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Effects of caloric restriction on peroxisome proliferator-activated receptors and positive transcription elongation factor b expression in obese rats

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Abstract. – OBJECTIVE: To investigate the effect of caloric restriction (CR) on expressions of peroxisome proliferators-activated receptors (PPARs) and positive transcription elongation factor b (P-TEFb) (including cyclin-dependent kinase 9 (CDK9) and cyclin T1) protein in visceral adipose tissue of obese rats.

MATERIALS AND METHODS: Obese rats were induced by high-fat diet for 8 weeks. Then they were divided into three groups: Model (n=5), 50% Calorie Restricted (50% CR, n=5), Intermittent Fasting (IF) (eight cycles of 3-d fasting and 3-d refeeding, n=6) for 8 weeks. Biochemical parameters were measured. Protein and mRNA expression of Cdk9, cyclin T1 and PPARs were qualified in visceral adipose tissue.

RESULTS: A significant decline in fasting plasma glucose (FPG), homeostatic model assessment of insulin resistance (HOMA-IR), body weight, and visceral fat weight was observed in 50% CR group. The IF group exhibited a significant decrease in FPG, HOMA-IR, visceral fat weight. Both 50% CR and IF downregulated mRNA and protein expression of PPARγ and Cdk9, cyclin T1 and upregulated mRNA and protein expression of PPARβ.

CONCLUSIONS: These results suggest that the effects of 50% CR and IF on HOMA-IR, body weight, visceral fat weight, P-TEFb and PPARγ expression may be related to their protective potential on obesity.

Key Words
Caloric restriction, Intermittent fasting, Obesity, PPARs, P-TEFb.

Abbreviations

AT adipose tissue; CCNT1 cyclin T1; CDK9 Cyclin-dependent kinase 9; CR Caloric restriction; DM Diabetes mellitus; IR Insulin resistance; LDL-C Low density lipoprotein cholesterol; PPARα Peroxisome proliferators-activated receptor α; PPARβ Peroxisome proliferators-activated receptor δ; PPARγ Peroxisome proliferators-activated receptor γ; PPARs Peroxisome proliferators-activated receptors; P-TEFb Positive transcription elongation factor b; VF Visceral fat.

Introduction

Characterized by energy imbalance, obesity has become the most common nutritional disorder in industrialized countries[1]. It evolves chronic situation, which results in the failure of systems regulating the level of energy reserves in adipose tissue. Abdominal adiposity has been associated with increased risk of insulin resistance (IR), type 2 diabetes mellitus (T2DM) and cardiac vascular disease (CVD)[2,3]. From a cell-biological aspect, obesity is defined as the expansion of adipose tissue in the body, which is caused by an increased size of adipocytes (hypertrophy) as well as an increased number of adipocytes (hyperplasia). Hyperplasia of the adipocytes is initiated by adipogenesis of the mesenchymal stem cells or preadipocytes[4]. Adipogenesis has been a primary target for anti-obesity strategies, and it is regulated by a complex network of transcription factors, such as the PPARγ[5]. Positive transcription elongation factor b (P-TEFb), composed of cyclin T1 and cyclin-dependent kinase 9 (CDK9), has been implicated in regulating the differentiation of several cell types, such as adipocytes and skeletal muscle cells[6,7]. The positive effects of CDK9 on the differentiation of 3T3-L1 cells are mediated by a direct interaction with phosphorylation of PPARγ, which is the master regulator of this process. PPARγ-CDK9 interaction leads to increased transcriptional activity of PPARγ and increased adipogenesis[5]. Caloric restriction (CR) is one of the primary intervention tools to weight loss and health maintenance. It is specifically defined as a reduction in energy intake well below the amount of calories that would be consumed ad libitum (≥ 10% in human studies and usually ≥ 20% in rodent species[8]. Today, many diets commonly referred to as CR in the literature involve intermittent feeding

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and fasting cycles (IF), also known as every-other-day feedings, or food restriction (FR) without micronutrient supplementation[9]. CR has been proved to exert diversified effects as following: weight loss, reducing visceral adipose tissue, precluding insulin resistance associated with aging[10]. CR also results in a metabolic and transcriptional reprogramming of the adipose tissue, which reduces adiposity by altering the gene expression profile[11]. By increasing the expression of adipogenic factors, and maintaining the differentiated state of Adipocytes, CR improves insulin sensitivity[12]. Although the exact underlying mechanisms of adipogenesis are still debatable, it is widely accepted that the transcription factor peroxisome proliferator-activated receptor γ is a master regulator[13]. However, to our knowledge, analysis of the effects of CR in adipogenesis is limited. Seldom research reported the changes in the visceral fat by different kinds of caloric restriction. In this research, to understand the molecular basis of CR-associated adipocyte differentiation, we performed gene and proteome analysis of visceral fat from 5-week-old male rats fed AL or subjected to 50% CR and IF for 8 weeks. Also, we compared the effects of ad libitum feeding (AL), 50% CR and IF on body weight, abdominal fat accumulation, metabolic profile, protein and mRNA expression of PPARs and P-TEFb, and we detected the genes were transcribed and translated; the translated proteins carried out the reduction in adiposity. On the basis of this finding, we investigated the effects of different protocol of CR on the metabolism and adipogenesis of visceral fat.

Materials and Methods

Experimental Animals and Materials

A total of 40 male Wistar rats aged 5 weeks were generated by Guangdong Medical Laboratory Animal Center. Rats were caged individually under SPF condition on a 12 h day/night cycle at 20-22°C. The experimental animals were allowed to acclimatize for 7 days.

Diet Protocol

The animals were divided into two groups: SFD (standard fat diet) group (n=5) and HFD (high-fat diet) group (n=35). Rats in SFD group were fed ad libitum with standard fat diet (Guangdong Medical Laboratory Animal Center, contains 14% fat, 46% carbohydrate and 30% proteins, 10% supplemented with minerals and vitamins, caloric value 3.09 kJ/g) and HFD group was fed with a high fat diet containing 42% kcal from saturated fat, 42.7% kcal from carbohydrate and 15.2% from proteins, caloric value 3.79 kJ/g (Shanghai Laboratory Animal Co. Ltd., Shanghai, China) for 8 weeks. Water was always available ad libitum. Food intake and body weight were measured daily. The obese rats were assigned randomly to model group (n=5), 50% CR group (n=5) and IF group (n=6). Rats in model group were fed HFD *ad libitum*, the stage was continued for 8 weeks (56 days). On the 57th day, those rats whose weights were more than 20% of average weight of SFD group were judged as obesity models. 16 obese rats were divided into subgroups: model group (n=5), 50% CR group (n=5) and IF group (n=6). 50% Caloric restriction was accomplished by 10% reduction every day in the daily food intake of model group until 50% reduction was achieved. Rats in IF group were fasted for two days and fed HFD ad libitum for five days as a cycle, and continued the cycle for 8 times. Model group was continually fed with HFD *ad libitum*. At the end of the experiments, after 12 h fasting, rats were euthanatized by intraperitoneal injection of 60 mg/kg nembutal (Nanjing reagent Co., Ltd, Nanjing, China). Blood was collected via the postcava puncture, and plasma was stored at -80°C. All animal experiments were approved by the Ethics Review Committee for Animal Experimentation of First Affiliated Hospital of Sun Yat-sen University and performed in accordance with the NIH "Principles of Laboratory Animal Care" (NIH publication No. 86-23, revised 1985).

Assays

Fixed tissues were processed routinely, embedded in paraffin, and sectioned. 5 mm sections were stained with hematoxylin eosin (HE). Stained sections were scanned by microscopy with a CCD camera (Nikon, Tokyo, Japan). The size and density of adipocytes in the adipose tissues were determined using ''ImageJ 1.43u/Java1.6.0_22'' software. To avoid inter-rating variation, a single observer (Yu. H.) carried out the morphometric analysis. Fasting blood was used for assaying fasting plasma glucose (FPG) with one touch ultra blood glucose monitoring system (Life-Scan, Milpitas, CA, USA). Other metabolic parameters, including triglycerides (TG), total cholesterol (TC), high density lipoprotein-cholesterol (HDL) and low density lipoprotein- cholesterol (LDL) were determined by using standard laboratory assays. Insulin level was measured

using commercial ELISA's commercially available RIA kits. The homeostasis model assessment was used to calculate the insulin resistance index (HOMA-IR) as (fasting glucose level in mmol/l × fasting insulin level in mIU/l)/22.5.

RNA Extraction and Real-time Reverse Transcription-polymerase Chain Reaction (RT-PCR)

Total RNA was extracted from frozen visceral fat using RNA iso PLUS (TaKaRa Biotechnology, Dalian, China), and was purified using the Fast Pure RNA kit (TaKaRa Biotechnology, Dalian, China), according to the manufacturer's protocol. To obtain cDNA, 1 mg RNA was subjected to reverse transcription by Prime Script Reverse Transcriptase (TaKaRa Biotechnology, Dalian, China) with random hexamer primers. Real-time quantitative PCR was performed using the Applied Biosystems 7300 Real-time PCR system (Life Technologies, Waltham, MA, USA) with SYBR Premix ExTaqII (TaKaRa Biotechnology, Dalian, China), according to the manufacturer's instructions. Transcripts of CDK9, cyclin T1, peroxisome proliferators-activated receptorα (PPARα), peroxisome proliferators-activated receptor β(PPARβ), peroxisome proliferators-activated receptorγ (PPARγ) were amplified. TBP was used for normalization. Each assay was performed in duplicate and validation of PCR-runs was assessed by evaluation of the melting curve. The Real-time PCR measurement of individual cDNAs was normalized to the amount of β-actin RNA and analyzed by the 2ΔΔCT method[14]. Primer sequences are shown in Table I.

Protein Extraction and Analysis of Target Protein Levels by Western Blot

Frozen visceral fat tissues were lysed with lysis buffer and then boiled for 5 min and sonicated. Lysis buffer was composed of 50 mM tris-HCl (pH 6.8), 2% sodium dodecyl sulfonate (SDS) and 5% glycerol. Protein concentrations of the soluble fraction were determined using the bicinchoninic acid (BCA) protein assay kit. 5-20 mg proteins were subjected to sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred to nitrocellulose membranes. Membranes were blocked with 2.5% milk and 0.25% bull serum albumin (BSA) in 0.1% tween 20+tris-buffered saline (TBS-T) for 1 h at room temperature, and then probed with the appropriate primary antibodies overnight at 4°C. The primary antibodies for ATP-citrate lyase (Epitomics, Burlingame, CA, USA), ATP-citrate lyase pS455 (Epitomics, Burlingame, CA, USA) and β-actin (Sigma-Aldrich, St. Louis, MO, USA) were used. After washing with TBS-T, membranes were incubated with secondary goat anti-rabbit IgG antibody (Abcam, Cambridge, MA, USA) for 1 h at room temperature. The specific proteins were visualized with LAS3000 (Fujifilm, Tokyo, Japan) and the data were analyzed using Multigauge software (Fujifilm, Tokyo, Japan).

Statistical Analysis

The experiments were performed three times and demonstrated similar results.

Values were expressed as mean ± SD by replicating three times. Statistical analysis was performed with use of the Student's *t*-test. Differences with p-values <0.05 were deemed statistically significant.

Results

Changes of Body Weight, Lee's Index and VF-to-body Weight Ratio

50% CR remarkably reduced VF-to-body weight ratio (Table II, Table III). The control rats gained weight normally by 31.8% over a period of 8 weeks while a decrease in body weight of 17.03% in the 50% CR rats and an increase of 0.047% in the IF rats was observed.

Table I. List of primers for Real-time RT-PCR.

	Forward	Reverse
CDK9	5'- AGGCACATTCGGGGAAGTATTTA-3′	5'- GGTTGACCACATTCTCGTGCTTT-3′
CyclinT1	5'- TGTGAGCAAACGACCAAGTGAT-3′	5'- AGGCAACTGGGTCATTGTAGGA-3′
PPARα	5'- GACGCTGGGTCCTCTGGTT-3′	5'- TCAGTCTTGGCTCGCCTCTA-3′
PPARβ	5'- TCACACAACGCTATCCGTTT-3′	5'- TGCACGCCATACTTGAGAAG-3′
PPARγ	5'- TCCAAGAATACCAAAGTGCG-3′	5'- GCTTCAATCGGATGGTTCTT-3′
β-actin	5'- TGCTATGTTGCCCTAGACTTCG-3′	5'- TGCTATGTTGCCCTAGACTTCG-3′

Table II. Changes of body weights (g) ($\bar{\chi}$±s).

Weeks	Control	Model	50% CR	IF
0	302.1±11.7	368.2±12.8#	364.6±9.1	361.5±13.4
2	346.6±12.5	420.8±19.6#	360.2±13.2	397.8±15.6
4	363.4±20.7	444.0±28.0#	347.4±7.4※▲	414.6±22.5
6	379.3±17.8	455.6±18.2#	324.5±9.3※▲	395.1±17.9Δ
8	398.0±13.2	466.4±10.7#	302.6±16.5※▲	378.5±16.0Δ

#: p<0.05, model *vs.* control; ※: p<0.05, 50% CR *vs.* model; Δ: p<0.05, IF *vs.* model; ▲: p<0.05, 50% CR *vs.* IF.

Table III. Effect of CR on Lee's index, VF- to -body weight ratio ($\bar{x}$±s).

	N	Lee's index	VF-to-body weight ratio (%)
Control	5	294.40±17.00	9.3±0.89
Model	5	347.60±20.35▲	15.5±3.17▲
50% CR	5	249.00±14.66#	7.64±0.80#
IF	6	278.33±13.48#Δ	9.38±1.62#

#: p<0.05, 50% CR, IF *vs.* model; ▲: p<0.05, model *vs.* control; Δ: p<0.05, IF *vs.* 50%

Metabolic Profile

The glucose levels of IF rats were lower than that of CR 50% rats (p <0.05), and significantly lowers in comparison to that of model rats (p <0.05). We found a decrease in serum insulin levels throughout experimental period in IF and 50% CR, the difference between IF and model rats is significant (p <0.05). However, the difference between 50% CR and IF wasn't significant (p <0.05). We also detected a significant decrease of HOMA-IR in IF rats, but no significant differences between 50% CR and model rats were appreciated, indicating IF displayed a better effect on improving insulin resistance than 50% CR. There was a significant (p <0.05) increase in serum HDL-C and decrease in serum total cholesterol concentration in IF group of animals throughout the study, whereas the serum LDL-C concentration was found to be significantly lower in 50% CR as compared to model (p <0.05) (Table IV).

Effect of CR on PPARs and P-TEFb Gene Expression in Visceral Fat

CDK9, cyclin T1 and PPAR γ mRNA expression in model group were significantly increased when compared with those of control group. 50% CR and IF remarkably inhibited CDK9, cyclin T1 and PPAR γ mRNA expression in visceral fat and inverted the increased CDK9, cyclin T1 and PPAR γ mRNA expression to near that of control group but could not improve PPAR γ mRNA expression. PPAR β mRNA expression in model group was significantly decreased compared with those in control group. 50% CR and IF remarkably promoted PPAR β mRNA expression in visceral fat and restored the decreased PPAR β mRNA expression to near that of control group (Table V, Figure 1).

Effect of CR on PPARs and P-TEFb Protein Levels in Visceral Fat

50% CR and IF increased the expression of PPAR β and reduced the expression of CDK9, cyclin T1 and PPAR γ, but had no effect on PPAR α. It showed a tendency that caloric restriction augmented PPAR β and brought the declined PPAR γ, CKD9, cyclin T1 expression in visceral fat to near those of control group. Both 50% CR and IF could not influence PPARα expression (Figure 1).

Table IV. Effect of CR on metabolic profile ($\bar{\chi}$±s).

Characteristic	Control (n=5)	Model (n=5)	50% CR (n=5)	IF (n=6)
FBG (mmol/L)	6.18±0.96	11.26±1.26#	8.38±2.53Δ	4.31±1.17▲※
FINS (mIU/L)	3.80±0.98	6.30±2.03#	5.43±1.26	3.85±1.51▲※
HOMA-IR	1.33±0.52	2.85±1.72#	2.02±0.68	0.88±0.43▲※
TC (mmol/L)	1.19±0.61	1.30±0.22#	1.18±0.39	0.80±0.27▲
TG (mmol/L)	0.56±0.08	0.59±0.18#	.53±0.16	0.55±0.06
HDL-C (mmol/L)	0.51±0.07	0.37±0.04	0.45±0.10	0.56±0.17▲
LDL-C (mmol/L)	0.21±0.046	0.28±0.13#	0.076±0.04Δ	0.18±0.06

#: p<0.05, model *vs.* control; Δ: p<0.05, 50% CR *vs.* model; ▲: p<0.05, IF *vs.* model; ※: p<0.05, IF *vs.* 50% CR.

Table V. Effect of CR on PPARs and P-TEFb gene expression in visceral fat.

	N	cyclin T1	CDK9	PPARα	PPARβ	PPARγ
Control	5	1	1	1	1	1
Model	5	1.19±0.19#	1.25±0.15#	0.89±0.10	0.80±0.16#	1.17±0.13#
50% CR	5	0.56±0.12※	0.71±0.18※	0.82±0.12	1.06±0.19※	0.58±0.11※
IF	6	0.63±0.12※	0.62±0.14※	0.89±0.10	1.02±0.15※	0.52±0.15※

ΔCT (threshold cycle) =CT (target gene) -CT (β-actin), ΔΔCT=ΔCT (other gene) -ΔCT (control gene), relative fold=$2^{-\Delta\Delta CT}$, control gene is 1. Data are means±S.D.
#: $p<0.05$, model *vs.* control; ※: $p<0.05$, IF *vs.* 50% CR.

Histopathology of Adipose Tissue

50% CR and IF markedly reduced the size of lipid droplets. Because the unilocular lipid droplet occupies most of the cytoplasm of adipocytes, the size of the lipid droplet is thought to represent the cell size. The adipocyte size distribution was significantly wider in AL rats compared with CR rats. The percentage of large

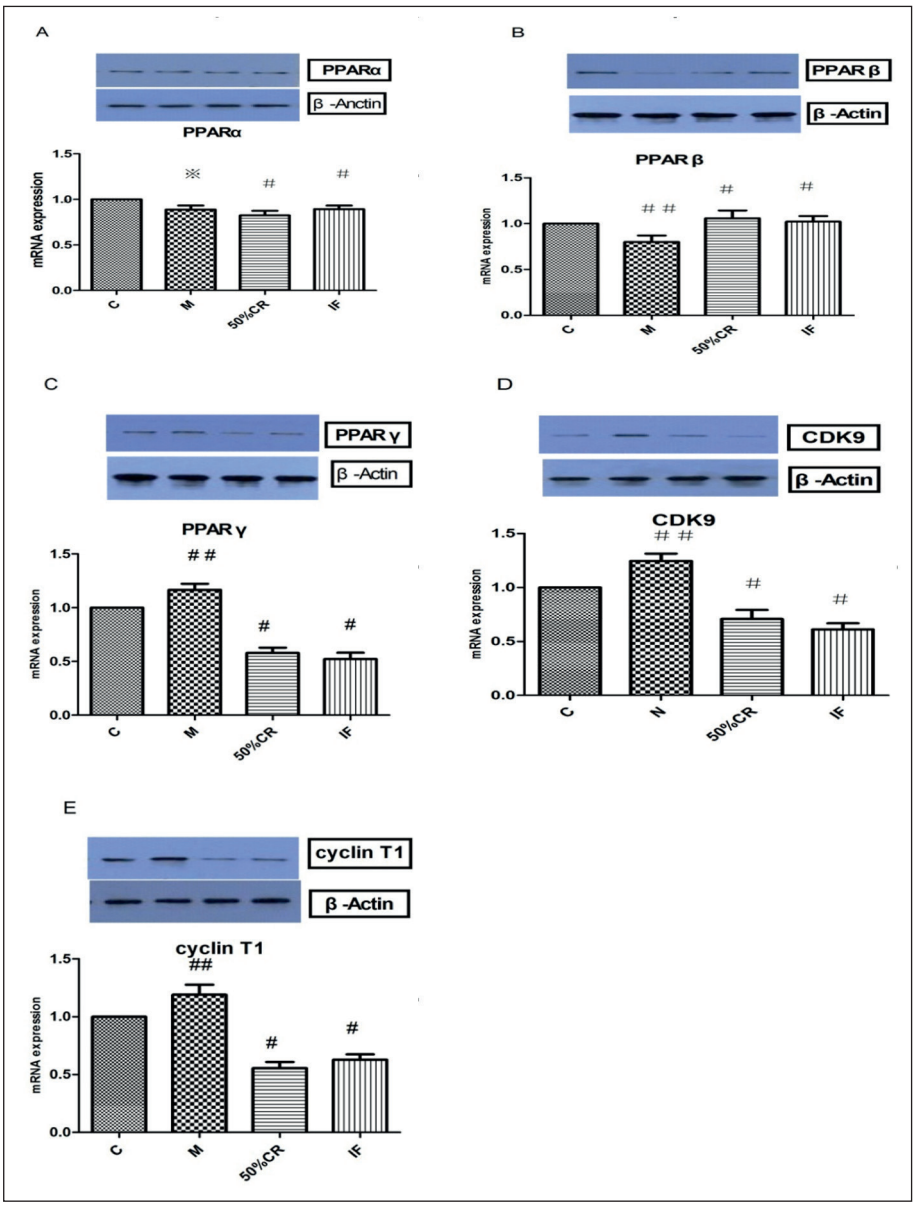

Figure 1. The gene expression and protein levels of cyclin T1, CDK9, PPAR α, PPAR β, PPAR γ. Groups: C, control; M, model; 50% CR, 50% caloric restriction; IF, intermittent fasting; ※: $p>0.05$, model *vs.* control; ##: $p<0.05$, 50% CR, IF *vs.* model; #: $p>0.05$,50% CR *vs.* IF.

adipocytes (>8000 mm^2) was 6.6% in AL rats and less than CR rats. In contrast, the portion of small adipocytes (<2000 mm^2) was approximately 50% in CR rats and 33% in AL rats. Consistent with the histological data, CR significantly reduced TG content in the fasted state. The changes in morphology were attenuated and the number of fat cells was increased in 50% CR and IF compared to that of model group (Figure 2).

Discussion

Recent overweight studies have demonstrated that caloric restriction can effectively improve the weight loss and reduce the risk of cardiovascular events[15]. However, due to poor compliance, long-term caloric restriction was very difficult for most of overweight people[16]. Recently, an alternative dietary approach, which was called intermittent fasting, has received increasing interest from researchers and the overweight patients. IF, also known as "every other day feeding", is a dietary protocol in which animals alternately fast and have access to food at certain intervals. Differing from CR, IF only requires energy restriction for 1-3 d per week, and allows for ad libitum food consumption on the no restriction days[17]. Evidence showed that this approach may have beneficial effects similar to CR[18]. In our work, the short- and long-term effects were compared between these different diets approaches. Numerous evidence has shown that overweight and obese individuals achieved weight loss through caloric restriction[19].

A recent research showed total body weight as well as subcutaneous and gonadal adipose tissue mass were markedly reduced after 6 weeks of CR[20]. Similarly, in this study, we found that the body weights of the experimental animals subjected under 50% CR were significantly reduced when compared with model rats. This could be a result of less caloric intake by these animals. The significant increase in body weight in model rats was due to excess food intake. In this study, either IF or 50% CR rats gained less weight than control rats and showed reduced visceral fat. However, the reduction in visceral fat was greater in 50% CR rats than in IF, this may account for greater food intake in IF than that of 50% CR. Karbowska et al [21]reported the effect of total calorie intake was the same as IF (3-d food deprivation, 3-d refeeding) and CR groups. IF rats results in greater reduction in white adipose tissue mass than CR rats.

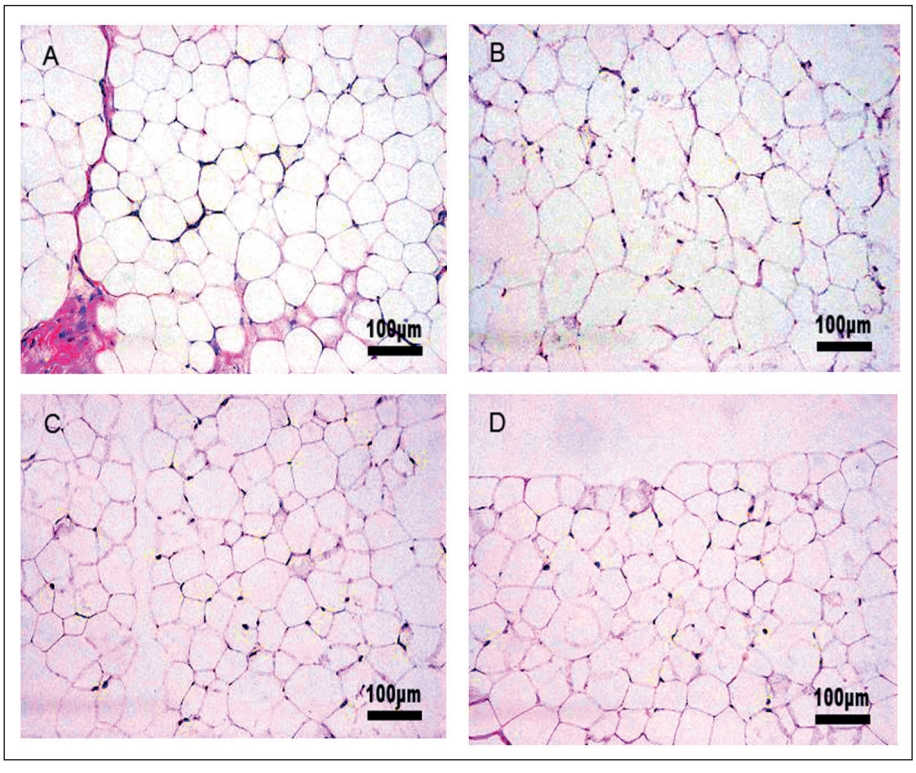

Figure 2. Effect of CR on hematoxylin eosin (HE) staining of adipose tissue in rats. A, control group; B, model group; C, 50% CR group; D, IF group.

Accumulating evidence suggested that even modest weight loss (5-7% of initial weight) helps to improve several diabetes risk parameters, including fasting glucose, insulin, and insulin sensitivity[22]. Positive association between obesity and the risk of developing type 2 diabetes mellitus has been also reported in numerous studies. Intra-abdominal fat accumulation has been associated with an increased risk of pre-diabetic conditions such as impaired glucose tolerance and insulin resistance[23]. Meta-analysis in this study further demonstrated that patients who fasted routinely had modestly lower average glucose levels[24]. Caloric restriction has also been reported to improve glycemic homeostasis, fasting glucose levels[25]. Reduction in fasting glucose were also assessed in 2 IF studies. Results from these studies demonstrated consistent improvement in insulin sensitivity after 3-24 weeks of treatment in normoglycemic and prediabetic subjects[26]. Anson et al[27] investigated glucose metabolism and enhanced neuronal resistance to stress in C57BL/6 mice subjected to *ad libitum* diet, IF, or limited daily food intake for 22 weeks. The IF group showed lower plasma glucose and insulin levels than the others. These findings were reproduced in male Wistar rats[28]. A significant decline in the FPG and FINS levels of IF rats in the present research reinforces this observation. There is a modest decrease in plasma FPG and FINS observed in 50% CR when compared to the model group, but only the decline of FPG levels was statistical. Chronic reduced-calorie diets have been demonstrated to enhance insulin sensitivity[29]. In the present study, significant decline of HOMA-IR index was also observed in the IF rats. The observed reduction in the IF rats may be caused by lower calorie consumption than that of model rats. We detected a significant decrease of HOMA-IR in IF rats, but no significant differences between 50% CR and model rats were observed, indicating IF displayed better effects on improving insulin resistance than 50% CR.

Obese individuals are frequently characterized by an impaired lipid profile, in which plasma triglycerides are raised, HDL-cholesterol concentrations are reduced and low-density lipoprotein apo B (LDL-apoB) levels are raised. This disturbed metabolic profile is more often seen in obese patients with a high accumulation of intra-abdominal fat and has consistently been related to increased risk of cardiovascular diseases[30,31]. Similarly, in the present research, we also observed IF significantly reduced levels of TG, and increased levels of HDL-C compared with model rats. However, we didn't detect any change in the levels of TG and HDL-C in 50% CR rats. In addition, we observed significant decrease in levels of LDL-C in 50% CR rats. The difference of the effects on lipid profile between 50% CR and IF needs to be further explored.

Epidemiological evidence indicated that abdominal fat is more likely to be related to negative health outcomes than the type of subcutaneous fat[32,33]. Metabolic abnormalities could be viewed as the result of impaired AT function. Abnormal AT is characterized by a large number of large fat cells, and a high level of local inflammation. Little is known about adipocyte differentiation in humans and its relation to development of obesity. There is a cross talk between the differentiation of adipocytes and metabolic control. Factors such as nutrition, stress or physical exercise are translated into proliferative stimuli. Adipogenesis involves two major tightly regulated events: pre-adipocyte proliferation, and adipocyte differentiation. The cross talk that exists between them determines the final adipocyte phenotype of the cell[34,35]. The lipid activated transcription factors PPARs, which belong to the nuclear receptor super family and play an important role in the differentiation of adipocytes and adipogenesis.

Among them, the adipose-specific isoform-PPARγ was significantly upregulated during the adipocyte differentiation[36]. PPARγ2 mRNA decreased in human subcutaneous adipose tissue resulted by a low caloric restriction[37]. This calorie restriction-induced downregulation of PPARγ was sustained throughout the 16 weeks of calorie restriction[38]. Another research showed that intermittent fasting, despite lower caloric intake, increased the expression of genes involved in lipid storage. In IF rats, PPARg2 mRNA levels were approximately two-fold higher than in the control group[21]. In the present work, 8 weeks of caloric restriction in both 50% CR and IF groups decreased the upregulated expression of PPARγ in the adipose tissue of obese rats. We inspected 50% CR and IF, may account for, at least in part, the weight loss and reducing of the visceral fat in obese rats, by downregulating PPARγ expression. PPARα expression is enriched in tissues with high fatty acid oxidation (FAO) rates such as liver, heart, skeletal muscle, brown adipose tissue, and kidney[39]. Masternak et al[40] reported no changes in both mRNA and protein levels of PPARα in the heart of mice by

30% CR for 19 months. Similarly, in the present study, there were no significant changes in levels of PPARα mRNA and protein in visceral adipose tissue.

Regulation of gene expression is executed primarily at the level of transcription of specific mRNAs by RNA polymerase II (RNAPII), typically in several distinct phases. Among them, transcription elongation is positively regulated by the positive transcription elongation factor b (P-TEFb), consisting of CDK9, cyclin T1 and T2[41]. Knockdown of CDK9 using siRNA decreased PPARs and cyclin T1 expression in 3T3-L1 cells. As Iankova et al[6] reported, this may be due to PPARγ-CDK9 interaction and result in downregulation of PPARγ expression.

Our investigation showed that CDK9 and cyclin T1 were downregulated by caloric restriction, which may result into the regulation of adipocyte differentiation and visceral fat reduction and weight loss. The effect of caloric restriction may account for, in a part, improving insulin resistance in obese rats. Histopathology of visceral adipose tissue is also a witness to support the speculation. Compared to model group, the number of fat cells in 50% CR and IF was more than that of model group after 8 weeks. The fat cell volume was smaller in 50% CR and IF than that of model group, suggesting that caloric restriction influenced adipocyte differentiation and resulted into changes in numbers and volume of fat cells in visceral adipose tissue. Limitations also existed in the present study. Firstly, the number of rats in every single group is limited, which will influence the reliability of the conclusion drawn from the data. Secondly, different from most researches on caloric restriction, both 50% CR and IF did not improve all the lipid profile, so it needs to be further investigated.

Conclusions

The results obtained in this study show that both intermittent fasting and 50% CR can regulate adipocyte differentiation, and as a result, improve the insulin resistance and reduce body weight as well as visceral fat by inhibiting expression of PPARγ, CDK9 and cyclinT1 in obesity Wistar rats. Obese rats exposed to 50% CR reduced more body weight and visceral fat than IF. However, IF improve the insulin sensitivity better than 50% CR even though there was more calorie consumption in IF than in 50% CR.

Conflict of Interest

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