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ORIGINAL ARTICLE

Effects of exercise and diet intervention on appetite-regulating hormones associated with miRNAs in obese children

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Abstract

Aims This study investigated the effects of exercise and diet intervention on appetite-regulating hormones and subjective appetite changes in obese children and examined expressions of specific key microRNAs (miRNA, miR).

Methods 16 obese children were included in a training program consisting of exercise and diet intervention for 6 weeks. Before and after the intervention, fasting blood was collected to determine appetite-regulating hormones (leptin, ghrelin, and orexin) and miRNA (miR-103a-3p and miR-200a-3p) levels; eating behavior of the children was reported using the Children Eating Behavior Questionnaire (CEBQ).

Results The level of orexin was significantly decreased ($P<0.05$), while ghrelin was significantly enhanced ($P<0.05$) after 6 weeks. The scores of food responsiveness (FR) and enjoyment of food (EF) of the CEBQ were significantly decreased ($P<0.05$) after intervention. The changes of leptin and that of SR were significantly correlated ($r=-0.455$, $P<0.05$), and the correlation between the alterations of orexin and that of EF was moderate with significance ($r=0.625$, $P<0.05$). miR-103a-3p expression was not statistically changed, while miR-200a-3p was significantly inhibited after 6-week intervention ($P<0.05$). The correlation between relative changes of miR-103a-3p and that of leptin and orexin were both with significant difference ($r=0.413$, $P<0.05$; $r=0.409$, $P<0.05$), whereas the alterations of miR-200a-3p were not correlative with hormones or appetite sensation.

Conclusion Exercise combined with diet intervention for 6 weeks was effective in regulating appetite sensations and hormones in obese children, and miR-103a-3p and miR-200a-3p might provide a foundation for target biomarkers of appetite trait in modulating the energy balance control by exercise and dietary intervention.

Level of evidence Level III, case–control analytic study.

Trial registration The trial was registered in ClinicalTrials.gov (NCT03762629).

Keywords Exercise · Diet · Obesity · Appetite

Introduction

Obesity in children usually develops from early age and predicts negative health consequence in adult life. Addressing appetite is a critical strategy to control food intake and thus impacts chronic energy imbalance. Exercise intervention is widely accepted as major way to increase energy expenditure during weight management, and habitual physical activity and appetite control are interconnected [1]. Long-term exercise has been shown to have the capacity to alter the sensitivity of the appetite-regulatory system [2, 3]. In addition, available evidences have proposed a regulatory role of diet modification on appetite [4, 5]; therefore, behavioral

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approach to combine exercise with diet restriction could better help appetite phenotype switching of obese children.

Appetite-regulating hormones are closely associated with appetite control and eating behaviors, among which circulating leptin, ghrelin, and orexin are mainly studied in obesity and reported to be involved during exercise-induced weight losing of obese population [6–8]. Leptin, produced in the adipose tissue, and ghrelin, produced in the stomach, are both able to signal to the hypothalamus to have opposite influences on food intake [9, 10], whose functions are shown to be impaired during the progression of obesity [11]. Orexin, mainly expressed in the hypothalamus, induced feeding behavior and was reduced in obesity [12, 13]. Circulating level of both peripheral appetite regulating hormones (leptin and ghrelin) and centrally released hormone (orexin) could represent the overlapping effects of obese subjects after different interventions.

microRNA (miRNA, miR) is a short non-coding RNA (18–25 nucleotides) that negatively influences the expression of the target mRNA via incomplete or complete binding to its 3′-untranslated region [14, 15]; these post-transcriptional modifications could better explain appetite adaptations to both exercise and obesity especially during childhood development prior to adulthood. Available findings have strongly posited miRNAs as potential candidates in exploring the mechanisms of energy balance control in hypothalamus [16], among which miR-200a-3p and miR-103a-3p have been causally implicated in the modulation of this process [17, 18].

Therefore, the current study was designed to examine the circulating levels of appetite-regulating hormones after exercise and dietary intervention in obese children and their relationship with key miRNAs, whose function have not been confirmed in human research. The Children Eating Behavior Questionnaire (CEBQ) was also conducted before and after the intervention to measure the changes of subjective appetite.

Methods and materials

Subjects and ethics statement

A total of 19 obese children without known severe cardiovascular or malignant diseases according to medical interviews were recruited from Shenzhen Sunstarasia Weight Loss Camp. Body mass index (BMI) ≥ 30 kg/m^2 were considered as obese. 16 obese children and their parents have completed all of the interventions and examinations. Ethics approval of this study was obtained from the Human Ethics Board of the Guangzhou Sport University and in accordance with the Declaration of Helsinki.

Exercise and diet intervention program

The exercise training program lasted for 6 weeks set out by exercise physiologist and monitored by qualified trainers. All obese children were subjected to series of trainings (1500–2500 kcal/day) comprised of moderate exercise (50–60% of maximum heart rate), high-intensity interval exercise (80–90% of maximum heart rate), and resistance training (12–15 repetition maximum), which were performed 5 h/day and 6 day/week. The dietary component of this intervention had a calorie intake ranged from 1300 to 2000 kcal/day according to individualized training and physiological conditions consisting of 50% carbohydrate, 25–30% fat, and 20–25% protein, which was designed by professional nutritionists. Total energy intake of a day was distributed in breakfast (30%), lunch (40%), and supper (30%). All examinations were conducted before and after the intervention program. Body composition was measured using a body composition analyzer (Inbody 370, Biospace, Korea). Venous blood was collected after overnight fast to determine the metabolic parameters.

Subjective appetite evaluation by the CEBQ

Eating behavior of the children was evaluated using the CEBQ by their parents, which has been validated in Chinese children population in a Chinese context [19, 20]. The CEBQ consists of 35 items in 8 dimensions: Food Responsiveness (FR), Enjoyment of Food (EF), Satiety Responsiveness (SR), Slowness in Eating (SE), Emotional Under-eating (EUE), Emotional Overeating (EOE), Desire to Drink (DD), and Food Fussiness (FF). SE and SR have been combined into one scale to represent the satiety responsiveness since they load to the same factor [21]. High score of FR and EF and low score of SR and SE reflect high level of appetite and thus used in the current study.

Plasma appetite hormone assays

EDTA blood samples were centrifuged for 10 min at 3000 rpm to obtain the plasma. Leptin, ghrelin, and orexin were determined using commercially available ELISA kits (EK-H11570, EK-H10925, and EK-H10983, Huayun Biotech, China) according to the manufacturer's protocols.

RNA extraction and quantitative real-time PCR

Plasma was collected avoiding contamination and removed carefully into a plastic sterile polycarbonate tube. miRcute Serum/plasma miRNA isolation kit (DP503, Tiangen, China) was used to isolate the total RNA according to the instructions.

All samples were spectrophotometrically measured (ND1000 NanoDrop, Thermo Scientific, USA) to determine quality of RNA and degree of haemolysis. Ratio of absorbance at 260 nm to that at 280 nm above 1.8 indicated high quality of RNA, and absorbance below 0.2 at 414 nm indicated low level of haemolysis, which were included in the study. After the addition of 25 fmol *C. elegans* cel-miR-39 (5′-UCACCG GGUGUAAAUCAGCUUG-3′) as spike in control of each sample, cDNA was synthesized from total RNA using Revert Aid First-Strand cDNA Synthesis Kit (K1622, Thermo Fisher, USA) and amplified in a reaction mixture containing 12.5 µl FastStart Universal SYBR Green Master (Rox) (04913914001, Roche, Switzerland), 2.5 µl of forward primer, 2.5 µl of reverse primers, 2.0 µl cDNA, and 8.0 µl DNase-free water. All PCRs were performed in triplicate and monitored using a detection system (Steponeplus, Applied Biosystems, USA), initiating at 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s, and 60 °C for 60 s. All samples were subjected to 95 °C for 15 s, 60 °C for 1 min, 95 °C for 15 s, and 60 °C for 15 s to draw the dissociation curve with threshold cycle (Ct) set below 35. The stem–loop reverse transcription (RT) primers and amplification primers for miR-103a-3p (RT: #ssD1282210530; forward: #ssD1282210530; reverse: #ssD089261711), miR-200a-3p (RT: #ssD809230227; forward: #ssD809230919; reverse: #ssD089261711), and C. elegans cel-miR-39 (RT: #ssD1083145001; forward: #ssD1083145002; reverse: #ssD089261711) were designed and purchased from Ribobio (Ribobio Co., Ltd, China). Synthetic *C. elegans* cel-miR-39 was the normalization factor to the raw Ct value obtained from each assay; the $2^{-\Delta\Delta Ct}$ method was used to calculate relative expressions of miRNAs.

Statistical analysis

Values are presented as means ± SD, with SPSS 13.0 software used for analysis. When the data were not normally distributed, log-transformation was applied. Paired-sample *t* tests were used to compare the data before and after the intervention. Correlation between relative changes was determined by Pearson correlation analysis. Linear dependence between two variables was estimated through correlation coefficient (*r*), whose value gives strength of linear relationship with 0.3, 0.5 and 0.8 being interpreted as poor, moderate and strong, respectively. $P < 0.05$ was considered significant. Figures were obtained using Sigma Plot 11.0.

Results

The effect of exercise and diet intervention on weight losing and metabolic parameters

Both BMI and body fat were significantly reduced by ~11.1% ($P < 0.001$) and ~17.6% ($P < 0.001$) after 6 weeks. Circulating levels of cholesterol, triglycerides, and low-density lipoprotein cholesterol (LDL-L) were also decreased with significance ($P < 0.05$), and high-density lipoprotein cholesterol (HDL-L) was significantly increased ($P < 0.05$). Fasting plasma glucose (FPG) was decrease but with no significant difference. Changes in anthropometric and metabolic parameters are summarized in Table 1.

The effect of exercise and diet intervention on appetite-regulating hormones

The levels of leptin and orexin were decreased after 6 weeks intervention with only statistical significance in orexin ($P < 0.05$), and the circulating level of ghrelin was significantly enhanced ($P < 0.05$) (Fig. 1).

The effect of exercise and diet intervention on subjective appetite sensations

The scores of FR and EF were significantly decreased ($P < 0.05$) after intervention. No significant change was found on SR and SE, even though they were observed increased compared with baseline (Table 2).

Table 1 Changes of anthropometric characteristics and metabolic parameters

	Baseline	Endline	*P*
Age (year)	12.74 ± 1.94		
BMI (kg/m^2)	27.74 ± 3.33	24.66 ± 3.11	< 0.001
Body fat (%)	40.00 ± 4.99	32.95 ± 4.66	< 0.001
Cholesterol (mmol/l)	4.23 ± 0.87	3.55 ± 0.57	< 0.001
Triglycerides (mmol/l)	0.77 ± 0.34	0.55 ± 0.19	0.001
HDL-L (mmol/l)	1.29 ± 0.18	1.40 ± 0.17	0.022
LDL-L (mmol/l)	2.22 ± 0.60	1.66 ± 0.41	< 0.001
FPG (mmol/l)	4.42 ± 0.47	4.16 ± 0.50	0.131

Data are provided as mean ± SD

BMI body mass index, *HDL-L* high-density lipoprotein cholesterol, *LDL-L* low-density lipoprotein cholesterol, *FPG* fasting plasma glucose

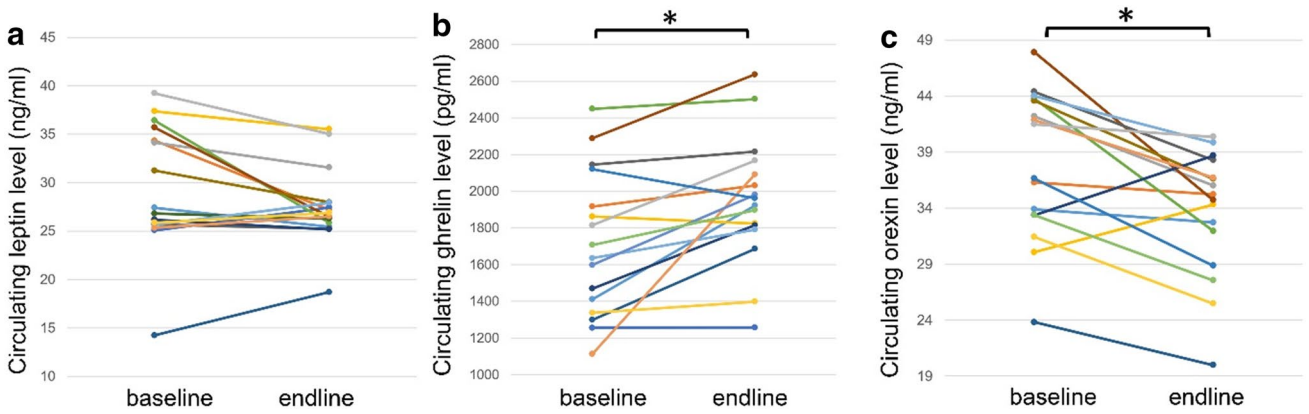

Fig. 1 The effect of exercise and diet intervention on appetite-regulating hormones: leptin (**a**), ghrelin (**b**), and orexin (**c**) ($n=16$). Asterisks indicate $P<0.05$

Table 2 Changes of subjective appetite sensation

	Baseline	Endline	*P*
FR	3.68 ± 0.91	3.08 ± 0.83	0.009
EF	4.01 ± 0.76	3.59 ± 0.71	0.041
SR	2.64 ± 0.56	2.87 ± 0.44	0.114
SE	2.25 ± 0.75	2.50 ± 0.50	0.101

Data are provided as mean ± SD

FR food responsiveness, *EF* enjoyment of food, *SR* satiety responsiveness, *SE* slowness in eating

The effect of exercise and diet intervention on circulating miR-103a-3p and miR-200a-3p

The expression of miR-103a-3p was not statistically changed, while miR-200a-3p was observed significantly decreased after 6 weeks intervention ($P<0.05$) (Fig. 2).

The correlation between relative changes of appetite-regulating hormones and that of subjective appetite sensation

There was no correlation between ghrelin and subjective appetite sensation; the changes of leptin and that of SR were significantly correlated ($P<0.05$) (Table 3 and Fig. 3a); the correlation between the alterations of orexin and that of EF was moderate with significance ($P<0.05$) (Table 3 and Fig. 3b).

The correlation between relative changes of miRNAs and that of appetite hormones and sensation

The correlation between relative changes of miR-103a-3p and that of leptin and orexin was both with significant difference ($P<0.05$) (Fig. 3c, d); there was also a potential correlation between change of miR-103a-3p and EF but with no significance. The alterations of miR-200a-3p were shown

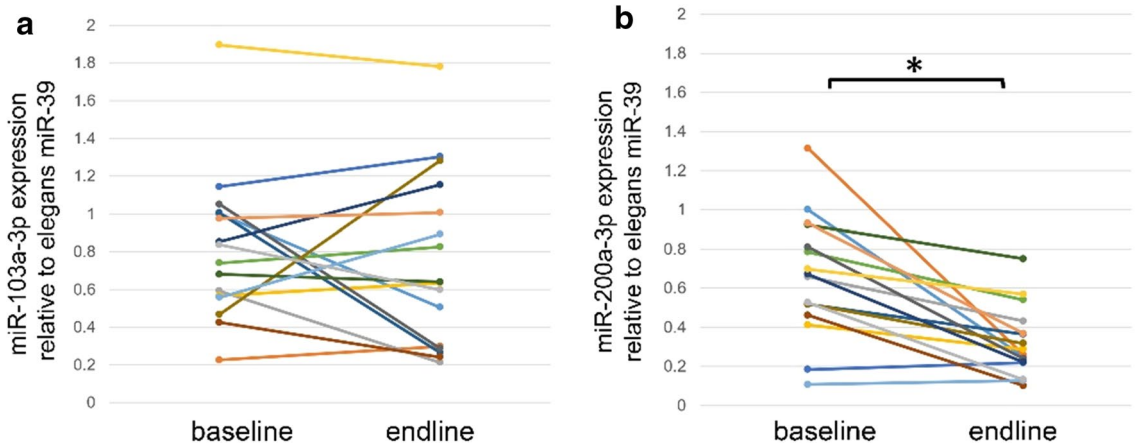

Fig. 2 The effect of exercise and diet intervention on circulating miR-103a-3p (**a**) and miR-200a-3p (**b**) ($n=16$). Asterisks indicate $P<0.05$

Table 3 Correlation with relative changes of appetite-regulating hormones

	Leptin		Ghrelin		Orexin	
	P	*r*	*P*	*r*	*P*	*r*
FR	0.762	− 0.082	0.726	0.095	0.940	0.020
EF	0.740	0.090	0.242	− 0.311	0.010	0.625
SR	0.036	− 0.455	0.269	− 0.294	0.501	− 0.181
SE	0.442	− 0.207	0.681	− 0.111	0.519	0.174

Relative change was calculated as endline–baseline

BMI body mass index, *HDL-L* high-density lipoprotein cholesterol, *LDL-L* low-density lipoprotein cholesterol, *FPG* fasting plasma glucose, *FR* food responsiveness, *EF* enjoyment of food, *SR* satiety responsiveness, *SE* slowness in eating

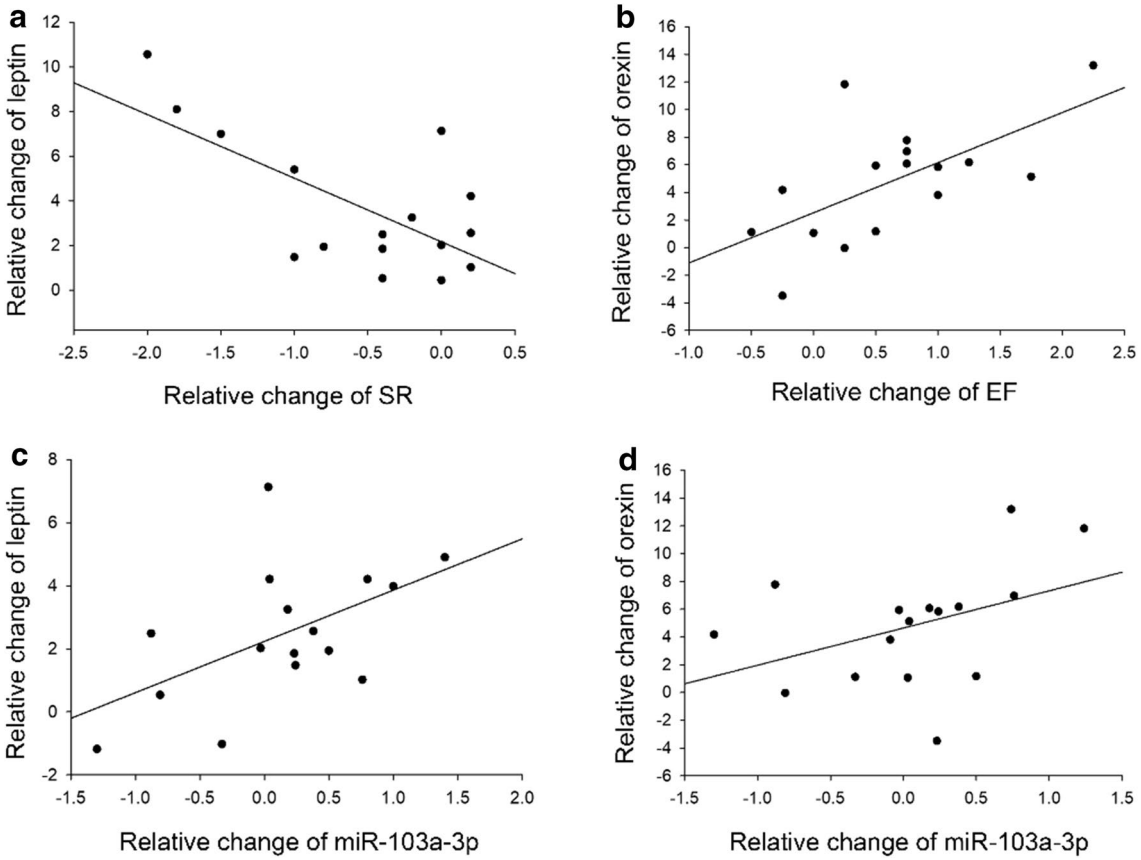

Fig. 3 Correlation of relative changes with significance: SR and leptin (**a**); EF and orexin (**b**); miR-103a-3p and leptin (**c**); miR-103a-3p and orexin (**d**)

to be not correlative with hormones or appetite sensation (Table 4).

Discussion

Both exercise and diet could influence the energy balance equation during weight management in obesity; behavioral approach to combine exercise with diet restriction could help change appetite phenotype of obese children, which may potentially be a key mechanism underlying weight control. Appetite and related hormones play important role in food intake regulation and in the occurrence of obesity or metabolic syndrome in children. Therefore, the current study conducted a 6 weeks of exercise and diet intervention program to investigate the effects on appetite-regulating hormones and subjective appetite changes in obese children. This study also indicates the association between the appetite-regulating hormones and miRNAs, which is closely related to appetite system modulation.

Table 4 Correlation with relative changes of miRNAs

	miR-103a-3p		miR-200a-3p	
	P	*r*	*P*	*r*
Leptin	0.012	0.413	0.611	− 0.138
Ghrelin	0.757	− 0.084	0.616	− 0.136
Orexin	0.015	0.409	0.646	− 0.124
FR	0.996	− 0.001	0.247	− 0.307
EF	0.167	0.363	0.656	− 0.121
SR	0.435	− 0.210	0.589	− 0.146
SE	0.514	− 0.176	0.865	0.046

Relative change was calculated as endline–baseline

BMI body mass index, *HDL-L* high-density lipoprotein cholesterol, *LDL-L* low-density lipoprotein cholesterol, *FPG* fasting plasma glucose, *FR* food responsiveness, *EF* enjoyment of food, *SR* satiety responsiveness, *SE* slowness in eating

After 6 weeks of intervention, both anthropometric and metabolic parameters of the subjects were shifted significantly, demonstrating an obvious effectiveness of this training protocol. Appetitive sensations of the obese children were scaled based on CEBQ, which has been reported strongly associated with childhood obesity [22]; four dimensions including FR, EF, SR, and SE were used in the current study. Food approach scales: FR and EF, generally enhanced with obesity status, were currently observed significantly decreased (~ 16% and ~ 10%) compared with the baseline. Since EF captures general appetite and FR detects maladaptive shift of interest to external food cues [23], the results indicate a negative change in subjective food preference and desire to eat. On the other hand, SR and SE scales evaluate the ability to ceasing eating or not initiating eating based on perceived fullness, which are considered to be low in obese individuals and thus contribute to overeat [24]. Our study observed SR and SE increased by ~ 9% and ~ 11% even though with no significance; the results indicate that there might be an improvement in the sensitivity to food cues and a decrease in eating speed of the obese children. Therefore, the data from CEBQ questionnaire showed a modified subjective appetite trait after exercise and dietary restriction.

Previous studies have suggested that the progression of obesity might be influenced by physical activity and appetite control, which are not independent of each other but interconnected [1]. The relationship between one bout of exercise and appetite has been examined in the obese; higher intensity exercise was observed in favor of appetite control regardless of the exercise duration [25, 26]. Even though increased energy intake is usually a compensatory strategy in response to exercise-induced energy deficit [27], long-term exercise could improve the sensitivity of the appetite-regulatory system [3]. Besides, the type, intensity, and duration of long-term exercise training may also modulate individual's appetite trait variously. In terms of dietary habit modification, it has been reported to be involved in the control of host appetite through modulating its gastrointestinal microbiome [4] and could be strongly linked to obesity development [5]. Total energy intake and macronutrient composition (including energy percentage from protein, fat, and carbohydrate) are factors that contribute to energy expenditure and thus have been intervened in the current study.

The regulation of appetite is complex, involving multiple tissues and organs to form a feedback loop and to govern eating behaviors; circulating regulating hormones could convey appetite signals between systems and represent the overlapping effects of obese subjects after interventions. The present research investigated the changes of peripheral appetite-regulating hormones (leptin and ghrelin) and centrally released hormone (orexin), which might be involved in hunger and satiety signaling during weight loss after lifestyle modifications of exercise and diet.

In the current study, exercise and dietary intervention did not significantly change the circulating level of leptin; in accord with this result, previous study also reported that lifestyle changes including eating and physical activity did not affect overall leptin level [28]. On the other hand, a negative correlation between the change of leptin and SR was observed, suggesting that there might be a potential role of leptin on food satiety responsiveness after 6 weeks of intervention in obesity. Instead of energy expenditure in humans, leptin could properly participate in controlling energy intake [29]. Another peripheral appetite-regulating hormone, ghrelin, was observed significantly enhanced by 6-week interventions of combined exercise and dietary intervention. Circulating ghrelin level is generally reported to be downregulated in the obesity compared with the lean, and dietary alteration-induced weight loss was observed accompanied by increased ghrelin level in the blood [30].

Since leptin resistance in obesity is due to receptor insensitivity or downregulation and there is no obvious change of leptin after 6 weeks in this study, modulation of ghrelin and orexin seems to be pivotally associated with modified feeding behaviors in responses to different stimuli [31]. Jackson et al. [7] recently reported that 8 weeks of exercise training did not affect the fasting level of leptin and ghrelin in overweight or obese female participants, whereas Kang et al. [6] observed leptin decreased and ghrelin increased in middle-aged obese females. Most studies show that interventions for weight management in children achieve a decrease in leptin and increase in ghrelin [32]. For example, Roberts et al. found that 2-week intervention program consisting of diet and exercise significantly reduced leptin level of obese children [33]. What's more, Eloumi et al. demonstrated that the exercise combined with energy restriction decreased plasma leptin level more significantly compared with exercise or energy restriction alone [34].

Levels of orexin in the central nerve system and peripheral tissues from obese animals and human subjects were reduced [13, 35], and there is a protective role of orexin against obesity. Our study found that the intervention had a down-regulatory effect on circulating orexin, whose relative alteration was significantly correlative with EF. Researchers have provided proof that orexin neurons critically mediated physical activity and increased energy expenditure even without changes in food intake [36]; at the same time, physical exercise produces an increase of orexin in both cerebrospinal fluid and peripheral blood and thus improves the function of hippocampus [8].

miRNAs have been implicated in appetite and energy balance control; different expressions of miRNA would be adjusted by various stimuli and pathological conditions. The current study examined the circulating levels of miR-103a-3p and miR-200a-3p, which have been proved to be critically related to hypothalamic function and affect overeating during obesity development [37]. miR-103a-3p attenuated the obesogenic phenotype of mice through stimulating the activity of the mammalian target of rapamycin (mTOR) pathway within the arcuate nucleus of the hypothalamus [18], which has not be confirmed in human experiment. Circulating miR-103a-3p of the obese children was not significantly changed after interventions in this research. This result could be explained by the different influences of exercise and diet on miR-103a-3p. Since previous study reported that plasma miR-103a-3p of young healthy men was present at higher level after chronic endurance training compared with baseline [38]; in high-fat diet-induced prediabete and type-II diabetes, miR-103a-3p showed elevated expression in peripheral blood of rats [39], and low-fat diet down-regulated circulating miR-103a-3p of diet-induced obesity mice [40]. On the other hand, the over-expression of miR-103a-3p in liver or fat was shown to have negative influences on stabilization of the insulin receptor, insulin signaling activity, and related glucose uptake [41]. Therefore, the circulating level of miR-103a-3p could not be a sensitive marker for delineating appetitive alteration. However, miR-103a-3p might have participated in regulating feeding behavior considering its relative change was observed to be significantly correlated with both leptin and orexin.

miR-200a-3p could be another target for the treatment of obesity since it was up-regulated in both hypothalamus and liver in fat-fed rats after leptin being blockaded [42] and over-expression of miR-200a-3p in the hypothalamus impaired insulin and leptin signaling in obesity [17]. The results of the current study show that circulating miR-200a-3p level was reduced after 6 weeks of intervention, suggesting that combining exercise with energy restriction would have a beneficial effect on normalizing circulating miR-200a-3p level. Even though Zhao et al. [43] reported that 6 weeks of high-intensity intermittent swimming training enhanced all members of miR-200 family in rat brain, exercise modality, intensity, duration, and frequency would specifically influence the result. As for the diet factor, scarce study has reported except for that miR-200-3p of plasma was up-regulated after one high-fat meal in healthy women [44]. Collectively, circulating miR-200a-3p level could be a negative appetitive indicator after exercise and dietary intervention.

There are two main limitations in the current study. First, we did not include a control group, which made it difficult to claim that the appetite and related hormones were restored in obese children compared with that in the normal weight. Even though previous study has concluded that body adiposity of participants would not modify appetite response to chronic exercise intervention [45], overweight or obesity (characterized by high level adiposity) may have displayed diverse hormone change patterns induced by long-term exercise and dietary restriction. Second, the examined circulating profiles of miR-103a-3p and miR200a-3p could be masked by other tissues, which are both not only predominantly expressed in the brain but also highly abundant in colon and epididymis of human [46]. Therefore, more thorough analyses of circulating miRNA pattern are encouraged to establish sensitive markers for appetite monitoring.

What is already known on this subject?

Appetite control in obesity by lifestyle modifications combining exercise training with dietary restriction is associated with circulating hormones.

Specific miRNAs could provide a unique perspective for understanding the interaction between environmental factors and internal stimuli in the development of obesity during childhood, which has been barely studied in human subjects.

What does this study add?

This study validated the association between appetite regulation and exercise and dietary intervention in obese children.

miR-103a-3p and miR-200a-3p were potentially involved in energy balance during obesity management of children, whose roles should be further characterized to help enhance the effectiveness of lifestyle strategies on this health problem.

Conclusion

Exercise combined with diet intervention for 6 weeks might be effective in regulating appetite sensations and hormones in obese children. miR-103a-3p and miR-200a-3p might provide a foundation for target biomarkers of appetite trait that

are influenced by behavioral modifications such as exercise and dietary intervention in weight management.

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Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

Ethical approval All procedures involving human participants were in accordance with the Declaration of Helsinki and its later revised standards. The Human Ethics Board of the Guangzhou Sport University approved the study prior to the study commencing.

Informed consent Informed consent was obtained from parents of all the participants before they were included in the study.

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