Dietary curcumin supplement promotes browning and energy expenditure in postnatal overfed rats

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Research

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Abstract

Background: Early postnatal overfeeding could result in metabolic imprinting that decreases energy expenditure following with white adipose tissue (WAT) gain throughout life. This research was to investigate whether curcumin (CUR) supplement could promote WAT browning and activate thermogenesis in postnatal overfed rats.

Methods and results: This study adjusted the size of litters to three (small litters, SL) or ten (normal litters, NL) to mimic early postnatal overfeeding or normal feeding from postnatal day 3. From postnatal week 3 (weaning period), the SL rats were fed with a standard diet (SL) or a diet supplemented with 1% ($SL_{1\%\ CUR}$) or 2% ($SL_{2\%\ CUR}$) CUR for ten weeks. At postnatal week 13, SL rats with 1% or 2% CUR supplement had lower body weight and less WAT gain, had increased lean mass ratio, and their glucose tolerance and blood lipid levels had recovered to normal when compared to SL rats that did not receive the supplement. Moreover, increased respiratory exchange ratio (RER) and heat generation were consistent with expression levels of uncoupling protein 1 (UCP1) and other browning-related genes in the subcutaneous adipose tissue (SAT) of the $SL_{2\%\ CUR}$ rats but not of the $SL_{1\%\ CUR}$ rats. In addition, 2% CUR dietary supplement enhanced the serum norepinephrine (NE) levels in SL rats, with the upregulated mRNA levels of β3-adrenergic receptor (β3-AR) in SAT.

Conclusion: Dietary CUR supplement attenuates body weight gain and metabolic disorders in SL, which might be induced by promoting browning of SAT and energy expenditure. Moreover, the benefits were more obvious in SL with 2% CUR supplement.

1. Background

Obesity is a chronic metabolic disease owing to long-term energy metabolism imbalance and has become increasingly prevalent[1]. Numerous studies about different nutrition exposures across species have shown that affected by obesogenic factors in early postnatal period could cause energy metabolism disturbance in the subsequent life process[2], but the mechanism remains to be elucidated. Artificial increase or decrease the pups in a litter is an ideal method to simulate neonatal over- or poor-feeding, respectively[3]. Studies have shown that the breast milk intake of small litters (SL) (three to four pups) is increased than that of normal litters (NL) (eight to twelve pups). Also, triglyceride (TG) content of breast milk from SL mothers was significantly rich, compared with NL group[4, 5].

Our previous study indicated that lower energy expenditure levels occurred in SL rearing (3 pups) rats and this situation could persist to adulthood[6]. Therefore, on the basis of developmental plasticity, exploring the possibility of regaining energy metabolism balance and discovering its mechanism could provide potential clues for pursuing solutions for obesity caused by early overfeeding.

Adipose tissue is among the critical organs to regulate homeostasis of whole-body energy metabolism[7]. White adipose tissue (WAT) is a primary organ to store excess energy intake, while brown adipose tissue (BAT) activates non-shivering thermogenesis, in which energy is dissipated as heat[8]. As one of the

principles to treat obesity is to cause the increase in energy expenditure, activating BAT and increasing its heat production is considered as a potential treatment for obesity[9, 10]. In fact, BAT in adults is absent. Fortunately, third adipocytes, named beige adipocytes have been discovered in WAT and they express uncoupling protein 1 (UCP1), a thermogenic marker, similar to BAT. In addition, Peroxisome proliferator-activated receptor γ coactivator-1a (PGC1a), a critical regulatory factor of energy metabolism and mitochondrial biosynthesis, is highly expressed in brown and beige adipocytes [8]. Moreover, PGC1a acts as a cofactor that will combine peroxisome proliferators-activated receptors g (PPARg) with positive regulatory domain containing 16 (PRDM16) to increase UCP1 level in WAT[11-13]. A growing number of scientific researchers are attempting to exploit the induction of WAT browning in addition to inducing thermogenesis in BAT to address energy metabolism disorders and help individuals maintain a healthy body weight.

Curcumin (CUR) derived from turmeric is a natural flavonoid component and a safety food additive[14]. Many studies have proved that CUR has the ability to against inflammatory, cancer, neurodegenerative and cardiovascular diseases[15, 16]. Recently, Wang et al. found that a dietary CUR supplement improved insulin sensitivity and decreased adipose tissue mass both in high-fat diet (HFD) [17] and obese/diabetic mice[18]. A dietary CUR supplement has been shown to relieve low-grade chronic WAT inflammation and upregulate BAT UCP1 expression in HFD mice[19]. In addition, CUR has been reported to be an anti-adipocyte dietary bioactive component which could modulate early stage of adipocyte differentiation[20]. All these findings suggest that a dietary CUR supplement might be a potential strategy to control obesity by WAT browning.

It is well known that the early postnatal period is one of the critical stages of WAT development. Overnutrition in early life (fetal or newborn periods) can impact adipocyte proliferation, differentiation and energy homoeostasis in adulthood [21]. CUR has the ability to prevent obesity and metabolic disorder, but its effects on postnatal overfeeding remains unknown. We hypothesized that adding a CUR supplement to the postweaning diet could promote WAT browning and energy expenditure, thereby preventing obesity and metabolic disorders in postnatal overfed rats induced by SL rearing.

2. Methods

2.1 Animals

All trials conducted on rats in the research got approval of Ethics Committee of Nanjing Medical University (Permit No. 1905046) and all procedures were conducted in accordance with the Guidelines for Use and Care of Laboratory Animals of Nanjing Medical University. Female Sprague-Dawley rats on gestational day 14 (Nanjing, Jiangsu, China) were obtained from Animal Core Facility of Nanjing Medical University (Nanjing, Jiangsu, China) and raised in a standard environment (a 12/12-h light/dark cycle, 22 ± 2℃, 40%~60% humidity). Tap water and food were freely available to all rats.

2.2 Experimental design

Artificially SL rearing (three to four rat pups per litter) allows raised breast milk availability and induces postnatal overfeeding, which mimics over-nutrition during suckling in humans[3]. For rats, postnatal week 3 (W3) is weaning period, puberty occurs at W6-8, and adulthood is W9 and afterwards [22].Our previous studies found that metabolic dysfunctions caused by postnatal overfeeding took place early at W3 and persisted to W13-16[6, 23]. Therefore, this study selected W3 and W13 as the two key experimental time nodes to explore the effects of postnatal nutritional environments on the health status of adults.

The experimental protocol is shown in Figure 1. Briefly, we randomly assigned male rat pups to three (small litters, SL) or ten (normal litters, NL) per litter to simulate early over or normal feeding respectively from postnatal day 3[24, 25]. At W3 (weaning period), the NL rats were fed with a standard diet (NL group, n = 9), and the SL rats were fed with either a standard diet (SL group, n = 6) or a diet supplemented with 1% ($SL_{1\%\ CUR}$ group, n = 9) or 2% CUR ($SL_{2\%\ CUR}$ group, n = 6) until W13. CUR was purchased from Oranika Health Products (95% standardized CUR extract, Richmond, British, Canada). We monitored the weights and food intakes of rats weekly at a fixed time point. The specific experiments at each time point are shown in Figure 1.

2.3 Magnetic Resonance Imaging (MRI)

The 7T Bruker BioSpec 70/20USR scanner was used in this study. Before the MRI scanning, we weighted the rats and then used 5% Isoflurane for anesthetic induction and 1-2% Isoflurane for maintenance dose. Anesthetics were mixed in compressed air and delivered by a nasal mask at a speed of 1 liter per minute. After judged to be anesthetized, the rats were placed in the head-holder of scanner stably to assure magnet could position reproducibly. We monitored the respiratory rate of rats to keep around 60-80 breaths per minute during experimental period. This study selected the axial sections from liver to bladder for the analysis of adipose tissue and used two sets of multi-slice spin-echo sequence (TR = 3604.5 ms, TE = 33.0 ms) to obtain 35 T2-weighted anatomical axial slices per rat for W3 rats or 70 for W10 rats, with the thickness of 1.50 mm. We set the field of view as 4.77 x 4.50 cm^2 (W3 rats) or 7.03 x 6.63 cm^2 (W10 rats), and matrix size as 256 x 256, three averages per slice.

Next, we manually looped the target adipose tissue samples of each section of the images and calculated the pixel areas (Image J software, National Institutes of Health, USA). The fat surface area per section was multiplied by the inter-section distance to yield the corresponding fat volume. Next, converting total body fat volume (cm^3) to mass (grams) by multiplying 0.9196 g/cm^3, density of adipose tissue[26], and the percent of body fat was roughly estimated by (body fat mass/body weight) * 100%. The body weight minus body fat mass was the lean mass. Finally, the lean mass percentage was calculated as (lean mass/body weight) * 100%.

2.4 Intraperitoneal glucose tolerance test (IPGTT)

This experimental scheme followed the protocol designed in previous study[27]. In short, the rats were given fasting but water freely overnight at W3 and W13. We collected a small drop of rat tail vein blood, then determined the level of glucose using a glucose meter (Accu-Chek, Roche Diagnostics, Mannheim,

Germany). Next, we measured the blood glucose levels of rats at 30-, 60-, 90- and 120-min time points following intraperitoneal administration of D-Glucose (2.0g per kilogram body weight). Then area under the curve (AUC) of glucose was calculated.

2.5 Energy expenditure

At W13, rats were housed in the metabolic chamber individually. The whole-body metabolic rate of rats was recorded through an indirect calorimetry and locomotor activity monitoring system (TSE Phenomaster, TSE, Germany), including oxygen consumption (VO2), carbon dioxide production (VCO2). [28]. We placed rats in metabolic chambers for 72 hours to acclimate and then detected per 15 minutes for the duration of an additional 24 hours. The parameter values obtained during the final 24-h were used for statistics and then calculated the respiratory exchange ratio (RER) (the ratio of VCO2 versus VO2). Furthermore, the heat production of rats was monitored closely during recording period closely. Water and food were freely available to all rats in each metabolic chamber.

2.6 Serum and tissue collection

Following fasted overnight, rats were weighted and anaesthetized with 300 mg chloral hydrate per kilogram, intraperitoneal administration at W3 and W13. Then, we collected blood samples from left ventricle and centrifuged at 2000×g, 4°C for 15 minutes. The supernatant was collected and saved at -80°C for subsequent biochemical analyses. The BAT and three main types of WAT (i.e., subcutaneous adipose tissue (SAT), epididymal adipose tissue (EAT) and retroperitoneal adipose tissue (RAT)) were rapidly isolated, rinsed with normal saline and then weighed. 4% paraformaldehyde was used to fix a portion of SAT for subsequent section and stain, and put the rest into liquid nitrogen for quick freezing and saved at -80℃ for later experiments.

2.7 Serum measurements

The content of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), total cholesterol (TC), TG, high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) were measured using an automatic biochemical analyzer (7100, Hitachi, Japan). Also, serum norepinephrine (NE) and insulin were detected by ELISA Kits (Rat Norepinephrine ELISA Kit CSB-E07022r, Rat Insulin ELISA Kit CSB-E05070r, CUSABIO, China).

2.8 Hematoxylin and eosin (H&E) staining

This study fixed SAT using 4% paraformaldehyde overnight at room temperature, followed by sectioned after paraffin embedded. H&E staining was undergone on the basis of a standard protocol. Four arbitrary fields of view per rat were analyzed by Image J to estimate the adipocyte area.

2.9 RNA isolation and reverse transcription quantitative real-time PCR (RT-qPCR)

Each frozen SAT was homogenized in TRIzol (TAKARA, Japan) to isolate total RNA respectively following the manufacturer's instructions. The extracted total RNA was quantified by spectrophotometry at OD260. Agarose gel electrophoresis was used to analyze the integrity of total RNA. According to the manufacturer's recommendation, this study prepared cDNA by M-MLV reverse transcriptase (TAKARA, Japan) using 1 ug extracted total RNA. The experiment utilized Quant Studio 3 real-time PCR instrument (Applied Biosystems, USA) to amplify cDNA by the SYBR Green master mix (Vazyme, China).Target genes expression levels were calculated in the way of $2^{-\Delta\Delta Ct}$[29], and normalized to the average of the housekeeping genes glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The sequence of all primers involved in this study is shown in Table 1.

Table1 Primer sequences used for mRNA quantification by RT-qPCR

	Forward primer 5'-3'	Reverse primer 5'-3'
Ucp1	ACTGTACAGAGCTGGTGACA	TGGTAGAGAGTTGATGAATCTCGT
PGC1a	GTGGATGAAGACGGATTGCC	TTCTGAGTGCTAAGACCGCT
PRDM16	GGACAGTGACAGAGACAAAAGC	CTGTGAATAGAAGGCCGGTA
TMEM26	TTGCCATGGGCTAGAATCCG	TAAAGGCCTGTGCAGCTACC
PPARγ	ATCAGGTTTGGGCGAATG	TTTGGTCAGCGGGAAGGA
b3-AR	TGCTGTTCCTTTGCCTCCAA	TAGCTACGACGAACACTCGA
GAPDH	GGCTCTCTGCTCCTCCCTGTTCTA	CGTCCGATACGGCCAAATCCGT

2.10 Western blotting

Rat SAT was homogenized in precooled RIPA buffer contained a protease inhibitor cocktail. Following centrifugation at 12000×g, 4°C for 15 minutes, the supernatant was collected. The concentration of proteins was detected using the BCA protein assay kit (Thermo Fisher Scientific, Rockford, IL, USA) following the manufacturer's instruction. Equal amount of total protein was loaded in each lane of 10% SDS-polyacrylamide gel electrophoresis, separated, then transferred onto polyvinylidene difluoride membranes (Bio-Rad, USA). The membranes were blocked with 5% dry non-fat milk for 2 hours at room temperature, and probed with anti-UCP1 (Abcam, USA) or anti-GAPDH (Proteintech, China) antibodies overnight at 4°C, then washed the membranes 5 times with phosphate-buffered saline and 0.1% Tween 20 (PBST) for 6 min each. Next, the membranes incubated with a horseradish peroxidase-conjugated secondary antibody for 1 hour at room temperature and then washed again with PBST (5 washes for 6 min each). The reaction was determined using a chemiluminescence system (ChemiDoc XRS+, Bio-Rad, USA). The band intensity was analyzed by Image Lab (Bio-Rad, USA).

2.11 Statistical analyses

All data were normally distributed and presented as the mean ± standard error of the mean. Significant differences between groups at W3 were analyzed using Student's unpaired *t*-test and at W13 using one-way analysis of variance (ANOVA) and followed by a post hoc least significant difference (LSD) *t*-test. A p-value < 0.05 was considered to be statistically significant.

3. Results

3.1 Bodyweight, food intake and liver enzymes

As shown in Figure 2, the weight and ingestion of rats changed with age in all groups. Compared with NL rats, body weight of SL rats was significantly high from postnatal W3 until W13. The weight gains in both $SL_{1\%\ CUR}$ and $SL_{2\%\ CUR}$ rats were lower compared to SL rats after intervention for 4 weeks (Fig. 2A). The food intake of NL and SL rats is basically equal after W6 and the rats fed CUR did not have difference in food intake in comparison to the SL rats (Fig. 2B).

Next, we determined if the CUR supplementation had a significant effect on liver function of rats. The SL rats showed a marked increase in their serum AST compared with NL at W3, but no significant difference was observed in serum ALT (Fig. 2C and D). Moreover, the levels of serum AST and ALT in SL, $SL_{1\%\ CUR}$ and $SL_{2\%\ CUR}$ groups were not different from those in the NL group at W13 (Fig. 2C and D).

3.2 Body composition, adipose tissue mass and size

The differences of body composition between groups at W3 and W10 are shown in Figure 3. The fat volume and body fat percentage of SL rats were higher than those of NL rats at W3 and W10. Compared to SL rats, $SL_{1\%\ CUR}$ rats had decreased fat volume and body fat percentage at W10 (Fig. 3A). The fat volume and body fat percentage in $SL_{2\%\ CUR}$ rats were significantly lower than those of SL rats and similar to those of NL rats at W10, but this was not true for the $SL_{1\%\ CUR}$ rats (Fig. 3B and C).

The lean mass in SL rats with or without CUR supplement was more than that in the NL rats at W10 and the concentration of 2% was more obvious (Fig. 3D). However, the opposite was true for lean percentage as shown in Figure 3E.

Similarly, BAT and WAT (i.e., SAT, EAT and RAT) weights in SL rats were higher than NL at W3 and W13 (Table 2). The results showed that the three main types of WAT weight of $SL_{1\%\ CUR}$ and $SL_{2\%\ CUR}$ rats were lower than those of SL rats, but there was no difference of BAT weight among SL, $SL_{1\%\ CUR}$ and $SL_{2\%\ CUR}$ rats (Table 2). Furthermore, the mass ratio of BAT to WAT in SL rats was lower than that in NL rats at W3 and W13, while that of both $SL_{1\%\ CUR}$ and $SL_{2\%\ CUR}$ rats was close to normal (Table 2). Histologically, the average surface area of subcutaneous white adipocyte in SL rats was larger than that in the NL rats at W3 and W13. As expected, the average surface area of subcutaneous white adipocyte in SL rats with CUR intervention ($SL_{1\%\ CUR}$ and $SL_{2\%\ CUR}$) was diminished markedly (Fig. 4A and B).

Table2 Adipose tissue weights in rats at W3 and W13

	NL	SL	$SL_{1\% CUR}$	$SL_{2\% CUR}$
W3				
BAT(g)	0.11 ± 0.01	0.20 ± 0.02^{a}		
SAT(g)	0.56 ± 0.05	1.58 ± 0.04^{a}		
EAT(g)	0.08 ± 0.00	0.25 ± 0.03^{a}		
RAT(g)	0.08 ± 0.00	0.30 ± 0.03^{a}		
BAT/WAT	0.17 ± 0.02	0.09 ± 0.01^{a}		
W13				
BAT(g)	0.29 ± 0.02	0.37 ± 0.31^{a}	0.32 ± 0.04	0.31 ± 0.31
SAT(g)	4.43 ± 0.37	9.39 ± 1.27^{a}	5.64 ± 0.32^{ab}	5.74 ± 0.50^{ab}
EAT(g)	3.60 ± 0.34	7.20 ± 0.33^{a}	4.56 ± 0.26^{ab}	4.50 ± 0.42^{b}
RAT(g)	2.54 ± 0.18	7.37 ± 0.89^{a}	3.57 ± 0.31^{ab}	4.77 ± 0.48^{ab}
BAT/WAT	0.028 ± 0.002	0.0160 ± 0.002^{a}	0.024 ± 0.003^{b}	0.022 ± 0.002^{b}

All values represent means ± SEM. $^{a}p < 0.05$ versus NL, $^{b}p < 0.05$ versus SL. Student's unpaired *t*-test at W3 and one-way analysis of variance (ANOVA) at W13 were performed. n=6-9 in each group.

3.3 Glucose homeostasis and serum lipids

This study conducted IPGTT to infer the insulin sensitivity of rats. The results showed that the AUC for plasma glucose of SL rats was larger in comparison to NL at weaning (W3) and adulthood (W13), suggested that SL rearing could impair the glucose tolerance of rats. the AUC of the $SL_{1\% CUR}$ and $SL_{2\% CUR}$ rats was decreased in comparison to SL rats and was similar to NL at W13 (Fig. 5A and B, D and E). Consistently, the serum insulin of SL rats is higher than that of the NL rats, but the serum insulin in the $SL_{1\% CUR}$ and $SL_{2\% CUR}$ rats recovered to normal levels (Fig. 5C and F).

Moreover, compared with NL rats, serum HDL-C levels of SL rats were decreased at W3, and there were no obvious differences in serum TC, TG, and LDL-C levels between NL and SL rats (Table 3). Notably, the serum TC, TG, and LDL-C levels of SL rats were all evidently higher than those of NL rats at W13, while those of both the $SL_{1\% CUR}$ and $SL_{2\% CUR}$ rats were reduced in comparison to the SL rats and close to normal levels. There were no differences in the level of serum HDL-C among groups at W13 (Table 3).

Table 3 Serum lipid biochemical parameters in rats at W3 and W13

	NL	SL	$SL_{1\% CUR}$	$SL_{2\% CUR}$
W3				
TC (mg/dl)	2.14 ± 0.09	2.00 ± 0.15		
TG (mmol/L)	0.59 ± 0.13	0.64 ± 0.13		
HDL-C (mmol/L)	0.59 ± 0.01	0.46 ± 0.05[a]		
LDL-C (mmol/L)	0.52 ± 0.04	0.55 ± 0.30		
W13				
TC (mg/dl)	1.59 ± 0.07	1.85 ± 0.07[a]	1.54 ± 0.08[b]	1.47 ± 0.06[b]
TG (mmol/L)	0.63 ± 0.05	1.23 ± 0.13[a]	0.66 ± 0.09[b]	0.57 ± 0.04[b]
HDL-C (mmol/L)	0.38 ± 0.01	0.36 ± 0.03	0.34 ± 0.03	0.37 ± 0.02
LDL-C (mmol/L)	0.41 ± 0.03	0.61 ± 0.03[a]	0.44 ± 0.04[b]	0.47 ± 0.02[b]

All values represent means ± SEM. [a]$p < 0.05$ versus NL, [b]$p < 0.05$ versus SL. Student's unpaired *t*-test at W3 and ANOVA at W13 were performed. n=6-9 in each group.

3.4 Energy expenditure

At W13, the VO_2 (Fig. 6A and B), VCO_2 (Fig. 6C and D), RER (Fig. 6E and F), and heat production (Fig. 6G and H) of SL rats were all lower than those of NL. Compared to the SL, the levels of VO_2, VCO_2, RER, and heat production were improved significantly in the $SL_{2\% CUR}$ rats but not in the $SL_{1\% CUR}$ rats.

3.5 Expression of browning-regulated genes in SAT

To evaluate the effect of curcumin on WAT browning, the expression levels of genes involved in the transformation of white adipocytes into beige adipocytes in SAT were detected. In comparison to NL, UCP1 expression of SL rats was inhibited at W3 and W13 but was significantly upregulated by dietary CUR supplementation in the SL rats. This trend became more obvious in the 2% CUR supplement rats at W13 (Fig. 7A-C). Subsequently, the mRNA levels of PGC1a in SAT were consistent with that of UCP1 (Fig. 7D).

In addition, PRDM16 mRNA level was decreased in SL rats, whereas expression levels of TMEM26 and PPARg revealed no obvious difference between the NL and SL rats at W3 (Fig. 7E). At W13, in comparison to NL rats, PRDM16, TMEM26 and PPARg expressions of SAT were suppressed in the SL rats and enhanced in the SL rats treated with dietary CUR, as expected. Likewise, the mRNA levels were enhanced more obvious in the $SL_{2\% CUR}$ rats (Fig.7F).

3.6 Serum NE levels and β3-ARgene expression in SAT

The thermogenic response mediated by UCP1 is regulated mainly by sympathetic nervous system through releasing NE and ligating to the β3-AR on membrane of adipocytes. This study investigated whether CUR affected the release of NE together with its receptor expression. As illustrated in Figure 8A,

at W3 and W13, the serum NE level of SL rats was significantly lower than that of NL. Moreover, the serum NE reached a normal level only in the $SL_{2\%\ CUR}$ rats. The pattern of β3-AR mRNA expression in SAT among groups was similar to their serum NE (Fig. 8B).

4. Discussion

Numerous nutritional programming related studies have proved that the suckling period nutritional environment could affect weight and energy homeostasis into adulthood[30, 31]. In this research, the postnatal overfeeding rat model which is induced by SL rearing could result in several metabolic dysfunction including higher body weight and WAT mass, increased serum lipids and insulin resistance at weaning and adulthood. These results were in accordance with our previous researches[6, 32].

Rats fed a post-weaning diet supplemented with CUR exhibited lower body weight, less fat mass, higher energy expenditure and improved glucolipid metabolism during adulthood compared to a standard diet in SL-reared rats. Furthermore, brown-like adipocytes with high UCP1expression emerged in the SAT of these rats with the CUR intervention. These data suggest that a dietary CUR supplement could stimulate the development of WAT browning and might be a strategy to increase energy expenditure for preventing obesity induced by postnatal overfeeding.

CUR's capacity as an anti-obesity nutraceutical that increases weight loss and lowers fat mass has been verified in adulthood obesity models [18, 33]. This study first to exhibit this effect in overfed rats in the SL-rearing model. Recently, several important window phases from fetal formation to childhood have been identified and studies found that effective intervention during these phases could have a profound impact and may prevent the progress of obesity and type 2 diabetes. [34]. This study proved that dietary CUR supplementation after weaning ($SL_{1\%\ CUR}$ and $SL_{2\%\ CUR}$ rats) could significantly reduce body weight, adipose tissue mass, and adipocyte volume of rats. Many metabolic diseases are increasing in parallel with the occurring of obesity in youth, including type 2 diabetes, insulin resistance and hyperlipidemia[35, 36]. Interestingly, this study initially found that dietary CUR supplementation was also useful in improving glucose intolerance and hyperlipidemia induced by postnatal overfeeding.

Keeping energy intake and consumption in a balanced state is the basis for maintaining a healthy weight. The rationales of treatments for weight loss usually include reducing total energy uptake and increasing energy expenditure[37]. In the present study, dietary administration of CUR (1% or 2% diet) raised energy consumption without affecting food intake in SL rats, which suggests that CUR could enhance energy metabolism rather than inhibit energy intake. Several studies has shown that CUR has the ability to up-regulate the basal metabolic rate which could lead to higher energy consumption [38]. Unsurprisingly, this study observed a raise in the energy consumption of SL rats with a diet containing 2% CUR.

Adipose tissue, as a caloric reservoir, plays a critical character in regulating the balance of systemic energy metabolism[7]. Induction of WAT browning could increase energy consumption and help to alleviate metabolic disorders[9, 10, 39]. Upregulation of UCP1 expression is closely related to raised

energy consumption and adaptive thermogenic and is usually used as an indicator during BAT activation and WAT browning[40]. In rodents, WAT depots have different propensities to form beige adipocytes[41]. Induction of WAT browning occurs easily in subcutaneous depots compared with visceral mesenteric or epididymal depots[42, 43]. In this study, we mainly observed the browning feature of CUR in SAT and found that 2% dietary CUR supplement could increase both UCP1 mRNA and protein expression of SAT in SL rats. Transcription of UCP1 requires coactivators, including PGC1a, PRDM16 and PPARg[9], which returned to normal levels in $SL_{2\%\ CUR}$ rats. Moreover, transmembrane protein 26 (TMEM26)[8], a specific beige-selective gene that can distinguish beige adipocytes from brown or white adipocytes in adipose tissues, was upregulated in the SAT of SL rats with a diet containing 2% CUR. To sum up, these findings suggested that CUR may act as a thermogenic activator to induce SAT browning, which could partly explain the benefits of the dietary CUR intervention on metabolic disorders in obese rats resulted from postnatal overfeeding.

Furthermore, this study observed the serum NE and β3-ARmRNA expression in the SAT of rats. The release of NE from the adrenal medulla and sympathetic terminals in WAT is mandatory for the immediate activation of existing beige adipocytes and the differentiation of beige adipocytes from their precursors[44]. NE binds to β3-AR expressed in brown/beige adipocytes and then activates c-AMP pathway-dependent mitochondrial UCP1, which is a critical mediator of adaptive thermogenesis in brown and beige adipose tissue [8]. The β3-AR signaling pathway is stimulated by several factors in WAT; the most effective is chronic cold exposure, which could induce the browning process[45].

In this study, we found that the SL rats experienced a decrease in serum NE and β3-AR mRNA expression levels in their SAT at both W3 and W13, but this could be reversed by feeding them a 2% CUR dietary supplement post-weaning. In vitro, our previous study have found that the browning marker genes expression was remarkably up-regulated following treatment of CUR in preadipocytes, and the increase was suppressed by β3-AR antagonist[46]. Taken together, our findings support that CUR-induced SAT browning may be associated with sympathetic stimulation through the norepinephrine-β3-AR pathway.

The effects of functional diets are closely related to the dose administered[47]. Generally, orally ingested CUR is metabolized in the liver and small intestine, and liver enzymes (AST and ALT) are usually used to detect oral toxicity[48]. In this study, no significant difference was found in the serum AST and ALT in the $SL_{1\%\ CUR}$ or $SL_{2\%CUR}$ rats compared to the NL rats, which suggests that neither dosage of CUR adopted in current research caused liver injury.

In previous studies, the major intervention for CUR in obese animal models was oral administration, including gavage and dietary supplementation. The concentrations used in the latter range widely, from 0.1% to 3%. In this study, we provided the SL rats with two different doses of CUR (95% standardized CUR extract) to understand the effects of dose difference. The doses set in the current study are 1% and 2%, referring to the concentration of dietary CUR (95% standardized CUR extract) used in previous studies on obese mice models induced by high-fat diet and Western diet, which were 1% and 3%, respectively.

In the present study, the changes of body weight, serum chemical parameters, adipocyte surface area, and energy expenditure as well as expression of browning-related genes in the $SL_{2\%CUR}$ rats were slightly better than those of the $SL_{1\%CUR}$ rats, but there were no significant differences in these obesity indicators and browning genes between the $SL_{1\%\ CUR}$ and $SL_{2\%CUR}$ rats. Therefore, dietary 2% CUR supplementation might be an ideal dose for treating postnatal overfeeding-induced obesity. However, whether a higher dose of CUR could lead to a stronger effect still needs further research.

5. Conclusions

A post-weaning diet supplemented with CUR significantly improved obesity and metabolic disorders in overfed rats induced by SL rearing, probably through triggering energy metabolism remodeling and upregulating the expression of browning-regulated genes in SAT. Importantly, CUR stimulated the browning program possibly regulated by NE/β3-AR. Further studies investigating other thermogenic organs and their molecular pathways are still required. Based on these findings, we concluded that CUR as a natural and edible plant could be a new viable strategy to fight against postnatal overfeeding-induced obesity and related metabolic disorders.

List Of Abbreviations

β3-AR: β3-adrenergic receptor

ALT: Alanine aminotransferase

ANOVA: Analysis of variance

AST: Aspartate aminotransferase

AUC: Area under the curve

BAT: Brown adipose tissue

CUR: Curcumin

EAT: Epididymal adipose tissue

GAPDH: Glyceraldehyde-3-phosphate dehydrogenase

HDL-C: High-density lipoprotein cholesterol

HFD: High fat diet

IPGTT: Intraperitoneal glucose tolerance test

LDL-C: Low-density lipoprotein cholesterol

LSD: Least significant difference

NE: norepinephrine

NL: Normal litters

PGC1a: Peroxisome proliferator-activated receptor γ coactivator-1a

PPARg: Peroxisome proliferators-activated receptorsg

PRDM16: Positive regulatory domain containing 16

RAT: Retroperitoneal adipose tissue

RER: Respiratory exchange ratio

SAT: Subcutaneous adipose tissue

SL: Small litters

TC: Total cholesterol

TG: Triglyceride

TMEM26: Transmembrane protein 26

UCP1: Uncoupling protein 1

VCO2: Carbon dioxide production

VO2: Oxygen consumption

WAT: white adipose tissue

W3: Postnatal week 3

W10: Postnatal week 10

W13: Postnatal week 13

Declarations

Ethics approval and consent to participate

All animal studies were performed following the guidelines established by the University Committee on the Use and Care of Animals and were overseen by the Unit for Laboratory Animal Medicine at Nanjing Medical University (IACUC: 1905046).

Consent for publication

Not applicable

Availability of data and materials

Data are all contained within the article.

Competing interests

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Authors' contributions

XZ and XL designed the study. XZ, SD and QY performed the experiments. XZ analyzed data. XZ and XL wrote the paper, and CM, NZ and WZ reviewed the manuscript. All authors had final approval of the submitted and published versions.

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Figures

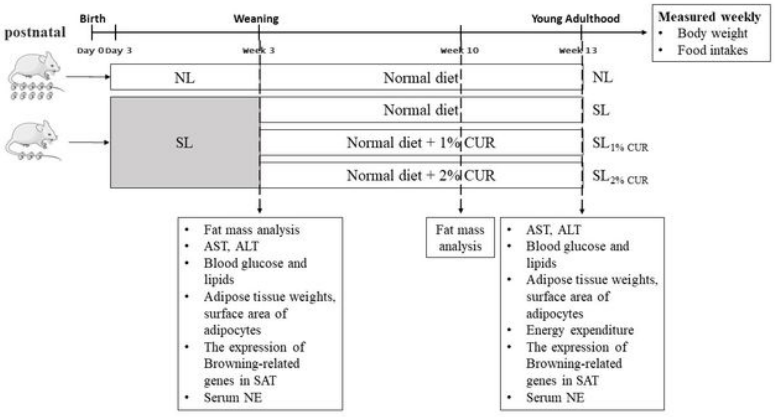

Fig.1

Figure 1

Schematic overview of the experimental design.

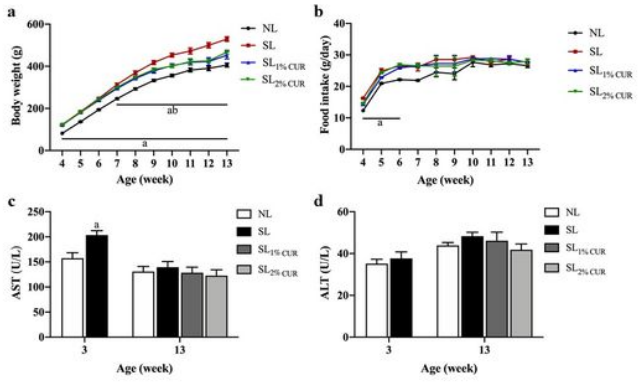

Fig.2

Figure 2

Body weight (a) and food intake in rats (b) from W4 to W13. Serum levels of aspartate aminotransferase (AST) (c) and alanine aminotransferase (ALT) (d) in rats at W3 and W13. All values represent means ± SEM. ap < 0.05 vs. NL, bp < 0.05 vs. SL. Body weight and food intake were analyzed by one-way analysis of variance (ANOVA), AST and ALT levels were analyzed by Student's unpaired t-test at W3 and one-way analysis of variance (ANOVA) at W13. n=6-9 in each group.

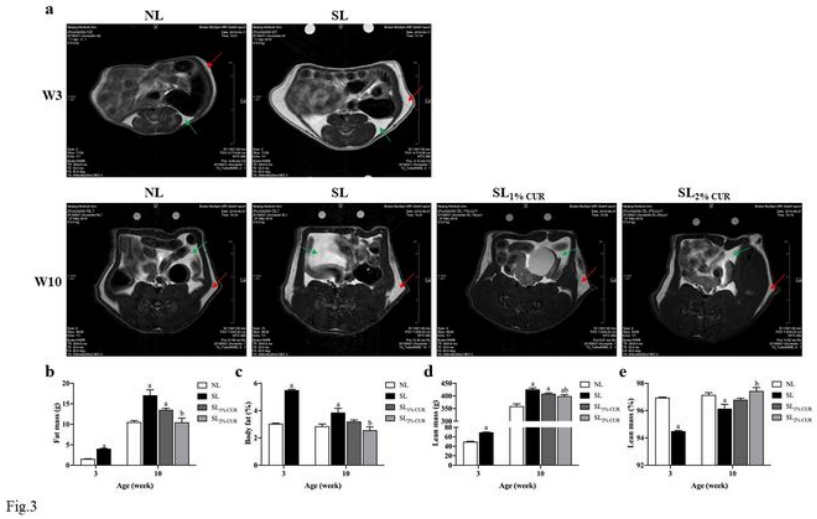

Fig.3

Figure 3

MRI analysis of body fat in rats at W3 and W10 (red arrow pointing to subcutaneous adipose tissue and green arrow pointing to visceral adipose tissue) (a) and followed by calculating fat mass (b), body fat percentage (c), lean mass (d) and lean percentage (e).All values represent means ± SEM. ap < 0.05 vs. NL, bp < 0.05 vs. SL. Statistical analysis was performed using Student's unpaired t-test at W3 and ANOVA at W10. n=3-4 in each group.

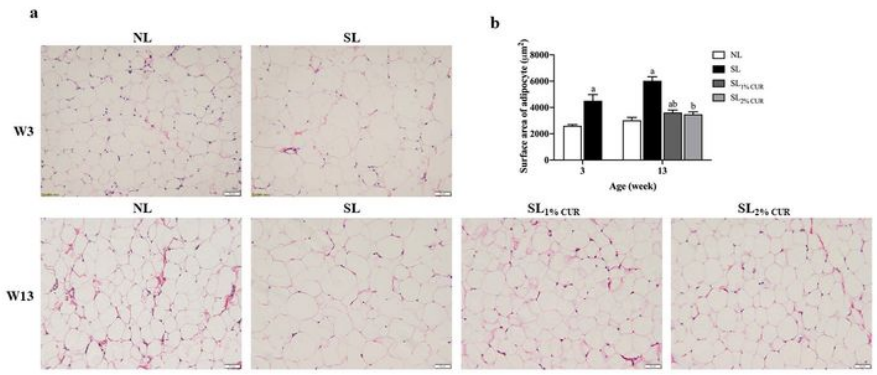

Fig.4

Figure 4

Hematoxylin and eosin (H&E) staining in sections of subcutaneous adipose tissue in rats (200×) (a) and determine the surface area of adipocyte (b) at W3 and W13. All values represent means ± SEM. ap < 0.05 vs. NL, bp < 0.05 vs. SL. Statistical analysis was performed using Student's unpaired t-test at W3 and ANOVA at W13. n=6-9 in each group.

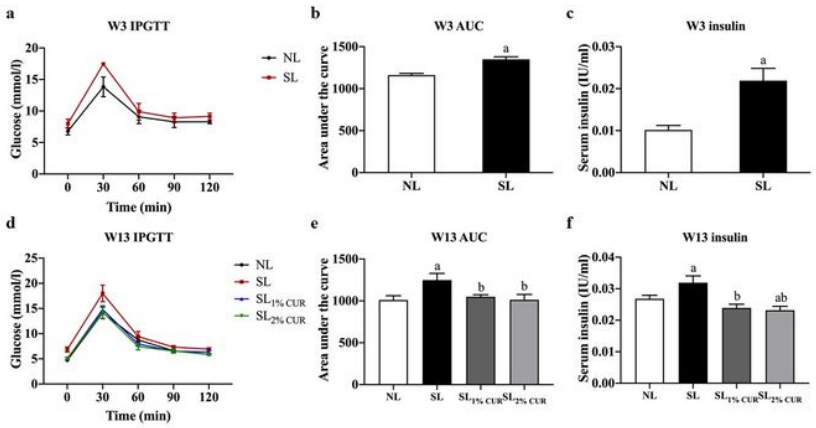

Fig.5

Figure 5

Intraperitoneal glucose tolerance test (IPGTT), area under the curve (AUC) and serum levels of insulin in rats at W3 (a-c) and W13 (d-f). All values represent means ± SEM. ap < 0.05 vs. NL, bp < 0.05 vs. SL. Statistical analysis was performed using Student's unpaired t-test at W3 and ANOVA at W13. n=6-9 in each group.

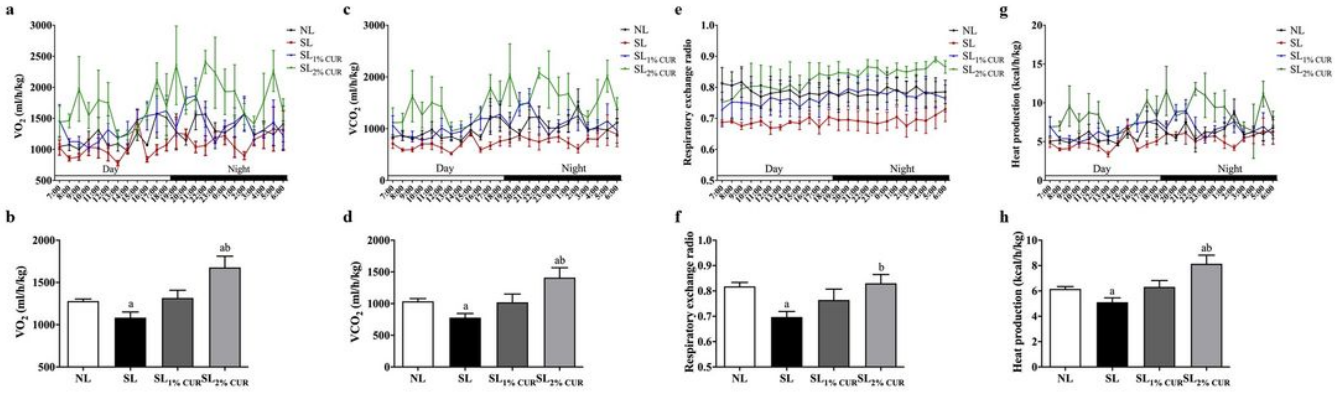

Fig.6

Figure 6

The whole-body metabolic rate including oxygen consumption (a and b), carbon dioxide production (c and d), respiratory exchange radio (e and f) and heat production (g and h) in rats at W13. All values represent means ± SEM. ap < 0.05 vs. NL, bp < 0.05 vs. SL. ANOVA was performed. n=4 in each group.

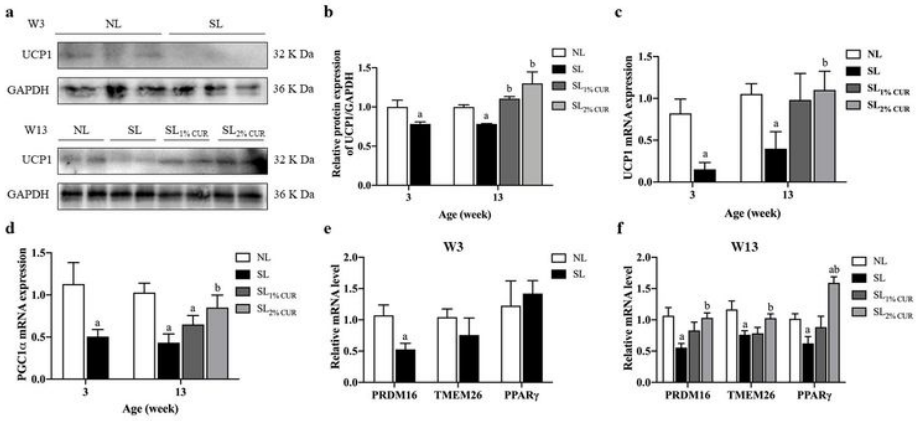

Fig. 7

Figure 7

Protein expression of UCP1 (a and b), mRNA expression of UCP1 (c) and PGC1α(d) of subcutaneous adipose tissue in rats at W3 and W13. mRNA expression of PRDM16, TMEM26 and PPARγ of subcutaneous adipose tissue in rats at W 3 (e) and W 13 (f). All values represent means ± SEM. ap < 0.05 vs. NL, bp < 0.05 vs. SL. Statistical analysis was performed using Student's unpaired t-test at W 3 and ANOVA at W13. n=6-9 in each group.

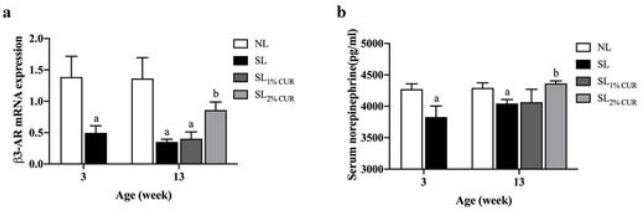

Fig. 8

Figure 8

Serum levels of norepinephrine (a) and mRNA expression of β3-AR of subcutaneous adipose tissue (b) in rats at W3 and W13. All values represent means ± SEM. ap < 0.05 vs. NL, bp < 0.05 vs. SL. Statistical analysis was performed using Student's unpaired t-test at W 3 and ANOVA at W13. n=6-9 in each group.