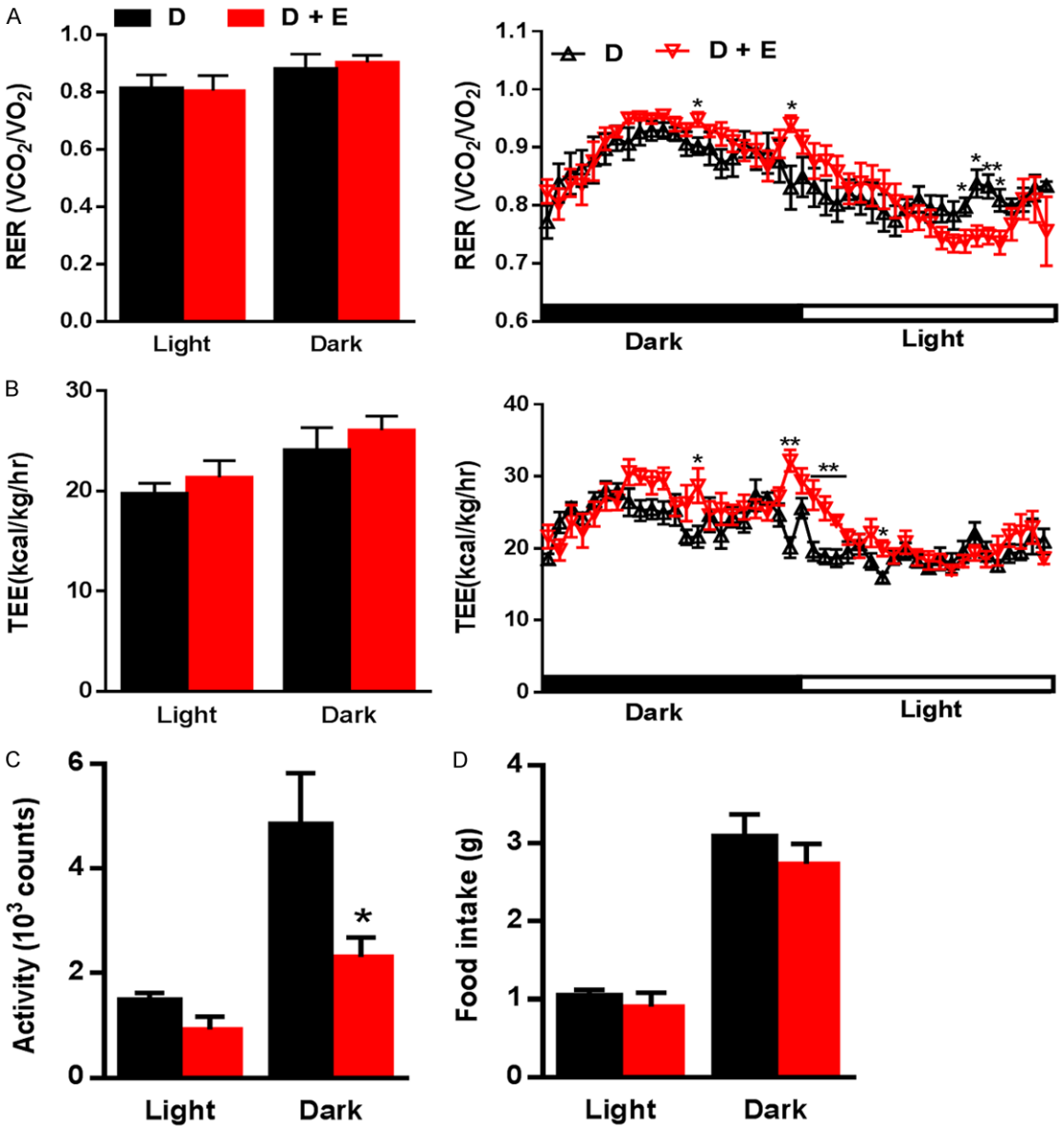

Figure 3. Comparison of RER (A), TEE (B), voluntary activity (C), and food intake (D) between D group and D+E group (n=6 in each group). Note: *vs. D group, *P<0.05; **P<0.01. D: standard diet group; D+E: diet+exercise group; TEE: total energy expenditure, RER: respiratory exchange rate.

period of passive exercise at night in the diet plus exercise group.

Correlation analysis between changes in morphological, blood lipids, energy homeostasis, and ECS components

We analyzed whether changes in morphology, blood lipid indexes, and energy homeostasis were related to changes in ECS composition after weight loss in the D and D+E groups (**Figure 4**). We found that only the FBG level was positively correlated with brain AEA contents (r=0.522, P=0.026) and negatively correlated with brain relative CB1 mRNA (r=-0.735, P=0.003) transcription levels; other ECS components were not correlated with morphological, blood lipids or energy homeostasis indicators after weight loss.

Discussion

Previous studies have shown that a combination of diet and physical exercise may have a

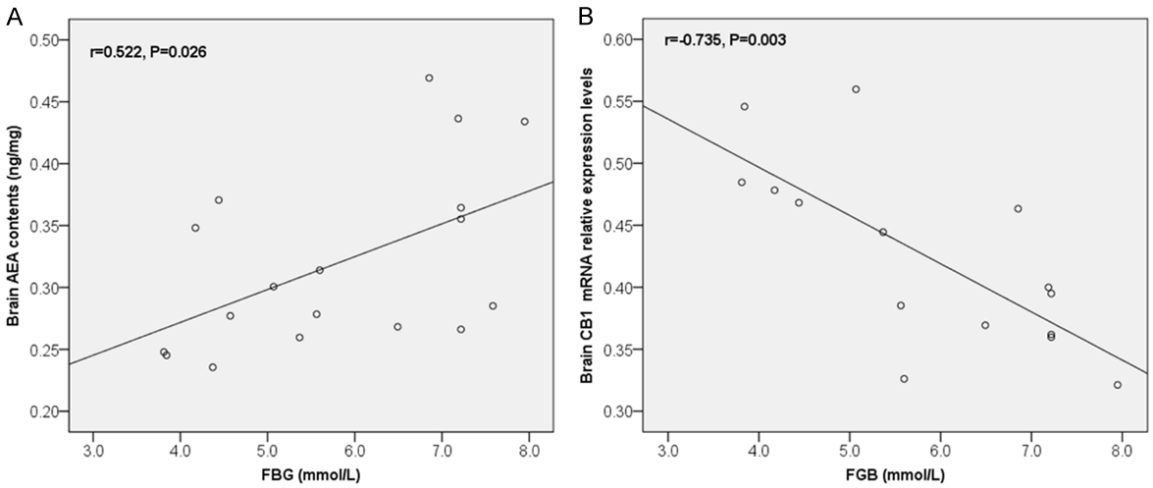

Figure 4. Pearson correlation analysis between brain AEA contents (A), brain CB1 mRNA relative expression levels (B) and FBG levels in D group and D+E group (Two groups as a whole, n=18 in A and n=15 in B). AEA: anandamide; CB1: Endocannabinoid receptor 1; FBG: Fasting blood glucose.

higher effect on weight loss, fat loss, and cardiopulmonary fitness compared to diet alone [19, 24]. However, the two interventions (diet alone and combination of diet and exercise) have a different effect on blood lipids. Compared with diet alone, the combination of diet and exercise can greatly improve HDL-C, fasting blood glucose, and insulin levels; in contrast, diet alone is more effective in reducing TG and LDL-C [25]. In this study, we found that a combination of diet and exercise might induce more significant weight loss and fat loss compared to diet-only intervention.

Obesity is accompanied by over-activation of various ECS components in different internal organs. The existing studies reporting on the diet effect on ECS are somehow inconsistent. For example, Engeli *et al.* showed that a diet, which induces 5% weight loss, has no significant effect on serum 2-AG, AEA levels, and CB1 and FAAH mRNA levels in subcutaneous adipose tissue [13]. Contrary, Bennetzen *et al.* found that dietary intervention leading to 10% of weight loss can increase the 2-AG in gluteal and abdominal adipose tissue and decrease the transcriptional expressions of CB1, FAAH and MAGL in gluteal adipose tissue, but has no effect on AEA [14]. These data suggest that dietary interventions may have different effects on ECS components in various organs. In this study, we found that diet alone and a combination of diet and exercise may improve cannabinoid levels, increase the expression of cannabinoid receptors, and metabolic enzymes; yet, an evident increase of CB1 transcriptional expression in liver, epididymal fat tissue, and gastrocnemius was found in diet intervention group compared to D+E group. The decrease of cannabinoid synthase and the upregulation of degradative enzymes in central and peripheral organs may account for the downward trend of cannabinoid levels in central and peripheral organs. However, the up-regulation of CB1 transcriptional expression in peripheral organs, such as liver, adipose tissue, and gastrocnemius muscle may be one of the persistent ECS components after diet-induced weight loss. We speculate that switching from HFD to the standard diet may trigger a protective response to counter the negative emotions, which, in turn, may be compensated by an increased peripheral CB1 signaling. With our results and previous reports on EC-like compounds that might persist after diet-induced weight loss [15], we further compared the similarities and differences of D and D+E intervention in the regulation of ECS.

Previous studies have already reported that the combined effect of diet and exercise on the over-activated ECS components is organ-specific during weight loss [2, 21, 26]. Di Marzo *et al.* [20] observed a decrease in 2-AG levels, but not AEA, which were correlated with a decrease in visceral adipose tissue (VAT) and triacylglycerol levels, following a 1 year nutritional and exercise interventions in viscerally obese men.

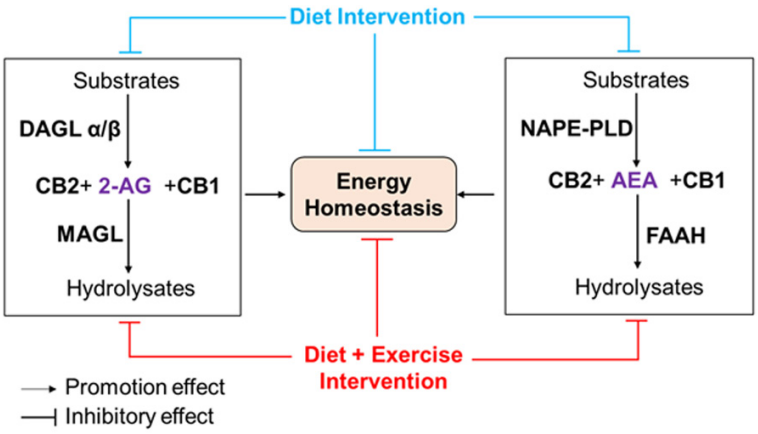

Figure 5. Both diet and diet combined with exercise intervention could produce comparable benefits in rebalancing the endocannabinoid system components and regulating energy homeostasis. Note: Blue, effects of Diet intervention; red font, effects of Diet+Exercise intervention.

Yet, only few studies assessed the effects of diet and exercise intervention on ECS components in the brain, liver, and skeletal muscles. In the present study, we found a similar effect when applying diet alone and a combination of diet and exercise on the ECS components in brain and peripheral organs; both interventions reduced the transcriptional expression of cannabinoid receptors, and synthase, and increased the expression of cannabinoid degrading enzymes. These results are consistent with Di Marzo *et al.* [26], which suggested that multiple interventions can rebalance ECS. Together with the current reports that basic EC levels may affect the metabolic benefits after multiple interventions-induced weight loss [21, 27], we believe that a combination of diet and exercise may not be superior to diet alone in terms of improving ECS in central and peripheral metabolic organs such as liver, white adipose tissue and gastrocnemius (**Figure 5**).

Moreover, studies have confirmed that both diet alone and a combination of diet and exercise may result in weight loss and metabolic profile improvement [25, 28]. Early research suggests that calorie restriction is associated with compensatory changes in energy expenditure [29]. Lopes *et al.* showed that a combination of diet and exercise-induced weight loss stimulates an increase of basal metabolic rate (BMR), a major influencing factor of total energy expenditure. In contrast, diet alone was not enough to produce this effect [24]. In this study, no significant difference was found in TEE between two interventions, thus suggesting that both diet and a combination of diet and exercise might have equivalent effect in improving energy homeostasis. Further analysis revealed that there was no difference in total energy intake and daytime activity levels between two groups of mice, while the nocturnal activity of mice in diet plus exercise group decreased evidently, which may due to the long-term passive exercise intervention at night. Yet, the higher BMR caused by diet and exercise intervention, which has been confirmed in previous studies [24], may compensate for the declined energy consumption caused by decreased nocturnal activity. Thus, the two groups showed comparable energy homeostasis after weight loss (**Figure 5**).

Subsequently, we explored whether the changes in ECS components are related to changes in blood lipids or energy homeostasis in obese mice treated with standard diet or a combination of standard diet and exercise. Unfortunately, a significant correlation was found only between brain ECS components and FBG. Glucose is the primary energy source for the brain [30]. In this study, the FBG levels returned to normal levels after the body weight was reduced. Previous studies have indicated that the AEA-associated endocannabinoid signaling can increase insulin resistance [10]. The positive correlation between FBG and brain AEA content suggested that central cannabinoids might be involved in the regulation of blood glucose stability in individuals with normal blood glucose after weight loss. In addition, both interventions reduced brain CB1 mRNA levels, which may indicate a rebalance of the central ECS signaling that tends to be over-activated in obesity [31]. Nevertheless, whether CB1 mRNA levels have higher or lower expression compared to normal healthy mice after weight loss still remains unknown. Finally, the negative correlation between FBG and CB1 mRNA expression suggests that, to a certain extent, lower central CB1 mRNA expression after weight loss is more beneficial to achieve a relatively high blood glucose levels within the physiological range. This is similar to previous reports suggesting that inhibition of CB1 expression con-

tributes to promoting blood glucose stability [30, 32]. Taken together, these results suggest that central AEA and CB1 signal may be involved in the regulation of physiological blood glucose stability after diet with or without exercise-induced weight loss.

This study also has a few limitations: 1) The levels of 2-AG and AEA in peripheral organs, such as liver, eWAT, and gastrocnemius, were not detected. 2) The detection of ECS components in the brain may not accurately reflect its function in different regions such as the hypothalamus, hippocampus, and arcuate nucleus. 3) Changes in transcriptional levels of ECS related enzymes do not necessarily reflect changes in protein translational levels. Thus, further studies should investigate ECS changes in these organs after weight loss.

Conclusion

In conclusion, we believe that both diet and diet combined with exercise intervention could produce comparable benefits in rebalancing the ECS components and regulating energy homeostasis. Central AEA and CB1 signal may be involved in the regulation of peripheral blood glucose stability after weight loss. Our results provide a new perspective on the effects of diet alone and a combination of diet-exercise-induced weight loss on lipid metabolism regulation pathway-ECS signal. Yet, future studies should focus on investigating the multiple comprehensive interventions-regulating central and peripheral ECS during weight loss.

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Disclosure of conflict of interest

None.

Address correspondence to: Dr. Qin Yang, International College of Football Tongji University, 1239 Siping Road, Yangpu District, Shanghai 200092, China. Tel: +86-021-55135072; E-mail: yangqinsus@163.com

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