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BASIC STUDIES

The expression of SIRT1 in nonalcoholic fatty liver disease induced by high-fat diet in rats

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Keywords
calorie restriction – high-fat diet – nonalcoholic fatty liver diseases – rats – SIRT1

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Abstract

Objective: SIRT1 is an NAD^+-dependent deacetylase and its enzymatic activity may be regulated by cellular energy. SIRT1 overexpression reduces the level of oxygen consumption, which is correlative with nonalcoholic fatty liver disease (NAFLD). To elucidate the role of SIRT1 on the development of NAFLD, we investigated the expression of SIRT1 in NAFLD induced by high-fat diet in rats and the effects of calorie restriction. *Methods:* Thirty-one male Wistar rats were divided at random into four groups. The rats in the normal control group NC ($n = 7$) and in the NAFLD model group HF ($n = 9$) were fed *ad libitum* with normal chow and high-fat diet, respectively, for 3 months, the rats in the calorie restriction (CR) group HCR ($n = 9$) were fed with a high-fat diet for 2 months and then 60% CR with normal chow for 1 month, and the rats in group CRH ($n = 6$) were firstly fed with 60% CR with normal chow for 1 month and then fed a high-fat diet for 2 months. At the end of the experiment, some parameters and expressions of SIRT1 were detected. *Results:* The rats in group HF displayed NAFLD. Compared with group NC, the expression of SIRT1 protein was significantly decreased ($P < 0.01$). However, the lower body weight and visceral fat mass of rats in group HCR were showed. Compared with group HF, CR increased the expression of SIRT1 in liver significantly ($P < 0.01$). Consequently, the ultramicropathology changes of NAFLD prominently improved in this group. Meanwhile, the rats in group CRH displayed higher expression of SIRT1 protein and very gentle pathology changes of NAFLD. *Conclusion:* The expression of SIRT1 is reduced significantly in NAFLD induced by high-fat diet in rats. CR increase-SIRT1 protein expression may be an important mechanism by which CR improves NAFLD.

Nonalcoholic fatty liver disease (NAFLD) encompasses a wide spectrum of liver damage, ranging from a pure fatty liver (steatosis) to nonalcoholic steatohepatitis (NASH) and liver failure. Risk factors associated with NAFLD include obesity, type 2 diabetes mellitus and hyperlipidemia. With the striking prevalence of obesity and type 2 diabetes mellitus worldwide, NAFLD is becoming a significant health problem. NAFLD affects 10–24% of the general population in various countries, the prevalence increases to 50% in diabetic and 76% in obese persons, and NAFLD is almost universal among diabetic people who are morbidly obese (1, 2). However, the pathogenesis that leads to NAFLD is not well elucidated, a popular mechanism is the 'two-hit' theory (3–5). Insulin resistance and oxidative stress are considered as major cause of NAFLD. Therefore, improving insulin sensitivity and oxidative stress has been a key strategy in the treatment of NAFLD. Unfortunately, till date, no medications have been proven to directly reduce or reverse liver damage independently of weight loss (1). Since, weight reduction is recommended in the first line among patients with NAFLD. It is well-known that calorie restriction (CR) is an important method to reduce weight. The mechanism responsible for the actions of CR, however, is still unknown. Recent studies indicate that SIRT1, a homologue of the yeast silencing information regulator 2, is a key gene involved in numerous effects of CR (6). SIRT1 is an NAD^+-dependent deacetylase and its enzymatic activity may be regulated by cellular energy. SIRT1 overexpression reduces the level of oxygen

consumption and relieves oxidative stress. The deacetylation of SIRT1 to too many substrates, such as Forkhead transcription factors (FOXO), p65 subunit of nuclear factor κ B (NF-κB), Ku70 telomeric protein (a DNA repair factor), peroxisome proliferators-activated receptor γ co-activator 1α (PGC-1α), maybe an important mechanism by which CR provides stress protection to cells. Cohen et al. (7) demonstrated that SIRT1 expression was higher in liver tissue of the CR animals when compared with expression in fed *ad libitum* animals. But whether or not the change of SIRT1 expression is associated with NAFLD is not yet reported. In our study, we established a rat model of NAFLD with high-fat feeding (8, 9). A strict CR for 1 month was performed according to different protocols. The changes of ultrastructure and SIRT1 expression in liver were observed to clarify the change of SIRT1 expression in NAFLD.

Materials and methods

Animals and diets

Normal male Wistar rats weighing 140–180 g were obtained from Experimental an Animal Centre of Tongji Medical College affiliated to Huazhong University of Science Technology. The normal diets were purchased from an Experimental Animal Centre of Hubei province. For high-fat diet making, 20% lard stearin (wt/wt), 10% sucrose and 0.1% bile salt were added into normal diet. The rats received either a normal diet with 18.94% of energy derived from fat, 31.67% from protein and 49.39% from carbohydrates or a high-fat diet with 50.55% of energy derived from fat, 15.72% from protein and 33.73% from carbohydrates. The rats were placed in special cages (3–4/cage) on a 12 h day/night cycle and provided *ad libitum* access to food and water, except when specified by experimental protocol. Animals were fasted for 12 h before any study. All protocols for animal experimentation and maintenance were approved by the Animal Ethics Committee in our university and carried out in accordance with the Institutional Guidelines.

Biochemical reagents

Glucose assay kits were purchased from Beihua Kangtai Clinic Reagent Company Limited (Beijing, China). Insulin assay kits were obtained from Jiuding Bioengineering Company Limited (Tianjin, China). Rabbit anti-rat SIRT1 (H-300) polyclonal antibody was from Santa Cruz Company (Santa Cruz, CA, USA). Mouse anti-rat β-actin monoclonal antibody and secondary antibody horseradish peroxidase conjugated goat immunoglobulin G (IgG) for immunochemistry were purchased from Boster Company (Wuhan, China). Enhanced chemiluminescence kit was purchased from Pierce Company (Rockford, IL, USA). Prestained Marker, SDS-polyacrylamide gel preparing kit, lysis buffer for Western, and BCA protein assay kit were supplied by Beyotime Biotechnology Company (Jiangsu, China). Other biochemical reagents were purchased from Dingguo Biotechnology Company Limited (Beijing, China).

Experimental protocol

Upon arrival, 31 male Wistar rats were fed with normal chow to acclimatize for 1 week. After acclimatization, the rats were randomly divided into four groups. Animals in the normal control (NC, $n = 7$) and NAFLD model group (HF, $n = 9$) were fed *ad libitum* with normal chow and high-fat diet, respectively, for 3 months. Rats receiving CR after high-fat feeding in the third group (HCR, $n = 9$) were fed with a high-fat diet for 2 months and then with 60% CR with normal chow for 1 month, while the rats in the fourth group (CRH, $n = 6$) were firstly fed with 60% CR with normal chow for 1 month and then high-fat diet for 2 months. The method of CR was performed as previously described (10). The rats receiving CR were housed singly in cages and fed *ad libitum* with normal chow for the few days. Food intake was measured each day in all rats and the average daily food intake of rat was obtained. The dietary caloric restriction was accomplished by 10% reduction in the average daily food intake every 5 days until a 40% reduction was achieved, then rats were fed with 60% CR for 1 month. At the end of the experiment, 1 ml blood was collected from the carotid artery. After performing the intravenous glucose tolerance test (IVGTT), all rats were sacrificed by an injection of an intraperitoneal (ip) overdose of sodium pentobarbital. Liver samples were quickly removed and immediately frozen in liquid nitrogen and stored at $-70\,^{\circ}C$ until analysis, or fixed in 4% buffered paraformaldehyde solution and 2.5% glutaraldehyde solution until use.

General characteristics observation

The food intake and body weight (BW) of rats in each group were observed weekly. After rats were sacrificed, epididymis fat and perirenal fat were isolated and weighed as visceral fat mass (VF). The percentage of VF to BW was calculated. Some biochemical parameters such as fasting plasma glucose (FPG), fasting

serum insulin (FINS), total cholesterol (TC) and triglyeride (TG) were tested. FPG was measured by the oxidation enzyme method and FINS by radioimmunoassay. TC and TG were automatically analysed with a multifunctional biochemistry analyser Olympus AU2700 (Olympus, Tokyo, Japan).

Insulin resistance evaluation

To evaluate insulin resistance of rats, insulin resistance index HOMA-IR and acute insulin response (AIR) were calculated because HOMA-IR and AIR were increased obviously in the early phase of insulin resistance (11, 12). Index HOMA-IR was computed as (FPG × FINS)/22.5. The IVGTT was performed to estimate AIR. Fasted 12 h at the end of the experiment, rats were anaesthetized with 1% sodium pentobarbital (60 mg/kg body weight, ip). Sterilized with routine method, rat skin was cut open. After the blunt dissection of musculi colli, the arteria carotis communis and external jugular vein, were exposed. The pedo-detaining cannulas were punctured into the artery and vein respectively, and then fast fixed. After 1 ml blood was collected from the arterial cannula, 50% glucose (0.4 g/kg body weight) was injected through the venous cannula immediately. After the glucose injection was over, blood samples of rat at different time points such as 1, 3, 5, 8, 15, and 30 min were collected through arterial cannula. The serum was separated by centrifugation and preserved at $-20\,^{\circ}$C until test. On the basis of FINS as the basic insulin secretion index, AIR was defined as the incremental area under the curve from the first to the eighth minute after the dextrose injection, divided by four (13).

Histopathological examination

Formalin-fixed and paraffin-embedded livers were processed routinely for haematoxylin and eosin. Tissues were also processed for electron microscopy with standard techniques. Rats were anaesthetised with 1% sodium pentobarbital (60 mg/kg body weight, ip) and killed after IVGTT. Small hepatic tissue fragments were excised and cut into 1 mm^3 sections. The fragments were immediately fixed in 2.5% glutaraldehyde and rinsed in 0.1 mol/l of phosphate buffer. After fixation in 1% osmium tetroxide and rinsing in 0.1 mol/l of phosphate buffer, the samples were dehydrated in a graded series of alcohol and embedded in pure epoxy resin. Ultrathin sections were cut with the Leica Ultracutuct slicer (Leica Microsystems, Wetzlar, Germany), stained with uranyl acetate and lead citrate, and examined using a FEI Tecnai G^2 12 transmission electron microscope (Shanghai, China).

SIRT1 protein expression by Western blot analysis

Samples of liver homogenate were analysed by the Western blot technique as described previously (14). Rabbit anti-rat SIRT1 (H-300) polyclonal antibody and mouse anti-rat β-actin monoclonal antibody were used to detect respective proteins. Frozen liver tissue (100 mg) was rapidly thawed and homogenized at 4 °C in 1 ml lysis buffer. Homogenates were centrifuged at 12 000 g for 20 min at 4 °C to yield supernatants. The protein concentrations were determined by a BCA protein assay kit. Equivalent amounts of proteins (30 μg) from homogenates were run in sodium dodecyl sulfate-polyacrylamide gel electrophoresis using an 8% acylamide resolving gel. Separated proteins were transferred to nitrocellulose membranes. Membranes were blocked in TBS-T (1 × TBS, 0.1% Tween-20) containing 5% milk for 1 h at room temperature followed by incubation with SIRT1 antibody (1:200) or β-actin antibody (1:400) at 4 °C overnight. Then, membranes were probed with their respective antibody labelled with horseradish peroxidase (1:4000) for 2 h at room temperature, and visualized using the enhanced chemoluminescence (ECL) reagent. After membranes were exposed to Kodak films for 5–10 min, the resulting images were quantitated by densitometry. Intensity of bands was analysed with Gel-Pro® analyzer 4.0 software. The results were expressed as a ratio of SIRT1 to β-actin densitometry.

Statistics

Results were expressed as mean ± SD and were analysed using one-way ANOVA. Student-Newman–Keul multiple-range test was then used to compare results between two groups. $P < 0.05$ was considered statistically significant. All statistical analyses were performed with the use of SAS 8.1 software.

Results

General characteristics of rats at the end of the experiment

The food intake of rats in group NC and HF remained quite stable and similar throughout the study period. At the end of the experiment, an unexpected death of one rat was found in group HCR. General characteristics of rats are summarised in Table 1. As expected, body weight was significantly lower ($P < 0.01$) in the HCR group compared with control rats and high-fat-fed rats. At the end of the experiment, there were no significant differences among group CRH, HF, and NC in body weight. However, VF of rats in group HF were much higher than that of other groups ($P < 0.01$),

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Table 1. Changes of characteristics in each group at the end of the experiment

Parameter	NC ($n=7$)	HF ($n=9$)	HCR ($n=8$)	CRH ($n=6$)
Baseline in beginning of the experiment				
BW (g)	168 ± 15	156 ± 13	159 ± 9	161 ± 14
FPG (mmol/l)	4.5 ± 0.49	4.3 ± 0.55	4.4 ± 0.38	4.7 ± 0.57
TC (mmol/l)	1.45 ± 0.32	1.52 ± 0.41	1.39 ± 0.56	1.49 ± 0.48
TG (mmol/l)	0.59 ± 0.13	0.66 ± 0.12	0.61 ± 0.10	0.57 ± 0.19
At the end of the experiment				
BW (g)	408 ± 44	374 ± 32	282 ± 19^{bd}	384 ± 25
VF (g)	9.0 ± 0.4	15.1 ± 4.1^{b}	2.5 ± 1.1^{bd}	15.9 ± 2.8^{b}
VF/BW (%)	2.2 ± 0.2	4.0 ± 1.1^{b}	0.9 ± 0.4^{bd}	4.2 ± 0.9^{b}
FPG (mmol/l)	4.4 ± 0.57	6.2 ± 1.46^{b}	3.8 ± 0.77^{d}	4.9 ± 0.51^{c}
TC (mmol/l)	1.41 ± 0.28	2.61 ± 0.29^{b}	1.06 ± 0.15^{bd}	2.06 ± 0.17^{bd}
TG (mmol/l)	0.67 ± 0.10	1.35 ± 0.21^{b}	0.45 ± 0.06^{ad}	0.93 ± 0.18^{bd}

[a] $P < 0.05$.
[b] $P < 0.01$ vs group NC.
[c] $P < 0.05$.
[d] $P < 0.01$ vs group HF.
NC, normal control.

Table 2. Changes of FINS, HOMA-IR and AIR in each group

Group	*n*	FINS (mU/l)	HOMA-IR	AIR (mU/l)
NC	7	13.09 ± 1.18	2.57 ± 0.35	55.81 ± 11.82
HF	9	29.22 ± 7.28^{b}	8.46 ± 4.53^{b}	93.84 ± 22.17^{b}
HCR	8	8.25 ± 2.42^{d}	1.41 ± 0.58^{d}	17.85 ± 6.72^{bd}
CRH	6	24.92 ± 4.28^{b}	5.50 ± 1.41^{ac}	77.92 ± 18.47^{ac}

[a] $P < 0.05$.
[b] $P < 0.01$ vs group NC.
[c] $P < 0.05$.
[d] $P < 0.01$ vs group HF.
FINS, fasting serum insulin; AIR, acute insulin response; NC, normal control.

suggesting that high-fat feeding led to abdominal obesity. Calorie restriction could significantly decrease VF of rats in group HCR. FPG, TC and TG of rats in group HF were increased prominently compared with that of other groups. The rats in group CRH had an obvious elevation in these parameters compared with control group because of the dramatic catch-up growth. However, these parameters of rats in group HCR were reduced significantly compared with that of group NC and HF.

Insulin resistance evaluation of rats in each group

To assess the effects of different feeding protocols on insulin resistance, IVGTT was performed in each group. FINS, HOMA-IR and AIR were used to evaluate the insulin resistance of rats (Table 2). Compared with other groups, the FINS, HOMA-IR and AIR of rats in group HF were increased greatly, suggesting the rats in this group had developed obvious insulin resistance. However, the FINS, HOMA-IR and AIR were reduced when rats in group HCR were fed with 1-month calorie restriction, suggesting insulin resistance in this group was obviously ameliorated. The rats in group CRH displayed higher FINS, HOMA-IR and AIR compared with group NC, but lower than that of group HF, suggesting the rats also existed in insulin resistance. Judged by these indexes, there was significantly different insulin resistance between group CRH and HF.

Changes of liver histology and ultrastructure

Photomicrographs of liver sections stained with haematoxylin and eosin are shown in Fig. 1. Liver sections from group NC rats were normal (Fig. 1A). The rats in group HF developed steatohepatitis and ballooning degeneration (Fig. 1B). The liver histological findings were almost normalised in rats of group HCR, which received 60% CR for 1 month (Fig. 1C). However, when rats in group CRH first received 60% CR for 1 month and then were fed with high-fat diet for 2 months, liver sections of rats showed very mild liver steatosis (Fig. 1D). No fibrosis was seen in any of the groups.

Ultrastructural changes of the liver in each group are shown in Fig. 2. In the rats of group NC, electron microscopy showed normal mitochondria and endocytoplasmic reticulum (Fig. 2A). In group HF, a higher number of lipid droplets accumulation (under lower power lens) and abnormal mitochondria could be observed. The mitochondrial variations were shown in sizes and shape, including swelling, rarefied matrix, breakage and loss of cristae and vacuolar degeneration.

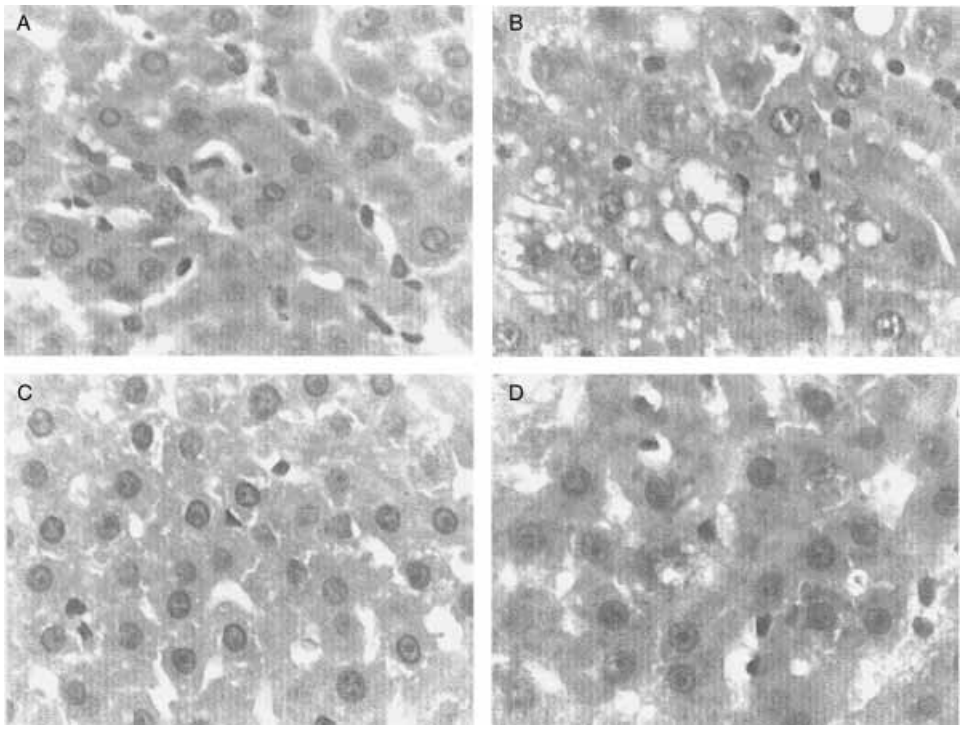

Fig. 1. Photomicrographs of liver samples stained with haematoxylin and eosin in each group**.** (A) Hepatic pathology of group NC, normal liver histology. (B) Hepatic pathology of group HF, steatohepatitis and ballooning degeneration. (C) Hepatic pathology of group HCR, the pathological changes of liver were obviously improved compared with group HF. (D) Hepatic pathology of group CRH, mild liver steatosis. The magnification of photomicrographs is $\times$ 200.

Hypermegasoma mitochondria were also observed. Furthermore, vesiculation and degranulation of endocytoplasmic reticulum could be seen in this group (Fig. 2B). However, after being fed with 60% CR for 1 month, the rats in group HCR had a significant improvement in the ultrastructure of liver cells. The lipid droplets and vesiculation of endocytoplasmic reticulum in liver cells were seldom seen, and structures of mitochondria were much clearer than that of group HF despite a little swelling (Fig. 2C). Strikingly, the rats in group CRH had similar ultrastructures with that of group NC, with the exception of slight swelling in the mitochondria (Fig. 2D).

SIRT1 protein expression of the liver in each group

As shown in Fig. 3, we analysed the expression of SIRT1 protein by Western blot analysis. Compared with group NC, the expression of SIRT1 in group HF was obviously decreased. But the expression of SIRT1 was elevated when the 60% CR for 1 month was administered in group HCR compared with group HF ($P < 0.01$). There were also significant differences in expression of SIRT1 protein between group CRH and NC ($P < 0.01$), but the expression of SIRT1 in group CRH was higher than that of group HF ($P < 0.05$).

Discussion

In this study, we investigated the expression of SIRT1 in NAFLD induced by high-fat diet and evaluated the treatment effects of calorie restriction. At first, we successfully induced NAFLD rat models by high-fat feeding. Indeed, progress in the understanding and treatment of NAFLD had been hampered by the lack of a practical experimental model that reproduces the key features of the disease. Recently, some studies had demonstrated that the high-fat feeding could reproduce the typical NAFLD (5, 8, 9, 15). Kraegen et al. (16) reported that rats fed with high fat diet for 3 days developed hepatic insulin resistance before the development of peripheral insulin resistance, suggesting feeding rats for a short duration would therefore provide an excellent model of NAFLD. Other studies suggested that the rats fed with a high fat diet for 3 weeks or 12 weeks also displayed typical characteristics of NASH (8, 9). In our rat models, VF of rats were much higher than any other group. Meanwhile, other general characteristics of rats such as FPG, FINS, TC

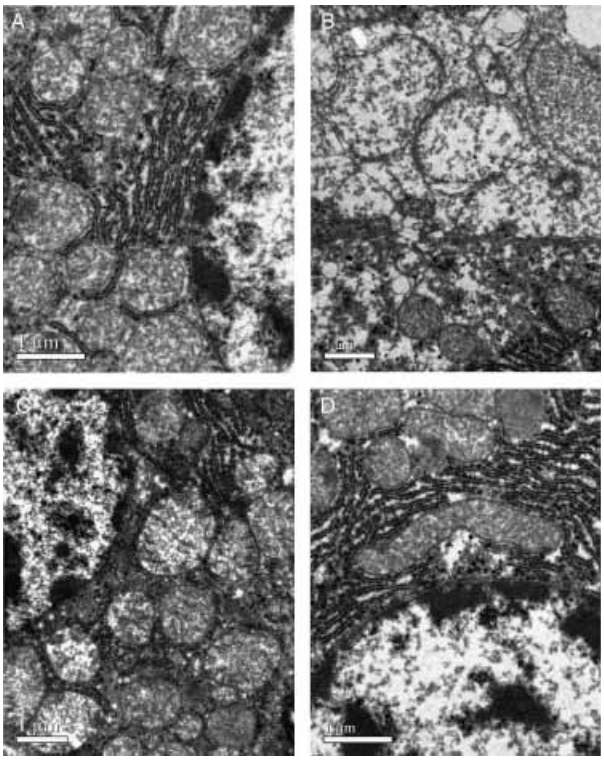

Fig. 2. Electron microscopy of hepatic ultrastructure of rats in each group. (A) Group NC showed the normal ultrastructure of liver tissure. (B) Group HF showed degenerative mitochondria changes with rarefied matrix and loss of cristae, and vesiculation and degranulation of endocytoplasmic reticulum. These changes were prominent in most of the mitochondria in this group. (C) Group HCR showed obvious improvement of hepatic mitochondria and endocytoplasmic reticulum. (D) Group CRH showed slight swelling in mitochondria and in normal endocytoplasmic reticulum. The plotting scale for magnification is 1 μm.

and TG in group HF were increased prominently. These suggested that high-fat feeding led to abdominal obesity, hyperinsulinemia and hyperlipemia. The obvious increase of HOMA-IR and AIR of rats in this group reflected that the rats had developed prominent peripheral insulin resistance. The liver histology and ultrastructure showed that the rats displayed steatohepatitis, ballooning degeneration, a higher number of lipid droplets accumulation, abnormal mitochondria and endocytoplasmic reticulum. Especially, the mitochondrial and endocytoplasmic reticulum variations were dramatic, suggesting indirectly the development of oxdiative stress and endocytoplasmic reticulum stress. All of these conformed to the pathology changes of NAFLD. Therefore, we can conclude that rats fed with the high-fat diet in our study developed typical example of NAFLD. Our study is consistent with above-mentioned researches. Meanwhile, results in this study further demonstrate that negative effects of a high-fat diet on the liver should not be ignored because some researchers proposed that dietary approaches to treat and prevent NASH and obesity should be a high-fat, high-protein and low-carbohydrate (Atkins type) diet (9, 17, 18).

Unfortunately, no medications have been proved to directly reduce or reverse liver damage independently of weight loss, but such medications would be desirable (1). Although many agents have shown promising results in preliminary pilot trials, however, there have been few treatment modalities examined in rigorous randomised double-blind placebo-controlled trials with adequate statistical power. It is well known that weight loss is associated with improvement in insulin

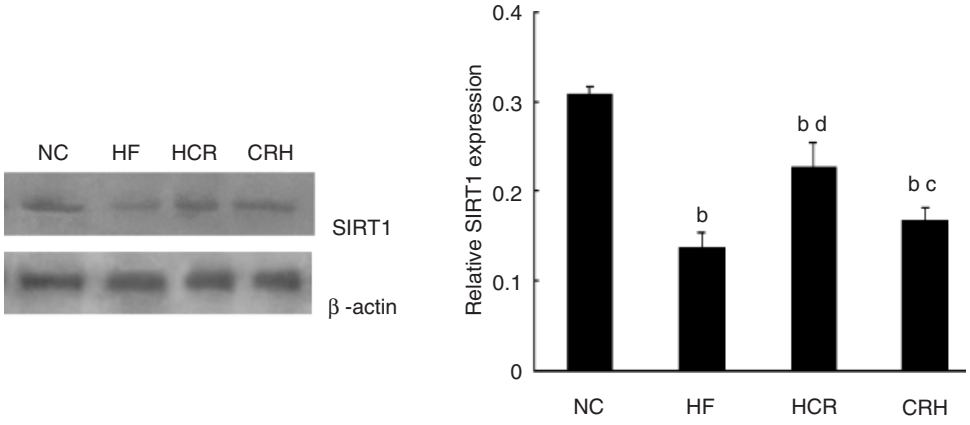

Fig. 3. SIRT1 protein expression of rat liver in each group. SIRT1 expression was assessed by Western blot analysis. The results were showed as a ratio of SIRT1 to β-actin densitometry. $^{b}P < 0.01$ vs group NC. $^{c}P < 0.05$, $^{d}P < 0.01$ vs group HF.

sensitivity and thus is a logical treatment modality for patients with NAFLD (19, 20). Weight reduction may be achieved by caloric restriction. Because it is comparatively safe, inexpensive and has other health benefits, CR should remain the first line among patients with NAFLD. But the evidence of efficacy and molecular mechanism of calorie restriction in patients with NAFLD is surprisingly scant. In our study, 60% CR with normal chow reduced some parameters such as VF, FPG, FINS, TC and TG and obviously ameliorated insulin resistance reflected by HOMA-IR and AIR of rats in group HCR. Consequently, the ultramicropathology changes of NAFLD improved prominently, suggesting that efficacy of CR in NAFLD improvement is clear and undoubted. But surprisingly, after rats displaying a catch-up growth in group CRH were fed with 60% CR firstly for 1 month and then fed a high-fat diet for 2 months, the changes of liver histology and ultrastructure of rats were much better than that of rats in group HF, although they had increased VF and insulin resistance compared with group NC. Catch-up growth is a risk factor for later obesity, type 2 diabetes and cardiovascular diseases (21), but we show here that CR can inhibit impairment of high-fat diet on liver in the short term at least.

Although CR can improve NAFLD significantly, patients with NAFLD seem more likely to have a diet high in saturated fats and cholesterol and low in fibre and anti-oxidants. Even under trial conditions, compliance is poor with 30–41% of participants dropping out, emphasizing the difficulty of maintaining weight loss through lifestyle change (19). Hence, it is important to address the molecular mechanism of CR in NAFLD treatment, and then we can imitate the effects of CR to treat NAFLD regardless of the strict and cruel calorie restriction. Recently, many researches suggest that the SIRT1 protein is a critical regulatory protein to resist stress damage and maintain cell survival in mammalian cell, and the deacetylation of SIRT1 protein is an important molecular mechanism of stress protection of CR (22–26). SIRT1 is an NAD^+- dependent deacetylase and its enzymatic activity may be regulated by cellular energy. It is already identified that SIRT1 is a key role in many cellular processes of mammalian *in vivo* (27–30): SIRT1 protein deacetylates p53, Tata box-binding protein-associated factor I of 68 kDa (TAFI68), p300 (CREB-binding protein homologue), p300/CREB-binding protein-associated factor (PCAF), myoblast determination protein (MyoD), Forkhead transcription factors (FOXO), p65 subunit of nuclear factor κ B (NF-κB), Ku70 telomeric protein (a DNA repair factor), peroxisome proliferators activated receptor γ coactivator 1α (PGC-1α), and so on. According to these substrates of SIRT1 protein, SIRT1 takes effects on gene silencing, cell cycle regulation, fatty acid metabolism, apoptosis and life-span extension. Furthermore, SIRT1 overexpression reduces the level of oxygen consumption linked to the generation of reactive oxygen species (ROS) and ROS levels correlate with NAFLD (31, 32). Some researchers suggest that the malfunction of liver mitochondria plays core role in the 'first and second hit' leading to NAFLD (32). As a key molecule to regulate biogenesis of mitochondria, the PGC-1α has an important effect in adaptability heat production of organism, formation of mitochondria and regulation of glucolipid metabolism. SIRT1 can regulate the activity of PGC-1α by deacetylization, furthermore, SIRT1 expression was reported to be induced by CR in rats (7). Therefore, it is rational to hypothesize that SIRT1 is involved in mechanism of CR in NAFLD treatment. We designed two CR groups to check our hypothesis: SIRT1 induced by CR should improve the NAFLD in HCR group or prohibit liver impairment from high-fat diet in CRH group. Our results partially confirm this hypothesis. In group HF, the expression of SIRT1 in rat liver was much lower than that of any other group. Consequently rats in this group displayed typical NAFLD. However, after rats in group HCR received 60% CR for 1 month, the expression of SIRT1 was increased and NAFLD was improved significantly. Surprisingly, when rats were firstly fed with 60% CR for 1 month following high-fat feeding, the expression of SIRT1 was higher than that in group HF and the pathology changes of NAFLD was very gentle in spite of increased VF and insulin resistance compared with group NC. Because CR reduces the electron and proton leakage of mammalian mitochondria to improve oxidative stress, meanwhile, it also increases the activity of PGC-1α to stimulate proliferation of mitochondria by deacetylization of SIRT1, it will make mitochondria increase ATP production but reduce oxygen consumption, decrease membrane potential and reduce active oxygen (28). We consider, in some extent, it was that CR increased SIRT1 protein expression in group CRH protected the liver of high fat feeding rats. Taken all together, these results show that changes of SIRT1 expression are certain exist in NAFLD and CR can improve NAFLD by increasing the expression of SIRT1. We suppose that the deacetylization of SIRT1 to PGC-1α is an important molecular mechanism by which CR can improve NAFLD.

In conclusion, this study indicates that the expression of SIRT1 is reduced significantly in NAFLD

induced by high fat diet in rats. CR-increase SIRT1 protein expression may be an important mechanism by which CR improves NAFLD. Thus we presume that the specific SIRT1 activating agent to stimulate SIRT1 expression or activity may also have a favourable effect on NAFLD by imitating contribution of CR. Further researches are needed to elucidate it.

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