Available online at www.sciencedirect.com
ScienceDirect
Metabolism Clinical and Experimental 60 (2011) 569–578
Metabolism
Clinical and Experimental
www.metabolismjournal.com

Lipid overaccumulation and drastic insulin resistance in adult catch-up growth rats induced by nutrition promotion after undernutrition

Lu-Lu Chen*, Xiang Hu, Juan Zheng, Wen Kong, Hao-Hao Zhang, Wei-Hong Yang, Su-Ping Zhu, Tian-Shu Zeng, Jiao-Yue Zhang, Xiu-Ling Deng, Di Hu

Department of Endocrinology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430022, China

Received 23 October 2009; accepted 24 May 2010

Abstract

This study was designed to explore the metabolic changes resulting from catch-up growth in adult (CUGA) induced by varying degrees of nutrition promotion after undernutrition and to confirm whether these changes are transient or not. The CUGA models were developed on rats refed on intakes of normal chow or high-fat diet after a period of caloric restriction. The growth of the rats measured by body weight and length stagnated during caloric restriction and then rapidly accelerated following refeeding. Catch-up growth in adult resulted in an increase in intramuscular and intrahepatic lipid content, visceral fat deposition, and insulin resistance, which is consistent with a transient rise in food efficiency during the early stage of refeeding. In addition, ectopic lipid deposition, visceral fat accumulation, and insulin resistance were more severe in rats refed the high-fat diet than rats refed the normal chow. These findings suggest that CUGA induced by rapid nutrition promotion could result in persistent lipid overaccumulation (increased visceral fat and ectopic lipid deposition) and drastic systemic insulin resistance. The effects of CUGA on metabolic characteristics are dependent on the type of diet that is used for refeeding, especially on the amount of fat intake.

1. Introduction

Insulin resistance (IR)–related diseases such as type 2 diabetes mellitus have become increasingly prevalent worldwide, placing a heavy burden on many health care systems. Recent health statistics have shown that this trend is higher in Asia than in other parts of the world [1,2]. Although nutrient excess has been recognized as the main cause of insulin-related diseases in developed countries [3], the same explanation cannot hold for Asian countries. In fact, the calories per capita and the dietary supply of fat in Asian countries are lower than in well-developed countries [4]. Therefore, it is difficult to explain the so-called Asia's problem with nutrient excess. Following the rapid economic development in recent years, Asian societies generally have undergone nutrition promotion while shifting from an environment of food scarcity to one where food is abundant. The nutrition level of Asian countries, which was previously lower compared with well-developed countries, has increased rapidly and remarkably in recent years [4]. In addition, the trend of dieting and weight-loss programs is on the rise, whereby people try to reach the sometimes unrealistic lean aesthetics health ideal. In reality, many of these attempts end in "yo-yo dieting" that consists of cycles of weight loss or nutrition deprivation followed by weight gain or nutrition promotion, leading to the development of IR-related diseases [5].

Essentially, nutritional promotion after undernutrition motivates catch-up growth (CUG) and increases the risks for IR-related diseases later in life [6]. Nutrition promotion can occur in prenatal, childhood, or adult life. Given that there are differences between immature and mature organisms, the pathogenesis and outcomes of CUG induced by nutrition promotion at different stages of life may also differ [7]. Most studies done on CUG in adult (CUGA) involve adult rats in which CUG is induced by refeeding after caloric restriction (CR) for 2 weeks [8]. Because it is reported that the majority of the effects of long-term CR on age-responsive gene expression can be produced after 4 weeks [9], the 2-week CR period might be a little short to relatively simulate the

Disclosure: The authors declared no conflict of interest.

* Corresponding author. Tel.: +86 27 85726082; fax: +86 27 85356365.
E-mail address: cheria_chen@126.com (L.-L. Chen).

0026-0495/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2010.05.014

undernutrition that occurs more commonly in the long term in human beings [10]. In addition, these studies assessed the insulin sensitivity by different methods in the same animal model; and discrepant conclusions were drawn [8,10,11]. It is also noteworthy that previous studies have not prospectively observed the dynamic changes of insulin sensitivity and lipid deposition in CUGA rodents during a longer period. Thus, there is still uncertainty as to whether the CUG phenomenon is transient or persistent. In our study, we prolonged the refeeding stage and performed the overall dynamic observations on the CUGA rats. Considering that the level and type of diets vary significantly across different regions of the world with economic conditions and dietary customs [12], a comparative study of the metabolic changes in CUGA induced by different types of nutrition promotion would be of great interest. Therefore, we attempted to develop a CUGA animal model by way of refeeding either with normal chow or with high-fat diet after modest CR (60% of that eaten by the ad libitum animals, which is considered as undernutrition without malnutrition previously [13]) to properly simulate the prevalent nutrition promotion after undernutrition and explore its effects on metabolism.

2. Materials and methods

2.1. Animals and diets

Healthy male Sprague-Dawley rats (aged 6 weeks, weighing 140-180 g) were obtained from the Laboratory Animal Center of Tongji Medical College, Huazhong University of Science and Technology. All of them were housed individually in stainless steel cages with free access to water and subjected to controlled temperature (22°C ± 3°C), lighting (lights on 6:00 AM to 6:00 PM), and relative humidity (50% ± 10%). All rats were adapted to surrounding environments for a week before the experiments. They were fed with either a standard rodent chow or a high-fat diet as scheduled (Fig. 1) once per day. The normal chow (13.68%, 64.44%, and 21.88% of calories derived, respectively, from fat, carbohydrate, and protein) was provided by the Laboratory Animal Center mentioned above. The high-fat diet (containing in terms of calories derived 59% fat, 20% carbohydrate, and 21% protein) was made as described previously by Chalkley et al [14]. During CR, the food intake of CUGA rats was restricted to 60% of that eaten by their ad lib–fed companions of the same weight. During refeeding, the CUGA animals were pair-fed with their weight-matched, ad libitum–fed controls (RNs vs NCs; RHs vs HFs). This is important to explore the diet shift (nutrition promotion) itself and to exclude any influence of gap between appetite and expenditure, which is likely to result in nutrient excess in these animals [15]. All the experimental procedures performed were approved by the Animal Ethics Committee in our university and were in accordance with the Hubei Province Laboratory Animal Care Guidelines for the use of animals in research.

2.2. Experimental design

2.2.1. Experiment 1: the effects of CUGA on rats by refeeding for different periods

The overall experimental procedure is illustrated in Fig. 1A. Animals were randomly allocated to 2 groups: RNs and NCs. Each group was then divided into 5 subgroups for 5 different sampling points, with 8 rats in each subgroup. The RNs were composed of CR subgroup (R4, maintained on CR for 4 weeks) and 4 CUGA subgroups (RN1, RN2, RN4, and RN8, refed with weight-matched normal chow for 1, 2, 4, and 8 weeks individually after CR for 4 weeks). The NC subgroups were age-matched controls of RNs mentioned above (fed ad libitum with normal chow for 4, 5, 6, 8, and 12 weeks, respectively).

2.2.2. Experiment 2: the effects of CUGA on rats by refeeding with normal chow or high-fat diet

The protocol for animal experimentation is shown briefly in Fig. 1B. The rats were randomly assigned to 4 groups (RNs, RHs, NCs, and HFs), and each was divided into 2 subgroups (n = 8) for 2 different sampling points as follows: 2 RNs (CUGA subgroups by normal chow: RN2 and RN4, refed with weight-matched normal chow for 2 and 4 weeks, respectively, after 4 weeks CR), 2 RHs (CUGA subgroups by high-fat diet: RH2 and RH4, refed with weight-matched high-fat diet for 2 and 4 weeks, respectively, after 4 weeks CR), 2 NCs (normal controls: fed ad libitum with normal chow for 6 and 8 weeks, respectively), and 2 HFs (high-fat diet controls: fed ad libitum with high-fat diet for 6 and 8 weeks, respectively).

All the animals in experiments 1 and 2 were prepared as described above for the following hyperinsulinemic-euglycemic clamp and other experiments as scheduled.

2.3. Energy intake, body weight, and food efficiency

We measured food intake and body weight of animals every day to calculate weekly energy intake and food efficiency by the following equations: energy intake = food intake (in grams) × energy density (in kilocalories per gram), food efficiency = total carcass body weight accumulated (in grams) × 100/total energy intake (in kilocalories) [16].

2.4. Body length and Lee index

We measured body weight and body length (nasoanal length) before sacrifice. Lee index was then calculated as described previously [17] from the following formula: Lee index = $\text{weight}^{1/3}$ (in grams) × 1000/nasoanal length (in centimeters).

2.5. Hyperinsulinemic-euglycemic clamp in conscious rats based on tail artery and vein catheterization technique

After an overnight fast, a 120-minute hyperinsulinemic-euglycemic clamp was conducted in conscious rats according to a modified form of the procedure described by Shang et al [18]. In short, tail artery and vein catheterizations were

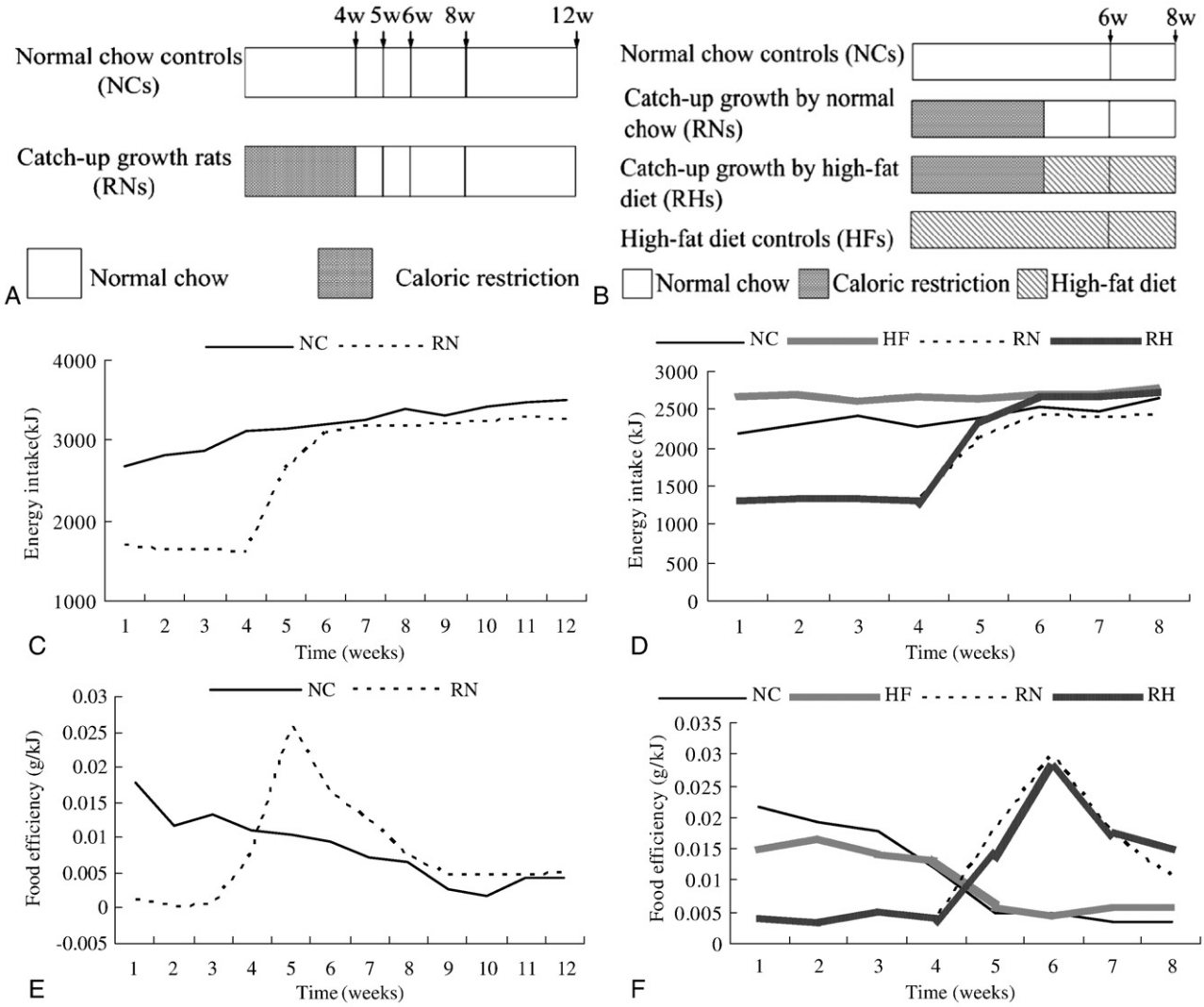

Fig. 1. Feeding regimen, energy intake, and food efficiency in CUGA rats by refeeding for different periods in experiment 1 (A, C, and E individually) or with different diets in experiment 2 (B, D, and F, respectively). The energy intake in RNs and RHs (C, D) was as much as 60% of their weight-matched NCs during CR and increased to 100% of their controls (RNs vs NCs; RHs vs HFs) while refeeding. The food efficiency (E, F) in RNs and RHs was sustained at around zero, increased remarkably early after refeeding, and began to decline while refeeding for 2 weeks.

performed with intravenous integrated catheters (24G × 19 mm; Weihai Jierui Medical Products, Weihai, China) filled with heparin-saline solution (50 IU heparin per milliliter 0.9% saline). The arterial catheter was used for blood sampling; and the venous catheter, for intravenous infusion. Fasting plasma glucose was measured using a glucose oxidase kit (Beijing Chemical Industry, Beijing, China) [19]. Plasma insulin level was estimated using a rat insulin enzyme-linked immunosorbent assay kit (Linco Research, St Charles, MO). A prime-continuous infusion of human insulin (Novolin R; Novo Nordisk, Tianjin, China) was maintained at a rate of 0.25 U/(kg h) to raise plasma insulin for 120 minutes, and 25% glucose solution was infused at variable rates and periodically adjusted to clamp the plasma glucose levels at approximately 4.5 mmol/L. Blood samples (20 μL) were collected every 5 minutes for plasma glucose determination using a glucometer (One Touch Ultra; Lifescan, Milpitas, CA). The average glucose infusion rate between the 60th and 120th minute ($GIR_{60\text{-}120}$) was used to evaluate insulin sensitivity. To estimate insulin-stimulated glucose transport activity in individual tissues, 2-deoxy-D-glucose (Sigma-Aldrich, St Louis, MO) was administered as a bolus (2 mmol/kg in saline) [20] 45 minutes before the end of the clamps (data not shown). All rats were kept quiet and conscious throughout the entire process.

2.6. Biochemical assays

Serum triglyceride (TG) and total cholesterol (TC) were determined with a glycerophosphate oxidase–phenol

aminophenazone kit and a cholesterol oxidase–phenol aminophenazone kit, respectively (Zhejiang Dongou Biological, Wenzhou, China) [21].

2.7. Body fat distribution

Subcutaneous (groin), perirenal, and epididymal white adipose tissues (SAT, PAT and EAT respectively) were carefully dissected out and weighed. Body fat percentage of various AT compartments was determined by dividing the weight of the calculated fat per animal by the carcass weight. The sum of PAT and EAT was considered as visceral fat [22].

2.8. Biochemical determination of tissue TG measurement

Tissue (skeletal muscle and liver) TG content was assayed using a method described previously [23]. Briefly, approximately 0.1 g of frozen tissue was homogenized in chloroform/methanol (1 mL, 2:1 vol/vol) over ice. Tubes with homogenates obtained were shaken for 4 hours before adding 0.6 mL of 0.6% NaCl. Samples were oscillated and subsequently centrifuged (2000 rpm for 10 minutes) to separate phases. The TG-containing organic bottom layer was separated and air-dried. The isolated lipids were resuspended in 250 μL ethanol and then determined spectrophotometrically at 570 nm with a glycerophosphate oxidase–phenol aminophenazone kit (Zhejiang Dongou Biological).

2.9. Statistical analysis

Results are presented as means ± SE. Statistical analyses were performed to assess the differences among the groups at the same sampling points using 2-tailed unpaired Student *t* test (experiment 1) or 1-way analysis of variance (experiment 2) followed by least significant difference (LSD) t test post hoc test for multiple comparisons with SPSS 11.5 software (SPSS, Chicago, IL). $P < .05$ was considered statistically significant.

3. Results

3.1. Food intake and energy efficiency

In experiments 1 and 2 (Fig. 1C, D), the average food intake of RNs or RHs rats were approximately 60% of that eaten by their weight-matched normal chow or high-fat diet controls (a little less than their age-matched controls) after refeeding. The energy efficiency values in both RNs and RHs (Fig. 1E, F) were sustained at around zero throughout CR and, following refeeding, rapidly increased to a level significantly higher (in the early 1∼2 weeks) than that in their controls ($P < .05$, RNs vs NCs; RHs vs HFs) and then declined 2 weeks later.

3.2. Overall changes in body weight, length, and Lee index during CR and refeeding

In experiment 1, the body weight and length (Fig. 2A, B) increased moderately in NCs, while in RNs, their growth showed no obvious changes during CR and increased rapidly after refeeding. However, the body weight and length in RNs were remarkably lower than those in NCs ($P < .05$). The Lee index in RNs (Fig. 2C) was obviously higher than that in NCs when refeeding for 1 and 2 weeks ($P < .05$). In experiment 2, the body weight and length in both RNs and RHs (Table 1) were significantly less than those in their controls ($P < .05$, RNs vs NCs; RHs vs HFs). However, the Lee index in RNs and RHs (Table 1) did not differ ($P > .05$) from their controls except in RN2.

3.3. Body fat distribution

In experiment 1, the body fat percentage of subcutaneous adipose tissue (SAT/BW) in RNs (Fig. 2D) was similar to their age-matched NCs ($P > .05$,), whereas that of visceral adipose tissue (VAT/BW) (Fig. 2E) was greater in RNs than their age-matched NCs after refeeding for 2 weeks ($P < .05$). The relative contribution of 3 fat subsets (SAT, PAT, EAT) to the total fat measured was calculated and represented as a stacked column chart in percentage (Fig. 2F). The contribution of the PAT subset gradually increased, whereas the SAT subset decreased, in the process of refeeding in RNs. In experiment 2, the SAT/BW and VAT/BW in RHs and HFs (Table 1) were greater than that in their age-matched NCs and RNs ($P < .05$).

3.4. Serum TG/TC concentrations

The serum TG was greater after refeeding in RNs in experiment 1 (Fig. 3A) and in RNs, HFs, and RHs in experiment 2 (Table 1) than that in their age-matched NCs ($P < .05$). In addition, the serum TG in RH2 was higher than that in RN2 ($P < .05$). Significant difference ($P < .05$) in serum TC (Fig. 2B) can be observed in RN1 in experiment 1 and in HF8 and RH4 in experiment 2 (Table 1) compared with their age-matched NCs ($P < .05$).

3.5. Changes in lipid deposition in skeletal muscle and liver

The intramuscular and intrahepatic TG content (Fig. 3C, D) was significantly greater in RNs than that in NCs after refeeding for 4 weeks in experiment 1 ($P < .05$). In experiment 2, the intramuscular TG increased remarkably (Table 1) in RH2 compared with NC6 and in RH4 compared with NC8 or HF8 ($P < .05$). Similarly, RHs showed elevated intrahepatic TG content (Table 1) compared with NCs or RNs ($P < .05$).

3.6. Insulin sensitivity

Among all experimental rats, no significant differences in fasting blood glucose concentrations could be observed (Fig. 4A, B). The fasting plasma insulin significantly increased in RN4 and RN8 in experiment 1 (Fig. 4C) and HF6, RN4, RH4, and HF8 in experiment 2 (Fig. 4D) compared with their age-matched NCs. The average $GIR_{60\text{-}120}$ at euglycemia was similar ($P > .05$) between RNs and NCs while refeeding for 1 and 2 weeks but significantly

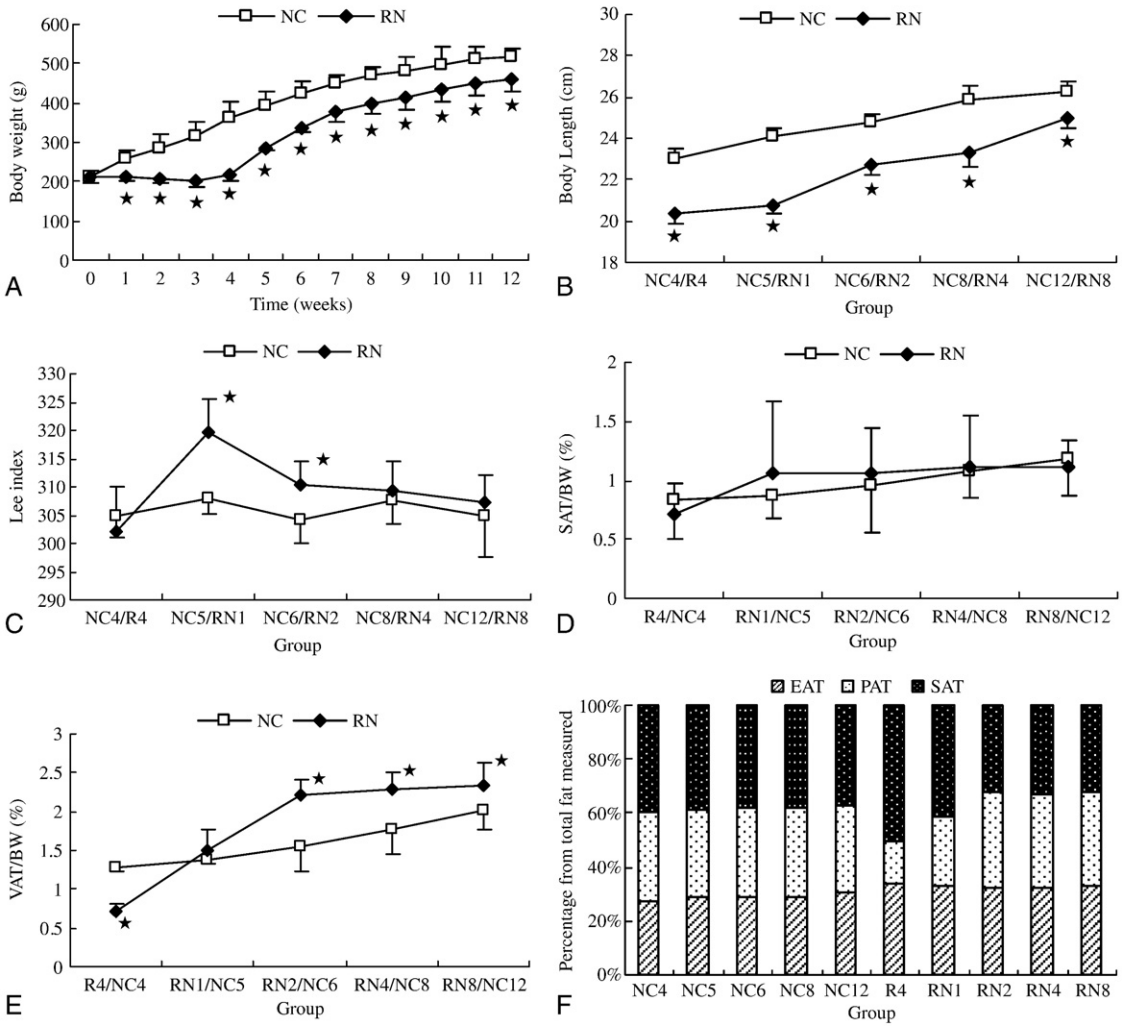

Fig. 2. Basic biological features of CUGA rats in experiment 1. The body weight (A) and body length (B) were markedly lower in RNs compared with NCs throughout the experiment 1. The Lee index was higher just in RN1 and RN2 compared with their controls (C). The body fat percentage of SAT (SAT/BW) displayed no difference between RNs and NCs (D). The body fat percentage of VAT (VAT/BW) increased dramatically while refeeding over 2 weeks in RNs compared with their NC controls (E). The contribution of the PAT subset gradually increased, whereas the SAT subset decreased, in the process of refeeding in RNs (F). ★$P < .05$ vs NC.

lower ($P < .05$) later (while refeeding for 4 and 8 weeks) in experiment 1 (Fig. 4E). In experiment 2 (Fig. 4F), the mean $GIR_{60\text{-}120}$ in RH4 was reduced markedly ($P < .05$) compared with their age-matched companions ($P < .05$, RH4 vs NC8, HF8, or RN4).

4. Discussion

The combined data from the 2 experiments described here show that prolonged CR and the subsequent refeeding could, respectively, induce growth arrest and accelerated growth in rats, which is associated with a transient but dramatic increase in food efficiency early after nutrition promotion. These rats are characterized by abnormal lipid metabolism (elevated plasma TG, VAT overaccumulation without significant increase in obesity, intramuscular and intrahepatic lipid deposition) and drastic IR (increased fasting plasma insulin and decreased steady-state GIR during the clamp). Furthermore, these metabolic changes persisted during our prolonged observation and were more severe in CUGA induced by high-fat diet than that by normal chow.

In this investigation of nutrition promotion after undernutrition, 7-week-old rats (sexually mature) were used, considering that CUGA in young adulthood is strongly associated with IR-related diseases [6]. The effects of varying degrees of chronic CR on metabolism in rats differ from one another. An approximately 60% CR, a dietary regimen low in calories without undernutrition [13], is widely used to decelerate the development of age-related diseases and reverse the adverse effects induced by overweight or nutrient excess in rats [24]. In human beings, the caloric level usually used in CR is a little higher than that in rats [25]. In fact, the present caloric level closely

Table 1
Changes in basic biological features and lipid metabolism of CUGA rats in experiment 2

6 wk	NC6	RN2	HF6	RH2
Body weight (g)	398.5 ± 20.6	331 ± 14*,†	387 ± 21.8	318.7 ± 16.4*,†
Body length (cm)	26 ± 0.2	24.83 ± 0.6*,†	25.5 ± 0.59	25 ± 0.35*,†
Lee index	301.4 ± 3.37	313.6 ± 5.84*	305 ± 3.85	303.3 ± 4.93
SAT/BW	1.042 ± 0.21	1.094 ± 0.28	1.7 ± 0.23*,‡	1.952 ± 0.44*,‡
VAT/BW	1.801 ± 0.17	2.32 ± 0.16*	4.8 ± 0.76*,‡	3.567 ± 0.85*,‡
Serum TG (mmol/L)	0.73 ± 0.09	1.203 ± 0.11*	1.38 ± 0.12*	1.546 ± 0.14*,‡
Serum TC	1.31 ± 0.07	1.634 ± 0.2	1.7 ± 0.08	1.594 ± 0.22
Intramuscular TG (mmol/kg tissue)	47.08 ± 14.4	62.27 ± 10	62.2 ± 9.96	98.04 ± 15.3*
Intrahepatic TG (mmol/kg tissue)	4.15 ± 0.37	8.247 ± 1.94	10.8 ± 1.59*	14.51 ± 1.46*,‡
8 wk	**NC8**	**RN4**	**HF8**	**RH4**
Body weight (g)	403.5 ± 13.7	354 ± 9.98*,†	409 ± 24.5	338.9 ± 5.34*,†
Body length (cm)	26.5 ± 0.43	25.86 ± 0.63*,†	26.6 ± 0.37	25.14 ± 0.28*,†
Lee index	303.6 ± 1.52	301.8 ± 2.84	308 ± 4.36	306.3 ± 2.84
SAT/BW	1.18 ± 0.27	1.197 ± 0.19	1.77 ± 0.4*,‡	2.12 ± 0.14*,‡
VAT/BW	1.845 ± 0.13	2.46 ± 0.43*	4.92 ± 1.08*,‡	4.06 ± 0.39*,‡
Serum TG (mmol/L)	0.782 ± 0.06	1.438 ± 0.11*	1.4 ± 0.05*	1.589 ± 0.06*
Serum TC	1.278 ± 0.02	1.654 ± 0.12	1.82 ± 0.09*	1.887 ± 0.09*
Intramuscular TG (mmol/kg dm)	44.26 ± 16	87.88 ± 8.43*	70.3 ± 5.21	110.3 ± 18.8*,†
Intrahepatic TG (mmol/kg dm)	5.295 ± 0.98	9.323 ± 0.3*	13.4 ± 2.32*,‡	15.72 ± 0.72*,‡

Data are expressed as mean ± SE.
* $P < .05$ vs NC.
† $P < .05$ vs HF.
‡ $P < .05$ vs RN.

resembles the undernutrition that previously occurred in developing countries. For example, the per capita food consumption (in kilocalories per capita per day) in East Asia was 1957 in 1964-1966 and 2921 in 1997-1999 and is expected to be 3060 in 2015 [4]. Therefore, approximately 60% CR was used in our CUGA rats. In addition, short- and long-term CR could evoke different phenotypic and genomic responses. It is reported that 4-week short-term CR could reproduce the majority of the effects of long-term CR on age-responsive gene expression, whereas 2 weeks could not [9].

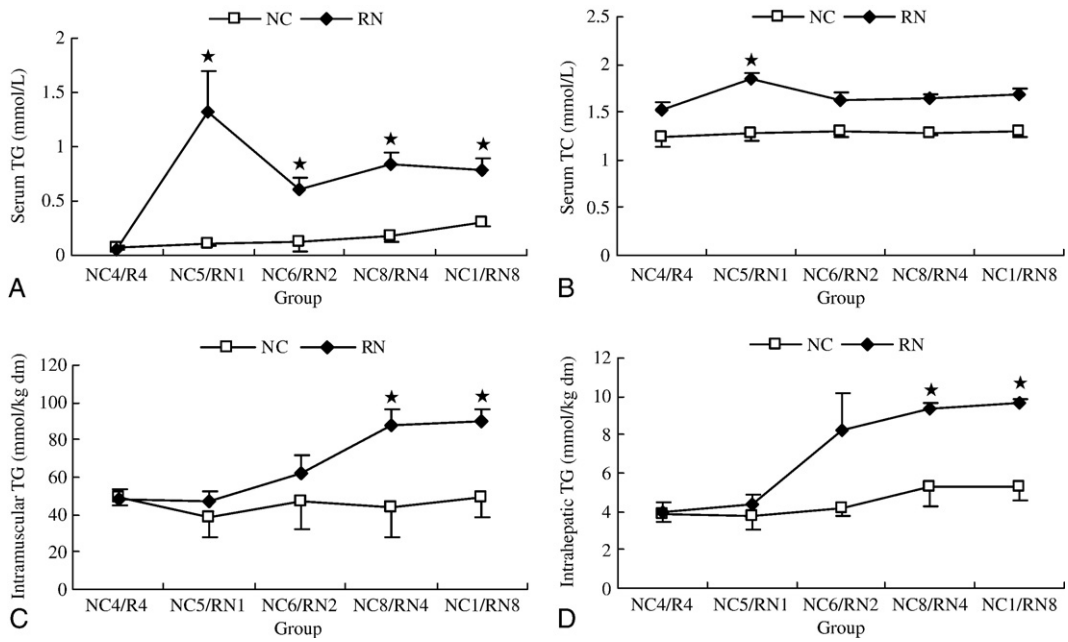

Fig. 3. The serum TG and TC and the intramuscular and intrahepatic TG content in experiment 1. The serum TG (A) was greater after refeeding in RNs, and the serum TC (B) is higher in RN1 compared with their controls. The intramuscular (C) and intrahepatic (D) TG content (C, D) was significantly greater in RNs than that in NCs over refeeding for 4 weeks. ★$P < .05$ vs NC.

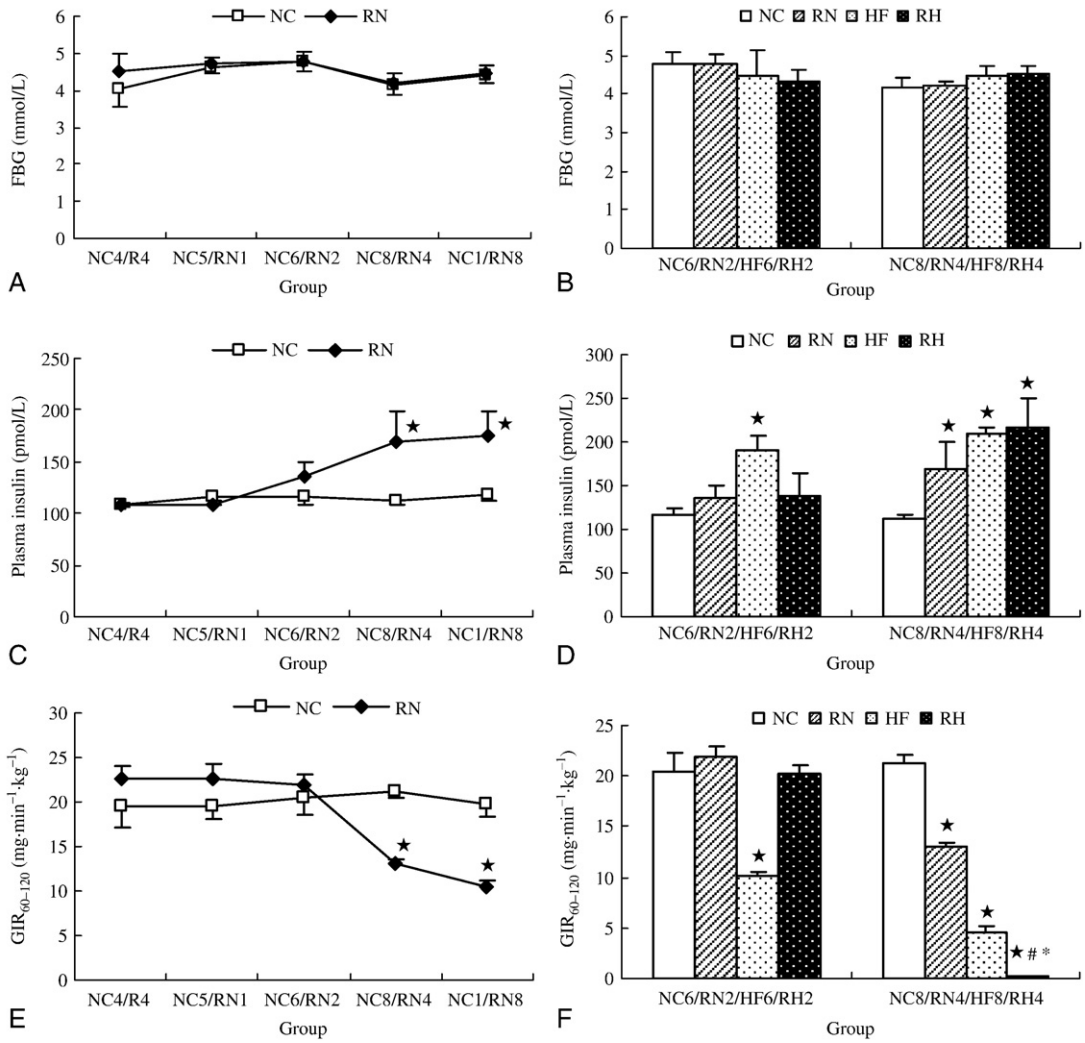

Fig. 4. Insulin sensitivity in experiment 1 (A, C, E) and experiment 2 (B, D, F). The fasting blood glucose did not differ among all groups(A, B); however, the fasting plasma insulin was greater in RN4 and RN8 in experiment 1 (C) and in RN4 and RH4 in experiment 2 (D) compared with their age-matched NCs. The $GIR_{60\text{-}120}$ decreased significantly in RN4 and RN8 in experiment 1 (E) and in RH4 in experiment 2 (F) compared with their age-matched companions (RNs vs NCs; RH4 vs NC8, HF8, or RN4). ★$P < .05$ vs NC; *$P < .05$ vs HF; #$P < .05$ vs RN. FBG indicates fasting blood glucose.

For instance, 4 weeks reproduced 100% of the long-term CR effects on xenobiotic metabolism et al [26]. As for refeeding, the level and variety of dietary intake vary significantly across different regions of the world according to economic conditions and dietary customs [12]. In China, the nutrition promotion might be characterized by the transition from semistarvation to subsistence level of consumption [12], whereas in some Mediterranean countries, their food habits have experienced a process of Westernization during these recent years. Even during the phenomenon of yo-yo dieting, the refeeding phase can be characterized by intake of different kinds of food ranging from healthy to fatty [15]. Thus, the CUGA rats were refed with either normal chow or high-fat diet in the stage of nutrition promotion. It is widely recognized that CUGA mainly embodies an accelerated recovery after growth arrest [10] and excessive fat accumulation due to a preferential recovery of body fat [6] in the process of nutrition promotion after undernutrition. Our results indicate that 60% CR could induce growth arrest and that the subsequent refeeding could cause accelerated growth and fat overaccumulation, which is consistent with the features of human CUGA described briefly above [6,10]. Hence, this work characterizes the 60% CR refeeding rats as a robust model for CUGA.

In this work, we observed the dynamic changes in the biological characteristics of these CUGA rats. It is interesting to note that food efficiency, which denotes the body weight increase per gram of food consumed, increased dramatically early after refeeding and gradually decreased in the process of nutrition promotion. This change of food efficiency in CUGA rats might suggest their thrifty metabolic profile during the nutrition promotion [6,15]. The thrifty metabolic profile is common in animals while they are exposed to undernutrition. The mechanism might be ascribed to decreased energy expenditure and the induction of an energy conservation mechanism during CR [27]. Changes in

behavior (locomotor activity et al) and gene expression (energy metabolism et al) induced by CR are likely to be involved in the formation of thrifty metabolic profile [9,26,28]. The elevation of food efficiency in CUGA rats could be explained by postulating that the adaptive thrifty energy metabolism formed during CR might be sustained for a certain period after refeeding while the body tries to replenish its stores [29].

In addition, we found that CUGA animals elicited a much higher degree of lipid accumulation, including greater visceral fat percentage and ectopic lipid deposition, which commonly results from nutrient excess [30]. It is worth mentioning that the food intake in CUGA rats was never more than their controls (RNs vs NCs). Therefore, the lipid overaccumulation could not be simply attributed to overnutrition, but to the nutritional shift itself. The substantial difference between CUGA rats and their controls being the early undernutriton, it might therefore be the ultimate source of the subsequent adverse effects in CUGA rats [6,15]. Thus, we tentatively put forward that CR and CR-induced thrifty metabolic profile mentioned above might be one of the possible mechanistic explanations for the excessive lipid deposition in CUGA rats [16]. The finding that lipid overaccumulation is associated with increased food efficiency supports this hypothesis. Furthermore, the lipid accumulation occurs in visceral fat, which is more closely related with IR [31], but not in subcutaneous fat, which might be protective factor in the IR-related disease [32]. The detailed mechanism is unclear, and further investigations are needed to confirm this. Importantly, the changes in visceral fat percentage and Lee index in CUGA rats are not exactly similar. This partial consistency between visceral fat percentage and Lee index in our findings might be explained by the fact that the former is a more sensitive indicator of fat accumulation to some extent [33]. In fact, this finding correlates with the discrepancy between BMI and percentage of body fat in Asians, where a higher percentage of body fat is observed, although the BMI is lower as compared with white populations [34,35]. Hence, more significantly, higher body fat rather than BMI or Lee index might be an important trait of CUGA. It is reported that the intramyocellular lipid by oil red O stain in rats refeeding 1 week after 2 weeks CR is not significantly different from that in normal chow controls [10]. In our study, the ectopic lipid deposition was observed while refeeding at 4 weeks but not before, which suggests that refeeding period might be an important factor in its formation. Somewhat surprisingly, the serum TG increased soon after refeeding began. This could be used to explain the appearance of the subsequent lipid overaccumulation and ectopic (intramuscular and intrahepatic) lipid deposition, which is similar to the abnormal lipid metabolism occurring in IR induced by excessive calorie intake [3]. Nevertheless, the reason for this rise in serum lipid is not completely clear. An explanation could be that the adaptive thrifty metabolic profile motivated during CR would not be able to conform to the subsequent rapid nutrition promotion and result in a relative nutrient excess in the organisms [6,15]. Further studies are still necessary to verify this.

In the current study, our findings indicate that the systemic insulin sensitivity is adversely and persistently affected in CUGA rats, which is consistent with the prevalence of IR-related disease in CUGA [6]. The drastic IR in CUGA rats might be explained by the increased visceral fat and ectopic lipid deposition due to thrifty metabolic profile and relative overnutrition mentioned before [15,36]. That the lipid overaccumulation appears before the IR makes this hypothesis plausible. This mechanism is analogous to speculations on explanations of the intrauterine growth retardation model, which is a commonly used model of prenatal CUG for experimental study [37]. Yet, the pathogenesis of intrauterine growth retardation and CUGA must be obviously distinct for the great metabolic differences between the fetus and the mature organism [7]. Thus, the detailed mechanism concerned with the impaired insulin sensitivity in CUGA must be complex; and more knowledge is required.

It is noteworthy that higher serum TG, greater lipid deposition in adipose and liver, and more severe systemic IR were observed in RHs compared with RNs. In addition, RHs showed higher intramuscular TG level and more severe IR compared with HFs, which were fed for 4 weeks more than RHs. These results suggest that the metabolic disorders mentioned above in CUGA rats depend on the types of diets used in nutrition promotion. The sustained thrifty metabolic profile formed during CR may be a good explanation to the phenomenon that high-fat diet refeeding promotes severe lipid overaccumulation and IR more rapidly.

As mentioned above, the sustained thrifty metabolic profile formed during CR might be an important causative factor in the etiology of lipid overaccumulation and its

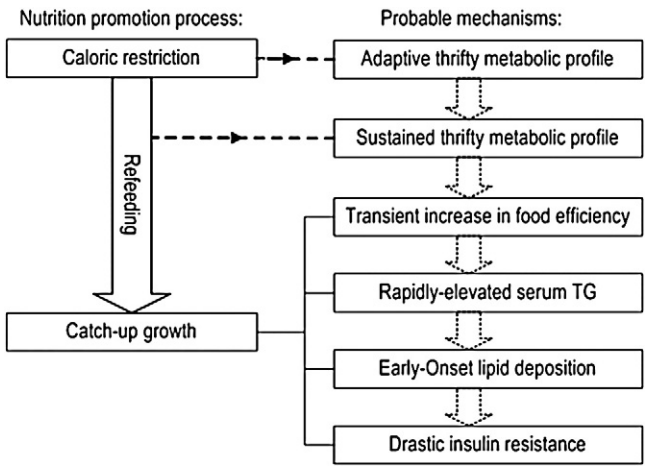

Fig. 5. Catch-up growth in adult induced by nutrition promotion is characterized by transiently lifted food efficiency, rapidly elevated serum TG, lipid deposition, and drastic IR (solid lines). The adaptive thrifty metabolism profile formed during CR and sustained thrifty metabolism profile while refeeding are likely to be the possible mechanisms to explain these consequences (shown with dotted line arrows).

consequent IR in CUGA rats. However, it is reported that the response to recovery from CR is strain-dependent divergent (Long-Evans and Sprague-Dawley rats), even though the ratio of oxygen consumption to food intake is probably lower in both of them as opposed to humans [38]. Thus, the use of animal models to mimic human beings has its limitations and requires caution.

The insulin sensitivity was assessed by different methods in similar animal models in previous experiments, and discrepant conclusions were drawn [8,10,11]. In brief, they developed CUGA rats by refeeding 1 week after CR for 2 weeks. The intraperitoneal glucose tolerance tests, homeostasis model assessment index, and hyperinsulinemic-euglycemic clamp were used to evaluate the insulin sensitivity in each article. Their data showed elevated plasma insulin-to-glucose ratio in intraperitoneal glucose tolerance tests and higher homeostasis model assessment index in refed rats than in controls, while no significant differences between refed and control groups were found in the glucose infusion rate during hyperinsulinemic-euglycemic clamp. It is well known that the hyperinsulinemic-euglycemic clamp is the criterion standard method with a high degree of accuracy to assess insulin sensitivity. We further found that the plasma glucose was clamped at approximately 145 mg/100 mL, which is approximately equal to 8 mmol/L and roughly equivalent to postprandial blood glucose but not fasting blood glucose (4∼5 mmol/L). In addition, the clamp was performed in rats generally anesthetized with sodium pentobarbital, which causes some degree of IR [39]. Thus, we performed our hyperinsulinemic-euglycemic clamps (plasma glucose was clamped at ∼4.5 mmol/L) in conscious rats without general anesthesia and increased stress (data not shown). Furthermore, we assessed the insulin sensitivity at several points during a prolonged period and observed the dynamic changes of these metabolic features. In our study, no significant IR was detected when refeeding 1 and 2 weeks after CR, while drastic systemic IR was found when refeeding 4 and 8 weeks. This work is different from the researches mentioned above [8,10,11]. Our data suggest that nutrition promotion could not cause IR instantly but after a certain period of refeeding in CUGA rats. Insulin resistance in CUGA rats was sustained after refeeding for 8 weeks, when the accelerated growth and fat recovery had slowed down for several weeks. Thus, it is plausible to assume that the IR induced by CUGA might not be transient but persistent.

Finally, it should be noted that we have not observed the metabolic features over an even longer term to confirm the persistent effects of CUGA. We only examined fat storage by Lee index and body fat percentage in this study. More complete experiments such as a prolonged monitoring of the model and dual-energy x-ray absorptiometry to assess the body composition or analyze the carcasses for water and nitrogen content, along with total body fat etc, are required and will be our next focus in the future. Nevertheless, our observations have important implications for the pathogenesis of IR induced by CUGA, which might differ from that exerted by simple nutritional excess. In addition, other interesting traits (including the transient increase in food efficiency and raised serum lipid) may be involved in this model; and further tests are necessary to clarify the mechanisms involved. Notwithstanding its limitations, we developed the animal model of CUGA induced by rapid and significant nutrition promotion. The observations obtained in this work allow us to conclude that the CUGA rats are characterized by a transient increase in food efficiency, lipid overaccumulation (increased visceral fat and ectopic lipid deposition), and sharp reduction in insulin sensitivity. We tentatively postulate that the increase in food efficiency may play an important role in the formation of lipid overaccumulation (elevated visceral fat and ectopic lipid deposition) and its consequent drastic IR on the basis of the order in which they appear (summarized in Fig. 5). These findings could have important implications in research on the etiology of CUGA and its consequences.

Acknowledgment

This work was supported by National Natural Science Foundation of China grant 30771035. We thank Ms Payal Buckoreelall for valuable help in revising the manuscript.

References

[1] Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes Care 2004;27:1047-53.
[2] Yoon KH, Lee JH, Kim JW, Cho JH, Choi YH, Ko SH, et al. Epidemic obesity and type 2 diabetes in Asia. Lancet 2006;368:1681-8.
[3] Wang MY, Grayburn P, Chen S, Ravazzola M, Orci L, Unger RH. Adipogenic capacity and the susceptibility to type 2 diabetes and metabolic syndrome. Proc Natl Acad Sci USA 2008;105:6139-44.
[4] Diet, nutrition and the prevention of chronic diseases. World Health Organ Tech Rep Ser 2003;916:i-viii, 1-149, backcover.
[5] Duarte FO, Sene-Fiorese M, Manzoni MS, de Freitas LF, Cheik NC, Garcia de Oliveira Duarte AC, et al. Caloric restriction and refeeding promoted different metabolic effects in fat depots and impaired dyslipidemic profile in rats. Nutrition 2008;24:177-86.
[6] Dulloo AG. A role for suppressed skeletal muscle thermogenesis in pathways from weight fluctuations to the insulin resistance syndrome. Acta Physiol Scand 2005;184:295-307.
[7] Warrington JA, Nair A, Mahadevappa M, Tsyganskaya M. Comparison of human adult and fetal expression and identification of 535 housekeeping/maintenance genes. Physiol Genomics 2000;2:143-7.
[8] Crescenzo R, Lionetti L, Mollica MP, Ferraro M, D'Andrea E, Mainieri D, et al. Altered skeletal muscle subsarcolemmal mitochondrial compartment during catch-up fat after caloric restriction. Diabetes 2006;55:2286-93.
[9] Dhahbi JM, Kim HJ, Mote PL, Beaver RJ, Spindler SR. Temporal linkage between the phenotypic and genomic responses to caloric restriction. Proc Natl Acad Sci USA 2004;101:5524-9.
[10] Cettour-Rose P, Samec S, Russell AP, Summermatter S, Mainieri D, Carrillo-Theander C, et al. Redistribution of glucose from skeletal muscle to adipose tissue during catch-up fat: a link between catch-up growth and later metabolic syndrome. Diabetes 2005;54:751-6.
[11] Crescenzo R, Samec S, Antic V, Rohner-Jeanrenaud F, Seydoux J, Montani JP, et al. A role for suppressed thermogenesis favoring catch-

up fat in the pathophysiology of catch-up growth. Diabetes 2003;52: 1090-7.
[12] Wang SQ, Shi N, Zhou L, Wu YT, Li T, Dong J. Brain-derived neurotrophic factor mRNA expressions in rat brain induced by short-term in vivo exposure to deltamethrin. Zhonghua Yu Fang Yi Xue Za Zhi 2003;37:33-6.
[13] Weindruch R. The retardation of aging by caloric restriction: studies in rodents and primates. Toxicol Pathol 1996;24:742-5.
[14] Chalkley SM, Hettiarachchi M, Chisholm DJ, Kraegen EW. Long-term high-fat feeding leads to severe insulin resistance but not diabetes in Wistar rats. Am J Physiol 2002;282:E1231-8.
[15] Jackman MR, Steig A, Higgins JA, Johnson GC, Fleming-Elder BK, Bessesen DH, et al. Weight regain after sustained weight reduction is accompanied by suppressed oxidation of dietary fat and adipocyte hyperplasia. Am J Physiol 2008;294:R1117-29.
[16] Bjorntorp P, Yang MU. Refeeding after fasting in the rat: effects on body composition and food efficiency. Am J Clin Nutr 1982;36:444-9.
[17] Balbo SL, Grassiolli S, Ribeiro RA, Bonfleur ML, Gravena C, Brito Mdo N, et al. Fat storage is partially dependent on vagal activity and insulin secretion of hypothalamic obese rat. Endocrine 2007;31:142-8.
[18] Shang J, Chen LL, Xiao FX, Sun H, Ding HC, Xiao H. Resveratrol improves non-alcoholic fatty liver disease by activating AMP-activated protein kinase. Acta Pharmacol Sin 2008;29:698-706.
[19] McCrimmon RJ, Shaw M, Fan X, Cheng H, Ding Y, Vella MC, et al. Key role for AMP-activated protein kinase in the ventromedial hypothalamus in regulating counterregulatory hormone responses to acute hypoglycemia. Diabetes 2008;57:444-50.
[20] Yamamoto N, Kawasaki K, Sato T, Hirose Y, Muroyama K. A nonradioisotope, enzymatic microplate assay for in vivo evaluation of 2-deoxyglucose uptake in muscle tissue. Anal Biochem 2008;375: 397-9.
[21] Chen LL, Zhang JY, Wang BP. Renoprotective effects of fenofibrate in diabetic rats are achieved by suppressing kidney plasminogen activator inhibitor–1. Vascul Pharmacol 2006;44:309-15.
[22] Chen LL, Hu X, Zheng J, Zeng TS, Zhang JY, Deng XL, et al. Dynamic changes of biological characteristics in modified catch-up growth rat model. Chin J Endocriol Metab 2009;25:438-40.
[23] Kim CH, Kim MS, Youn JY, Park HS, Song HS, Song KH, et al. Lipolysis in skeletal muscle is decreased in high-fat-fed rats. Metabolism 2003;52:1586-92.
[24] Koubova J, Guarente L. How does calorie restriction work? Genes Dev 2003;17:313-21.
[25] Dirks AJ, Leeuwenburgh C. Caloric restriction in humans: potential pitfalls and health concerns. Mech Ageing Dev 2006;127:1-7.
[26] Cao SX, Dhahbi JM, Mote PL, Spindler SR. Genomic profiling of short- and long-term caloric restriction effects in the liver of aging mice. Proc Natl Acad Sci U S A 2001;98:10630-5.
[27] Lane MA, Baer DJ, Rumpler WV, Weindruch R, Ingram DK, Tilmont EM, et al. Calorie restriction lowers body temperature in rhesus monkeys, consistent with a postulated anti-aging mechanism in rodents. Proc Natl Acad Sci U S A 1996;93:4159-64.
[28] Yamamoto Y, Tanahashi T, Kawai T, Chikahisa S, Katsuura S, Nishida K, et al. Changes in behavior and gene expression induced by caloric restriction in C57BL/6 mice. Physiol Genomics 2009;39: 227-35.
[29] Summermatter S, Mainieri D, Russell AP, Seydoux J, Montani JP, Buchala A, et al. Thrifty metabolism that favors fat storage after caloric restriction: a role for skeletal muscle phosphatidylinositol-3-kinase activity and AMP-activated protein kinase. FASEB J 2008;22:774-85.
[30] Houmard JA. Intramuscular lipid oxidation and obesity. Am J Physiol Regul Integr Comp Physiol 2008;294:R1111-6.
[31] Escriva F, Gavete ML, Fermin Y, Perez C, Gallardo N, Alvarez C, et al. Effect of age and moderate food restriction on insulin sensitivity in Wistar rats: role of adiposity. J Endocrinol 2007;194:131-41.
[32] Tran TT, Yamamoto Y, Gesta S, Kahn CR. Beneficial effects of subcutaneous fat transplantation on metabolism. Cell Metab 2008;7: 410-20.
[33] Stephens DN. Does the Lee obesity index measure general obesity? Physiol Behav 1980;25:313-5.
[34] Mandavilli A, Cyranoski D. Asia's big problem. Nat Med 2004;10: 325-7.
[35] Wang J, Thornton JC, Russell M, Burastero S, Heymsfield S, Pierson Jr RN. Asians have lower body mass index (BMI) but higher percent body fat than do whites: comparisons of anthropometric measurements. Am J Clin Nutr 1994;60:23-8.
[36] Lim S, Son KR, Song IC, Park HS, Jin CJ, Jang HC, et al. Fat in liver/muscle correlates more strongly with insulin sensitivity in rats than abdominal fat. Obesity (Silver Spring) 2009;17:188-95.
[37] Briana DD, Malamitsi-Puchner A. Intrauterine growth restriction and adult disease: the role of adipocytokines. Eur J Endocrinol 2009;160: 337-47.
[38] Evans SA, Messina MM, Knight WD, Parsons AD, Overton JM. Long-Evans and Sprague-Dawley rats exhibit divergent responses to refeeding after caloric restriction. Am J Physiol 2005;288: R1468-76.
[39] Clark PW, Jenkins AB, Kraegen EW. Pentobarbital reduces basal liver glucose output and its insulin suppression in rats. Am J Physiol 1990; 258:E701-7.