Involvement of PPARγ/FSP27 in the pathogenic mechanism underlying insulin resistance: tipping the balance between lipogenesis and fat storage in adult catch-up growth rats

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Abstract

Background: Catch-up growth in adult (CUGA) is characterized by visceral fat accumulation, ectopic lipid deposition and insulin resistance (IR). Here, we investigated the determinants of these pathophysiological consequences of CUGA.

Methods: Rats were divided into different groups: control rats were offered normal chow ad libitum (AL), while experimental rats were put on 4-week caloric restriction (CR) initially, followed by regaining weight-matched normal chow (RN) in the RN group. General characteristics of lipid metabolism, expression level of genes in visceral adipose tissue (VAT), and glucose infusion rate (GIR 60–120) by the hyperinsulinemic-euglycemic clamp were examined.

Results: After CR, percentage of abdominal fat mass (AFM%) was lower in the RN group than in the AL group but no difference was observed in serum non-esterified fatty acid (NEFA). Expression of fat-specific protein 27 (FSP27) was decreased in the RN group, while the expression of peroxisome proliferator-activated receptors γ (PPARγ), the key lipogenic gene, was increased. After refeeding, AFM% increased over time and serum NEFA persistently elevated in the RN group. Ectopic triglyceride contents were increased whereas insulin sensitivity was impaired. The expression of FSP27 did not follow the increase in the expression of PPARγ. Additionally, we observed a sustained increase in the expression of ATGL and CGI-58 in VAT in the RN group compared with the AL group after CR and refeeding, and a persistent shift-to-the-left of adipocyte size distribution accompanied by enhanced lipogenesis during CUGA.

Conclusion: The persistent CR-induced imbalance of lipogenesis/fat storage capacity might be responsible for the CUGA-associated metabolic disorders.

Keywords: Catch-up growth in adult, Visceral fat accumulation, Fat storage capacity, Lipid overflow, Insulin resistance

Introduction

Nowadays, insulin resistance (IR)-associated diseases present an explosive increase in prevalence in developing countries, especially in Asia. It is reported that developing countries have widely undergone rapid transition in nutritional status, which usually leads to catch-up growth (CUG) [2, 3]. Recent researches have emphasized that CUG, characterized by catch-up accumulation of fat, is a principal risk factor for IR-associated diseases later in life [4]. Therefore, CUG is extremely important to account for the epidemiological changes of IR-associated diseases in developing countries[1, 5].

Employing a rat model of catch-up growth in adult (CUGA) developed by caloric restriction (CR)-weight matched refeeding, we have previously observed that CUGA leads to visceral fat accumulation, ectopic lipid deposition and drastic IR within a short time during
refeeding after CR [5–7]. This raises a question as to why CUGA rats without high-fat diet exhibit phenotypes of classical IR. Although the precise mechanisms involved still remain poorly understood, recently, emerging evidences have revealed that CR remarkably enhances lipogenic potential but attenuates fat storage capacity [8, 9]. Fat storage capacity is the capacity of the adipocytes to accumulate triglyceride stores within the cells. It can be directly measured or indirectly assessed based on adipocyte morphology or X-ray imaging [8, 9].

Ectopic lipid deposition has been recognized as a critical pathogenic factor of IR [10–12]. Once increase in fat storage capacity is not in parallel with the increase in lipogenesis, it will most likely result in lipid spillover, ectopic lipid deposition and IR [13, 14]. One of the mechanisms may be white adipose tissue (WAT) dysfunction with lipid deposition [13, 15]. This is supported by findings showing that fat storage capacity of WAT in obese insulin-sensitive human subjects was elevated compared with obese insulin-resistant human subjects [14, 16, 17].

In mammals, WAT is currently considered as the main lipid storage depot, and is of crucial importance in modulating whole-body lipid flux [13, 15]. WAT exerts its regulatory action by efficient conversion of surplus nutrients to triglyceride, then storing triglycerides in lipid droplets (LD), and releasing fatty acids into the circulation through breaking down the stored triglycerides [15]. This metabolic flexibility in WAT is critical for whole-body lipid homeostasis [16, 18–20]. The lipogenic capacity of WAT is controlled mainly by PPARγ [21], while the storage capacity is regulated principally by FSP27 [22]. PPARγ belongs to the nuclear hormone receptor superfamily and can promote de novo lipogenesis by regulating target genes like fatty acid synthase (FAS) and lipoprotein lipase (LPL) [21]. It plays an important role in the lipogenesis of adipocytes [23]. FSP27 is localized on the surface of LD and is one of the LD-associated proteins [22]. It is a crucial factor for the regulation of the storage function of adipocytes [24]. Recent studies also reported that perilipin1 is required for FSP27-mediated promotion of lipid storage in unilocular LD of adipocyte through increasing lipid exchange and transfer. Decrease in perilipin1 expression partly impairs fat storage mediated by FSP27 [25]. This capacity of fat storage also can be reflected by adipocyte size [26]. As FSP27 expression is under the control of PPARγ [14], the efficient fat storage in WAT depends on the concerted expression of FSP27 and PPARγ [16]. Therefore, we evaluated the relative changes in lipogenesis and fat storage capacity through the change in expression of PPARγ and FSP27. Additionally, a recent study proposed that the enzymatic capacity for fatty acid synthesis determined insulin-stimulated 2-DG uptake in WAT [27]. We postulated that FAS activity might be an important factor in determining insulin-mediated glucose uptake in VAT during CUGA [28].

Moreover, to determine whether the imbalance of lipogenesis/fat storage capacity has any effect on lipid overflow and ectopic lipid deposition, lipolytic capacity in visceral adipose tissue (VAT), serum non-esterified fatty acid (NEFA), and ectopic triglyceride contents are widely accepted as critical risk factors. Adipose triglyceride lipase (ATGL), also known as triglyceride hydrolase, is of importance in the dysregulation of lipolysis and lipid spillover in obesity as well [29, 30]. Although ATGL and hormone-sensitive lipase (HSL) are not tightly coordinated in response to drastic changes in food supply during CR, it is reasonable to believe that ATGL is the major lipase at least in CR [31]. Its activity is associated with LD-associated proteins and co-activator comparative gene identification 58 (CGI-58) [32]. Under physiological conditions, FSP27 acts to constitutively limit the LD presence of ATGL [33], while perilipin1 inhibits ATGL activity via binding CGI-58 [34]. Normal lipid storage function, thus, is an essential prerequisite for sequestering triglycerides in LD away from ATGL and preventing lipid spillover and ectopic lipid deposition [14]. Emerging evidence has demonstrated that deficiency in expression of FSP27 and perilipin1 along with up-regulation of ATGL and CGI-58 are involved in lipid overflow and the development of IR in obesity [14, 29]. Therefore, we also evaluated the relative changes in lipogenesis and fat storage capacity through the change in the expression of ATGL and CGI-58.

In brief, given the pathogenic significance of early CR for CUGA during refeeding [7, 35], the possibility arises that a sustained imbalance of lipogenesis/fat storage capacity during visceral fat accumulation in CUGA may be involved in the pathogenesis of CUGA-associated ectopic lipid deposition and IR. To test this hypothesis, this study investigated the changes in lipogenic and fat storage capacity in VAT, as well as lipid overflow, ectopic lipid deposition and IR during CUGA.

Methods and materials

Animals
Six-week-old male Sprague-Dawley (SD) rats (body weight 170 ± 10 g, n = 80) were obtained from SJA Laboratory Animal Co. Ltd. (Hunan, China) and maintained under SPF laboratory conditions in the Laboratory Animal Center of Tongji Medical College, Huazhong University of Science and Technology [36]. All protocols were approved by the Animal Ethics Committee of our university and in accordance with Hubei Province Laboratory Animal Care Guidelines for the use of animals in research.
Experimental design
The experimental design (Fig. 1) was employed as described previously [5, 6]. Briefly, following acclimation for 1 week, rats were randomized to the normal chow ad libitum (AL) and CUGA by normal chow (RN) groups based on body weight. Then, each group was divided into five subgroups for five different sampling points, with 8 rats in each subgroup. The AL groups (fed ad libitum with normal chow for 4, 5, 6, 8, and 12 weeks respectively, AL₄, AL₅, AL₆, AL₈, and AL₁₂) were age-matched controls of the RN groups. The RN groups were submitted to different feeding conditions and composed of a CR subgroup (fed with 60% of weight-matched normal chow for 4 weeks, R₄), and four CUGA subgroups (refed with weight-matched normal chow for 1, 2, 4, and 8 weeks respectively after 4-week CR, i.e. RN₁, RN₂, RN₄, and RN₈). The average daily food intake in each group was measured by the difference in weight between the amount of food provided and the amount of food remaining over a 1-day period, which was used to calculate energy intake [7].

Serum assays
Serum NEFA was determined by a NEFA C kit (Wako Chemicals, Richmond, VA, USA). Fasting serum glucose was detected using a glucose oxidase kit (Roche Diagnostics GmbH, Rotkreuz, Switzerland). Serum insulin was quantified using a rat insulin ELISA kit (Millipore, USA).

Body composition
Dual-energy X-ray absorptiometry (DEXA) scan was used to measured body composition, as published [37]. DEXA scan was performed using a GE Lunar Prodigy Advanced System (GE Healthcare, Milwaukee, WI, USA) to detect lean body mass (LBM), body fat mass (BFM) and abdominal fat mass (AFM). The data were analyzed by Encore 2007 Small Animal Software (version 11.40.004). AFM can be distinguished using DXA by identifying it as a specific region of interest within the analysis program. Fat mass from this region was strongly correlated with weighted visceral fat mass and had been validated to be a useful predictor of visceral fat mass.

Hyperinsulinemic-euglycemic clamp and measurement of 2-deoxy-D-glucose uptake in vivo
A 120-min hyperinsulinemic-euglycemic clamp in conscious rats was carried out according to a protocol previously developed by our laboratory to evaluate systemic IR [5, 6]. The average glucose infusion rate was calculated in 60–120 min to reflect the degree of systemic insulin sensitivity. 45 min before the end of the clamp, 2-deoxy-D-glucose (Sigma-Aldrich, St Louis, MO, USA) was administered as a bolus (2 mmol/kg in saline) to evaluate insulin-stimulated glucose transport activity in VAT. The rate of 2-DG uptake into perirenal adipose tissue was measured using a non-radioisotope enzymatic assay [38].

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Fig. 1 Experimental design. As mentioned in the method section, briefly, the rats were randomized into the AL and RN groups. Then, each group was divided into five subgroups for five different sampling points, with 8 rats in each subgroup. The AL groups (fed ad libitum with normal chow for 4, 5, 6, 8, or 12 weeks, i.e. AL₄, AL₅, AL₆, AL₈, and AL₁₂ respectively) were age-matched controls of the RN groups. The RN groups were submitted to different feeding conditions and composed of a CR subgroup (fed with 60% of weight-matched normal chow for 4 weeks, also known as R₄), and four CUGA subgroups (fed with weight-matched normal chow for 1, 2, 4, or 8 weeks after 4-week CR, i.e. RN₁, RN₂, RN₄, and RN₈ respectively)
Sample collection
The rats were fasted for 10 h overnight and anesthetized by intraperitoneal injection of 10% chloral hydrate at 0.3 g/kg. The rats were quickly sacrificed after blood collection via cardiac puncture and the perirenal fat was quickly sampled.

Triacylglycerol contents in peripheral tissues analysis
Triacylglycerol contents in liver and muscle were determined as previously published [39]. Briefly, 100 mg of frozen tissues (liver or muscle) were homogenized with 1 mL of chloroform/methanol (2:1, vol/vol). Next, the tissue homogenates were rotated at room temperature for 4 h to facilitate total lipid extraction. Then, 0.6% NaCl was added before centrifugation for 10 min. The bottom layer was collected, air dried, and resuspended in ethanol. The triacylglycerol concentration was quantified with an enzymatic colorimetric test kit (Triacylglycerol GPO-PAP, Roche Diagnostics, Indianapolis, IN).

Fatty acid synthase activity measurement
FAS activities were measured in tisue lysates following previous instructions [40]. FAS activity was measured by mixing serial dilutions of FAS enzymes with the activity detection buffer containing 31.25 μM NADPH (Sigma, USA), 5 μM acetyl-CoA (Sigma, USA), 100 mM potassium phosphate (pH 7.0) and 1 mM DTT (Sigma, USA). The FAS assay was initiated by adding 10 μM malonyl-CoA (Sigma, USA). The conversion from NADPH to NADP was detected by the decrease in 340 nm absorbance using the SpectraMax M2 plate reader (Molecular devices, Sunnyvale, CA). Rates were normalized to the background rate of NADPH oxidation in the presence of acetyl-CoA. The specific FAS activity was expressed as nmol NADPH oxidized-min⁻¹·mg protein⁻¹.

Measurement of adipocyte size
Sections from perirenal fat (n = 4 rats/group) were fixed in 10% formalin and paraffin-embedded. Hematoxylin/eosin staining was performed on 4-in 10% formalin and paraffin-embedded. Hematoxylin/−expressed as nmol NADPH oxidized·min⁻¹·mg protein⁻¹ of the presence of acetyl-CoA. The specific FAS activity was measured to the background rate of NADPH oxidation in the (Molecular devices, Sunnyvale, CA). Rates were normal-ized to the background rate of NADPH oxidation in the (pH 7.0) and 1 mM DTT (Sigma, USA). The FAS assay was initiated by adding 10 μM malonyl-CoA (Sigma, USA). The conversion from NADPH to NADP was detected by the decrease in 340 nm absorbance using the SpectraMax M2 plate reader (Molecular devices, Sunnyvale, CA). Rates were normalized to the background rate of NADPH oxidation in the presence of acetyl-CoA. The specific FAS activity was expressed as nmol NADPH oxidized-min⁻¹·mg protein⁻¹.

Statistical analysis
Data were expressed as means ± SEM. Differences between the groups at the same sampling points were evaluated by unpaired student’s t-test. Distributions of adipocyte size were analyzed by the quantitative distribution method. The comparison of the distributions was determined by the Kolmogorov-Smirnov test. P < 0.05 was considered significant. Data were analyzed by SPSS software 13.0 (SPSS Inc., Chicago, IL, USA).

Results
Energy intake, body weight and visceral fat accumulation
As shown in Fig. 2a, during the experiment, the weekly energy intake of the rats in the RN group was lower than that of the AL group. During the caloric restriction period, the weekly energy intake of rats in the RN group was 60% that of rats with the same weight in the AL group. After restoring the diet, the weekly energy intake of the rats in the RN group quickly recovered to that of rats with the same weight in the AL group, but was slightly lower than that of the AL group (age matching). This resulted in a significant decrease in body weight.

Table 1 Nucleotide sequences of primers designed for quantitative PCR analysis

| Gene       | Sense primer (5'-3') | Antisense primer sequence (5'-3') |
|------------|----------------------|----------------------------------|
| PPARγ      | GCCCTTGTGGTGATTTATAGAG | GCAGCAAGTTGTCCTGGATGT            |
| FAS        | ACCTCACACTAGAAGGACCACAG | GTGGATCTGGCCTGGTTAT              |
| FSP27      | AAGGCACTAGTGCCCCAGCAG  | TCCACAGATTTGTCCTGCTCTTC          |
| Perilipin1 | GTACCTATGGCCCCTTCCTC  | CCACTGTGCTCGAGAGATA              |
| ATGL       | TGGGCAGACTTTCCAAGCCTTCT | CCAAGGAGATTCTCCTGCCTCC          |
| CGI-S8     | TGATCAAGAGAGACACTTCTC  | TCAGGGCCTGACTGAGACTC             |
| HSL        | ATGTGGAAGAGAGAGACCTGAA | TAGTGCTGGAGGTTGGTGTG             |
| LPL        | CAACTGGCGAAGAGAGAGAGA | CAGGAGTCTGCTCCCTTACCTAAGCTCT    |
| β-actin    | CACCGCGAGATACACCTTCT  | CCCATGCCACATCACCC                |
Changes of (a) energy intake; (b) body weight; (c) BFM% (BFM/BW); (d) AFM% (AFM/BW); (e) AFM/BFM ratio; and (f) LBM% (LBM/BW) over the course of CUGA (n = 8/group). Solid line/white square, AL groups; dotted line/black square, RN groups. *P < 0.05, **P < 0.01 versus the corresponding AL groups.

Fig. 2 The rat model of catch-up growth in adulthood (CUGA) was modeled using a diet that matched the weight of the rats. The rats in the calorie restriction period were given a feed amount that was 60% of the free-fed volume of rats in the AL group with the same weight, while those in the diet recovery period were given a feed amount that was 100% of the free-fed volume of rats in the AL group with the same weight.

Blood parameters, insulin sensitivity and ectopic lipid accumulation

As shown in Table 2, at the end of CR, serum NEFA in the R4 group was similar to that of the AL4 group. After refeeding, serum NEFA was markedly increased in the RN groups compared with the AL groups (P < 0.05). No differences in serum glucose were detected between the RN and AL groups. Notably, although no significant differences were observed in serum insulin between the R4 and AL4 groups at the end of CR, there was a tendency for serum insulin to be higher in the RN groups after refeeding. On week 4 and 8 of refeeding, serum insulin in the RN4 and RN8 groups was significantly increased in comparison with controls (P < 0.01). Consistently, a striking reduction of GIR60–120 at hyperinsulinemic-euglycemic clamp was observed in the RN4 and RN8 groups relative to their controls (P < 0.05). The HOMA-IR index was also increased in the RN groups, supporting the clamp results. Meanwhile, we also found that both intramuscular and intrahepatic TG contents were significantly increased in the RN4 and RN8 groups compared to the AL controls.
(P < 0.01). In brief, with the increase in NEFA during refeeding, RN rats showed a gradual increase in ectopic triglycerides and serum insulin level, but decrease in whole-body insulin sensitivity. Thus, we postulated that because of adequate energy supply at the refeeding stage, the enhanced NEFA exceeded oxidation in peripheral tissues, which then caused triglyceride accumulation in peripheral tissues and the development of IR.

### Changes of key regulatory factors involved in lipogenesis and fat storage capacity in VAT during CUGA

FAS activity in the RN groups overshoot controls after refeeding (P < 0.01), but there was no significant decrease at the end of CR (P > 0.05) (Fig. 3a). On the contrary, FAS gene expression was found to be markedly lower in the R4 group after 4 weeks of CR (P < 0.01), but after refeeding, FAS gene expression in the RN groups gradually increased over time. On week 2 of refeeding, FAS gene expression in the RN2 group was significantly up-regulated as compared with controls (P < 0.05) (Fig. 3b). A similar expression profile of FAS protein was observed (Fig. 3c and m). The results showed that although the increase in FAS activity is consistent with the elevation in FAS expression after 2 weeks of refeeding, the changes in FAS activity did not follow the changes in FAS expression at the end of CR and on week 1 of refeeding. Hence, the changes in FAS activity could only partially be explained by the changes in FAS expression, suggesting that additional posttranslational mechanisms are likely involved in the regulation of FAS activity. Interestingly, our results also showed that the change in insulin-mediated 2-DG uptake is paralleled by the change in FAS activity (Fig. 3d). Besides these, LPL has been considered a key regulators of lipid accumulation in adipocytes. As show in Fig. 3e, LPL gene expression significantly increased in response to 4 weeks of CR (P < 0.05), and it was more obvious in the RN groups than in their controls after refeeding (P < 0.05). Together, these results were in favor of the notion that during CUGA, enhanced lipogenesis might occur at the CR stage, and persisted after refeeding, which might be a pivotal factor for initiating and sustaining visceral fat accumulation.

Although the lipogenic capacity was enhanced, the triglyceride storage capacity in VAT was decreased relatively. The measurement of PPARγ gene expression indicated that it was increased by 0.41-fold in the R4 group compared with the AL4 group after CR (P < 0.05), and this was further aggravated by refeeding. At the end of experiment, PPARγ gene expression in the RN4 group was raised to 2.20-fold compared with the AL12 group (P < 0.01) (Fig. 3f). The PPARγ protein level was shown the similar tendencies (Fig. 3g, m). In contrast, FSP27 gene expression in the R4 group was dramatically reduced to 0.59-fold of the AL4 group by the end of CR (P < 0.01). However, refeeding only partially moderated this association. The lowered FSP27 gene expression in the RN groups was sustained till 2 weeks of refeeding. On week 8 of refeeding, FSP27 protein level in the RN8 group increased to 1.29 times that of controls (P < 0.01) (Fig. 3h, i, and m). The above results suggested that during CUGA, FSP27 gene expression lagged far behind PPARγ gene expression (Fig. 3j). In addition, after 4 weeks of CR, perilipin1 gene expressions and protein level were lower in the R4 group compared with the AL4 group, which decreased even further after refeeding (P < 0.05) (Fig. 3k, l, and m). Collectively, our findings lead to

### Table 2 Metabolic parameters, GIR60 at euglycemic clamp and ectopic triglyceride contents at the end of CR and during refeeding

| Parameter                      | Calorie Restriction | Refeeding |
|-------------------------------|---------------------|-----------|
|                               | AL4                 | R4        |
| Serum NEFA (μmol/l)           | 357.8 ± 34.4        | 349.1 ± 21.6 |
| Serum glucose (mmol/l)        | 4.90 ± 0.45         | 4.77 ± 0.35 |
| Serum insulin (pmol/l)        | 1046.2 ± 2.7        | 971.7 ± 10.0 |
| GIR60 (pmol/l)                | 7.05 ± 1.34         | 6.24 ± 1.79 |
| HOMA-IR                       | 3.11 ± 0.08         | 2.86 ± 0.15 |
| Intramuscular triglyceride (mg/g tissue) | 7.05 ± 1.34 | 6.42 ± 1.15 |
| Intrahepatic triglyceride (mg/g tissue) | 11.10 ± 1.94 | 9.66 ± 1.77 |

The data were represented as Mean ± S.E.M. n = 4/group. NEFA, nonesterified fatty acid; GIR60, average glucose infusion rate; AL, ad libitum normal chow; RN, catch-up growth in adult with normal chow. Statistical differences between AL and RN groups at the same experimental time point are indicated as follows: *P < 0.05, †P < 0.01
Fig. 3 Lipogenesis and fat storage capacity in VAT during CUGA. 

- **A**: FAS activity and expression of FAS mRNA (b) and protein (c). 
- **D**: 2-DG uptake in response to administration of insulin in hyperinsulinemic–euglycemic clamp. 
- **E**: Relative amounts of LPL mRNA. 
- **G**: Relative PPARγ mRNA expression. 
- **H**: Relative FSP27 (h) gene expression. 
- **I**: The relative protein levels of PPARγ (black triangle) and FSP27 (white triangle) standardized by controls. 
- **J**: Gene expression of perilipin1. 
- **K**: Protein expression of perilipin1. 
- **M**: Representative immunoblot analysis for FAS, PPARγ, FSP27 and Perilipin1. 

β-actin served as a loading control. All experiments consisted of n = 4/group. White bars, AL groups; black bars, RN groups. *P < 0.05, **P < 0.01 versus the corresponding AL groups.
the proposal that CR might be induce and sustain the imbalance of lipogenesis/fat storage capacity during CUGA.

Changes in gene expression of lipolytic enzymes in VAT during CUGA
Since ATGL gene and protein expression is highly correlated under several different experimental conditions, we only assayed ATGL mRNA level in this study. As shown in Fig. 4, ATGL gene expression was significantly elevated in the R4 group in comparison with the AL4 group as a result of 4 weeks of CR ($P < 0.05$). This was more evident in the RN groups than in their controls after refeeding ($P < 0.01$) (Fig. 4a). Comparatively, both gene expression (HSL, $P < 0.01$) and protein levels (Ser$^{660}$-phosphorylated HSL, $P < 0.01$) were also significantly increased in the R4 group compared to the AL4 group in response to 4 weeks of CR. After refeeding, this was even more apparent in the RN groups than in their controls (Fig. 4b, c). Moreover, expression of CGI-58 was consistent with ATGL gene expression. CGI-58 gene expression was enhanced by CR and increased further after refeeding in the RN groups relative to the controls ($P < 0.01$) (Fig. 4d). In brief, these results indicate that following refeeding, the lipolytic capacity in VAT was enhanced in the RN group, which displayed lipid overflow in their VAT.

Morphology of perirenal adipose tissue and the distribution of adipocyte size during CUGA
The morphology of H&E staining showed that adipocyte size tended to be smaller in the R4 group than in the AL4 group at the end of CR. After refeeding, the adipocyte size in the RN groups increased gradually over time, but it failed to exceed that of controls even after 8 weeks of refeeding (Fig. 5a). Quantitative measurement of cell profile area revealed that adipocyte size distribution in RN group exhibited a significant shift to the left. Four weeks after CR, the median adipocyte area in the R4 group was dramatically smaller than in the AL4 group ($1369.10 \mu m^2$ vs. $2867.07 \mu m^2$, $P < 0.01$). Four weeks after refeeding, adipocyte area distribution in the RN4 group still displayed a significant shift to small size relative to
controls (3369.00 μm² vs. 3964.60 μm², \(P < 0.05\)). Even eight weeks after refeeding, the mean adipocyte area in the RN₈ group could not exceed that of controls (3755.64 μm² vs. 3961.70 μm², \(P = 0.63\)) (Fig. 5b).

Taken together, we observed a persistent shift-to-the-left of adipocyte size distribution accompanied by enhanced lipogenesis during CUGA.

**Discussion**

The main findings of this study relate to the mechanisms linking visceral fat accumulation to ectopic lipid deposition and IR in CUGA. The results suggested that the pathophysiological consequences of CUGA are associated with (i) CR-induced and sustained enhancement of lipogenesis in VAT after refeeding, which results in visceral fat accumulation; (ii) CR-induced and sustained mismatch between lipogenesis and fat storage capacity in VAT during refeeding, which leads to ectopic lipid deposition and IR.

In this study, we initially investigated the changes in lipogenic capacity in VAT during CUGA. Our study showed that PPARγ expression in VAT was significantly higher in the R₄ group than in the AL₄ group after 4 weeks of CR. A previous study also found that 12-month CR markedly increased PPARγ expression in VAT [9], but another study reported that PPARγ expression in
VAT was declined after 2 weeks of CR [28]. These differential changes of PPARγ expression in VAT in response to CR can be attributed to the different duration of CR. The majority of the effects of long-term CR on responsive gene expression can be produced after 4 weeks and the 2-week CR period might be a little short to observe significant effects.

Notably, despite an increase in PPARγ expression in VAT in the R4 group, there were no differences in FAS activity and 2-DG uptake between the R4 and AL4 groups, and AFM% was found to be lower in the R4 group, suggesting that nutrition deficiency at the CR stage might be an important reason for the lack of lipogenic capacity in VAT. A similar finding of LPL gene expression further supported this suggestion. It could be hypothesized that CR increases lipogenesis to keep up with the demand to compensate for limited fuel supply, but a previous study suggested that in the context of nutrition shortage, this enhanced lipogenic capacity may be only an enhanced lipogenic potential [42], which will have to be verified in future studies. At the following refeeding period, we observed that PPARγ and LPL expression, FAS activity and 2-DG uptake in VAT in the RN groups were all higher than in controls, concomitant with which AFM% in the RN groups was rapidly increased in the refeeding period, we observed that PPARγ expression in VAT was rapidly increased, with the expression of PPARγ and LPL expression further supported by the result that serum NEFA was significantly higher in the RN groups than in the AL group at this stage. This might be ascribed to the nutrition deficiency, which promotes the release of NEFA from VAT to oxidize for energy in peripheral tissues, thereby preventing lipotoxicity.

In addition to the observation of similar expression profile of ATGL gene and protein of HSL phosphorylation during the following refeeding, we discovered that although the lipogenic capacity in VAT was enhanced in the RN groups, the triglyceride storage capacity was relatively insufficient (slowly increased FSP27 expression persistently increased in RN VAT during refeeding, triglyceride storage capacity in VAT was enhanced in the RN group after refeeding. This is further supported by the result that serum NEFA was significantly higher in the RN groups than in the AL groups at refeeding stage. Herein, during CUGA, RN rats showed increased lipolysis and lipid overflow in VAT. We speculated that despite an increase in triglyceride synthesis in RN VAT during refeeding, triglyceride storage capacity in RN VAT was relatively reduced, thus providing more contact surface area of LD for ATGL and HSL, which combined with up-regulation of ATGL, CGI-58 and HSL phosphorylation and down-regulation of perilipin1 facilitates lipolysis and lipid overflow.

The present study only examined perirenal fat and was considered to be representative of visceral fat, but future...
studies should directly examine the contribution of all types of visceral adipose tissues (mesenteric, omental, perirenal, and epididymal). In addition, since refeeding depends on an initially inadequate intake, which will produce their own acute and chronic adjustments in metabolism, this suggests that caloric restriction may be the initial reason for imbalance between lipid production and storage capacity. This will have to be further examined in future studies. Finally, given that %body fat and %AFM were increased while the mean cell size decreased, it seems that adipocyte numbers increased in the RN groups, which could be supported by the increase in PPARγ. These small cells might be more insulin-sensitive than those of the AL rats. Furthermore, a greater number of small cells might be expected to provide greater fat storage capacity. This is also a hypothesis that will have to be examined in future studies.

Based on our findings, we tentatively propose a hypothetical mechanistic rationale for the development of IR in CUGA. On one hand, at CR stage, in order to effectively compensate the limited energy supply, PPARγ expression increases in VAT, which leads to an increase in lipogenic capacity in VAT (“active storage energy”). At this time, FSP27 expression adaptability down-regulates, and lipid storage capacity of VAT decreases correspondingly. On the other hand, after refeeding, PPARγ expression persistently increases in VAT. At this stage, adequate nutrition supply turns CR-induced lipogenic potential into enhanced lipogenic capacity, causing the rapid accumulation of visceral fat. FSP27 expression cannot correspondingly up-regulate, resulting in insufficient capacity of lipid storage. Hence, the change in lipogenesis/lipid storage capacity in VAT adapts to CR and persists even in a new nutritional environment, which leads synthesized triglyceride to exceed triglyceride storage in VAT, causing lipid overflow and the development of IR during CUGA. This study assists us in understanding the underlying mechanisms of CUGA-associated metabolic disorders, and is of significance for the control and prevention of IR-associated disease in developing countries (Fig. 6).

In conclusion, this study reveals part of the mechanisms involved in the development of IR in CUGA from VAT dysfunction as triglyceride depot. The persistent CR-induced imbalance of lipogenesis/fat storage capacity might be responsible for the CUGA-associated metabolic disorders.

Abbreviations
AFM: Abdominal fat mass; AL: Ad libitum normal chow; ATGL: Adipose triglyceride lipase; BFM: Body fat mass; CGI-58: Comparative gene identification 58; CR: Caloric restriction; CUGA: Catch-up growth in adult; DEXA: Dual-energy X-ray absorptiometry; FAS: Fatty acid synthase; FSP27: Fat-specific protein27; HSL: Hormone-sensitive lipase; IR: Insulin resistance; LBM: Lean body mass; LD: Lipid droplets; LPL: Lipoprotein lipase; NEFA: Non-esterified fatty acid; PPARγ: Peroxisome proliferator-activated receptors γ; SD: Sprague–Dawley; VAT: Visceral adipose tissue; WAT: White adipose tissue

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L-LC designed the study, contributed to discussion and reviewed the manuscript. YL performed the research, analyzed the data, contributed to discussion and wrote the manuscript. XH contributed to the study design. SY conducted the research. JZ and L-XD contributed to the study design. All authors approved the final version of the manuscript. L-LC is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. We thank Yu Dia (Department of Nuclear Medicine, Union Hospital, Huazhong University of Science Technology) for excellent technical assistance in DEXA. We also thank Ms. Anita Regmi (Huazhong University of Science Technology) for her invaluable help in revising the manuscript.

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Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors’ contributions
YL and SY carried out the studies, participated in collecting data, and drafted the manuscript. YL, L-LC and XH performed the statistical analysis and participated in its design. JZ and L-XD contributed to the study design. L-LC helped to draft the manuscript. All authors read and approved the final manuscript.

Ethics approval
All protocols of animal treatment in present study were approved by the Animal Ethics Committee of our university and in accordance with Hubei Province Laboratory Animal Care Guidelines for the use of animals in research.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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