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Caloric restriction increases adiponectin expression by adipose tissue and prevents the inhibitory effect of insulin on circulating adiponectin in rats☆

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Abstract

Aging is associated with redistribution of body fat and the development of insulin resistance. White adipose tissue emerges as an important organ in controlling life span. Caloric restriction (CR) delays the rate of aging possibly modulated partly by altering the amount and function of adipose tissue. Adiponectin is a major adipose-derived adipokine that has anti-inflammatory and insulin-sensitizing properties. This study examined the effects of CR on adiposity and gene expression of adiponectin, its receptors (AdipoR1 and AdipoR2) in adipose tissue and in isolated adipocytes of Brown Norway rats that had undergone CR for 4 months or fed *ad libitum*. The study also determined plasma concentrations of adiponectin and insulin in these animals and whether insulin infusion for 7 days affects adiponectin expression and its circulating concentrations under CR conditions. CR markedly reduced body weight as anticipated, epididymal fat mass and adipocyte size. CR led to an increase in plasma free fatty acid and glycerol (both twofold), and adipose triglyceride lipase messenger RNA (mRNA) in adipose tissue and isolated adipocytes (both >2-fold). Adiponectin mRNA levels were elevated in adipose tissue and adipocytes (both >2-fold) as was plasma adiponectin concentration (2.8-fold) in CR rats. However, CR did not alter tissue or cellular AdipoR1 and AdipoR2 expression. Seven days of insulin infusion decreased adiponectin mRNA in adipose tissue but did not reverse the CR-induced up-regulation of circulating adiponectin levels. Our results suggest that the benefits of CR could be, at least in part, dependent on enhanced expression and secretion of adiponectin by adipocytes.
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Keywords: Caloric restriction; Adipose tissue; Adipocytes; Adiponectin; Insulin

1. Introduction

It has been recognized for over 70 years that caloric restriction (CR) feeding regimens extend the life span by delaying and/or retarding the aging process [1,2]. Although CR is considered as the most robust method to extend life span and to retard age-related pathologies in rodents [3], the precise mechanisms whereby CR feeding increases survival remain to be clarified. Growing evidence suggests that white adipose tissue is an important organ in the control of life span [4]. Adipose tissue, as the largest fuel reserve in the body, has an important role in the regulation of energy balance and glucose homeostasis [5,6]. In addition to fat storage, a series of proteins (adipokines) are synthesized and released by adipose tissue. Examples are leptin, tumor necrosis factor α, adiponectin and the recently described zinc-α2-glycoprotein of which some are strongly associated with insulin resistance and obesity [5,7].

It is recognized that CR feeding reverses aging-induced insulin resistance that is closely related to body fat mass [8,9] and influenced by adipose-derived adipokines. Prolonged CR feeding decreases fat mass, particularly visceral fat [10], and reduces plasma leptin and insulin concentrations in rodents [3]. The reduction of body fat is postulated to be a primary factor in CR-induced life span extension, as mice with less adipose tissue live longer even without food restriction [11]. It is well documented that with fat mass expansion, such as in obesity, there are significant increases in the production of adipokines that promote inflammation and insulin resistance [12]. Therefore, it is possible that CR opposes aging-related metabolic disorders through modulation of body fat accumulation as well as by adipokine synthesis and signaling.

Adiponectin (also known as apM1, GBP28, AdipoQ and ACRP30), a 30-kDa protein, is produced primarily by adipocytes and secreted into the circulation [13,14]. Adiponectin is one of the most abundant adipokines expressed by adipocytes, and its circulating concentrations decrease with increased body weight and especially increased adiposity [14–16]. Adiponectin has been suggested to have anti-inflammatory, antiatherogenic and antidiabetic properties [16]. The importance of adiponectin in enhancing insulin sensitivity is supported by evidence that it stimulates glucose uptake and fatty acid oxidation in myocytes and reduces hepatic glucose production [17,18]. These effects of adiponectin are mediated through activation of the AMP-activated protein kinase (AMPK) [18,19]. In addition, adiponectin may act centrally via the melanocortin pathway in the regulation of appetite [20].

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Adiponectin binds to two complementary receptors, AdipoR1 and AdipoR2, which are thought to transmit the insulin-sensitizing effects of adiponectin. [21–23]. AdipoR1 is a receptor that binds the smaller globular adiponectin associated with more extensive biological activity, whereas AdipoR2 binds full-length adiponectin [21]. AdipoR1 and AdipoR2 were found abundantly expressed in skeletal muscles and liver, respectively [21]. The presence of both receptors has also been reported in mouse adipose tissue and 3T3-L1 adipocytes [24,25]. Furthermore, messenger RNA (mRNA) levels of AdipoR1 and AdipoR2 are increased in skeletal muscle and liver in fasted mice but decreased in adipose tissue and skeletal muscle in obese *ob/ob* mice [24].

Although adiponectin expression in adipose tissue was up-regulated by CR in mice [3], less is known whether CR alters the production of adiponectin and its regulation in rats. This study examined in details the effects of CR on adiposity and gene expression of adiponectin and its receptors (AdipoR1 and AdipoR2) in adipose tissue and in isolated adipocytes in rats on CR or fed *ad libitum*. The study also determined circulating adiponectin and insulin levels in these animals. Finally, whether chronic insulin infusion affects adiponectin expression and its circulating levels under CR condition was explored.

2. Materials and methods

2.1. Animal and dietary manipulations

Male Brown Norway rats (substrain BN/SsNOlaHSD) of defined health status were purchased from Harlan Laboratories (21–28 days old). The rats were maintained under specific pathogen-free conditions on a 12-h light/dark cycle (0800–2000 h) at 22°C±1°C in the University Animal House. Rat and mouse standard maintenance diet (Special Diet Services, Essex, UK) was used, and water was provided *ad libitum* to all animals.

CR feeding was commenced 6 weeks after birth for 4 months. Rats were housed singly and fed 55% of *ad libitum* (control rats) intake. The restricted diet was preweighted and supplied between 1030 and 1100 h. CR rats consumed most of their food within approximately 5 h that they were effectively daytime meal eaters. The control rats were housed singly and given free access to food between 1000 and 1500 h to synchronized to the same feed/fast cycle as the rats on CR. Sentinel animals were used to monitor the health status of the animals throughout the study and the integrity of the barrier housing conditions. Survival data, husbandry details and growth curves have been published previously for this substrain of BN rats under these feeding regimens [26].

One group of the CR rats received insulin treatment by implanting subcutaneous mini osmotic pump (Alzet 2002; Charles River Laboratories, Wilmington, UK) under sterile conditions into subcutaneous pouches in the midscapular region of the animal's back for 1 week [27]. Pumps were filled aseptically with 200 μl of 27 μmol/l insulin in isotonic saline (pH <2) containing 5% (vol/vol) stripped rat plasma. Pumps containing 200 μl of isotonic saline were implanted in both control and CR animals to control for any artifacts induced by the surgical procedure. The pumps were primed in sterile isotonic saline at 37°C for at least an hour prior to surgery. The animals were killed a week later, and the surgical procedures had no discernible effect on body weight or food intake.

All animal studies were conducted in accordance with the Animal Procedures Act (1986), UK, for the ethical care and treatment of animals.

2.2. Adipose tissue and plasma collection

Four months after the initiation of CR feeding, rats were killed by cervical dislocation. Body weight was recorded for each animal, and blood was removed from animals via cardiac puncture using a heparinized syringe. The blood was centrifuged at 3000*g* for 5 min, and the plasma supernatant was removed and stored at −80°C. The epididymal fat pads were isolated and weighed. One fat pad was then rapidly frozen in liquid nitrogen and stored at −80°C until use; the other fat pad was used for histology and the fractionation of adipocytes and stromal–vascular (SV) cells.

2.3. Adipose tissue histology

Epididymal fat samples from control and CR animals were fixed in 10% neutral formalin for 24 h. After which, they were dehydrated in absolute ethanol, cleared in xylene and finally embedded in paraffin. The paraffin was cut into 5-μm sections, stained with Harris hematoxylin and counterstained with eosin. For quantification of adipocyte size, sections were analyzed using an Optiphot-2 microscope equipped with a digital camera (Nikon, Tokyo, Japan). The cell perimeter and sectional area were measured in 100 adipocytes per section (three sections for one rat and five animals per group), and data analysis was performed using the MCID Basic software (Imaging Research Inc., Ontario, Canada) for digital image processing [28].

2.4. Mature adipocytes and SV cell fractionation

One epididymal fat pad of each rat was placed into a universal tube with 5 ml isolation buffer [1.35 M sodium chloride, 47 mM potassium chloride, 25 mM calcium chloride, 12.5 mM magnesium sulphate, 100 mM HEPES, 12.5 mM sodium dihydrogen phosphate and 12.5 mM disodium hydrogen phosphate (pH=7.4)] with 1% albumin. The samples were then transferred to a sterile cell culture hood, and the fat pad was transferred into a fresh universal tube with 5 ml isolation buffer containing 3.5% albumin, 1 mg/ml collagenase and 0.1 mg/ml glucose (Sigma, Poole, Dorset, UK). The tubes were then sealed using parafilm and placed in a heated oven at 37°C with continuous rotation for 1 h. After the completion of collagenase digestion, the samples were passed through a 250-μm mesh into fresh universal tubes before centrifuging at 173*g* for 10 min at room temperature. At this stage, the mature adipocytes (floating to the top) were removed to a new tube and washed three times with 5 ml isolation buffer containing 1% albumin. Mature adipocytes were collected after the buffer was carefully removed with a syringe. The remaining buffer was then removed from the original tubes, and the SV pellet was left at the bottom. The pellet was then washed three times with isolation buffer containing 1% albumin and removed into a new tube. These two fractions were then stored at −80°C until analysis.

2.5. RNA extraction and complement DNA synthesis

Total RNA was extracted from tissues or cells using TRIzol (Invitrogen, Carlsbad, CA, USA). The RNA concentration was determined from the absorbance at 260 nm, and the purity checked by the ratio of absorbance at 260:280 nm. First strand DNA was reverse transcribed from 0.5 μg of total RNA using a Reverse-iT first strand synthesis kit (ABgene, Epson, UK) in a final volume of 10 μl.

2.6. Reverse transcriptase polymerase chain reaction

Gene expression in tissues and adipocytes was detected by reverse transcriptase polymerase chain reaction (RT-PCR). One microliter of RT reaction was amplified with 20 μM of each primer and 1.1 × Reddy Mix PCR Master Mix (ABgene) in a volume of 25 μl. The following primer pairs were used: AdipoR1, 5′-ACT GGC TGA AAG ACA ATG ACT ACC-3′ (forward), 5′-AGA AAT AGC ACA AAA CCA AGC AAA TG-3′ (reverse); AdipoR2, 5′-GCC TGT TTT AAG AGC ATC TTT AGA ATA C-3′ (forward), 5′-ATA TTT GGG CGA AAC ATA TAA AAG ATC C-3′ (reverse); β-actin, 5′-GAC AGG ATG CAG AAG GAG ATT ACT G-3′ (forward), 5′- GAG CCA CCA ATC CAC ACA GA-3′ (reverse); adiponectin, 5′-CCC CTG GCA GGA AAG GA-3′(forward), 5′- CCT ACG CTG AAT GCT GAG TGA T-3′ (reverse). PCR was performed on a thermal cycler (Hybaid, Teddington, UK), with an initial denaturation at 94°C for 20 s, annealing at optimized temperature and extension at 72°C for 30 s. The cycle number was 30 for AdipoR1 and AdipoR2 and 23 for adiponectin and β-actin. Negative controls (without template) were included in parallel. To confirm the identity of PCR products, the products were sequenced commercially (MWG, Ebersberg, Germany). PCR products were separated on a 1% agarose gel, and images recorded with a Kodak 1D Image Analysis System (Kodak Digital Science, Rochester, NY, USA).

2.7. Real-time PCR

Real-time PCR amplification was performed in a final volume of 25 μl, containing complement DNA (equivalent to 25 ng of RNA), optimized concentrations of primers, TaqMan probe FAM-TAMRA and a master mix made from qPCR core kit (Eurogentec, Glasgow, UK) using a Stratagene Mx3005P detector. The sequence of primers were as described above. The sequence of TaqMan probes were as follows: adiponectin, 5′-AGC CCG GAG AAG CCG CTT ACA TG-3′; AdipoR1, 5′-CCA CAG ACC ACC TAT GCC CTC CTT CCG-3′; AdipoR2, 5′-CAC AGA GAC GGG CAA CAT TTG GAC ACA TCT-3′. PCR amplification was performed in duplicate using a 96-well plate, and the PCR cycling conditions were as follows: 50°C for 2 min and 95°C for 10 min, followed by 40 cycles (95°C for 15 s, 60°C for 1 min). Blank controls without complement DNA were run in parallel. β-Actin was used as a reference gene. All samples were normalized to the β-actin values, and the results were expressed as fold changes of C_t value relative to controls using the $2^{-\Delta\Delta C_t}$ formula.

2.8. Measurement of plasma adiponectin and insulin by enzyme-linked immunosorbent assay

Plasma adiponectin concentrations were measured using an enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems, Abingdon, UK) according to the manufacturer's instructions. Plasma insulin levels were determined using an ELISA kit (DMG, Berlin, Germany).

2.9. Measurement of lipolysis

Plasma free fatty acids (FFAs) and glycerol levels were determined by using enzymatic colorimetric method with commercial kits: FFA (Roche Applied Science, Penzberg, Germany) and glycerol (Sigma).

2.10. Statistical analysis

A nonpaired Student's *t* test was used to test for statistical significance when comparing group means between control and CR groups. One-way analysis of variance coupled with Bonferroni test was used to compare group means when comparing three experimental groups. Data were expressed as means±S.E.M.. A probability level of $P<.05$ was considered statistically significant.

3. Results

3.1. CR reduces adiposity and increases lipid mobilization

The CR regimen significantly reduced the body weight as anticipated from previous studies ($P<.001$, Fig. 1A)[26] and epididymal fat mass (−54%, $P<.001$; Fig. 1B) of the control animals. CR also led to a significant reduction in cell size (Fig. 1C) with a decrease in adipocyte diameter (−17%, $P<.001$; Fig. 1D) and the sectional area (−33% , $P<.001$; Fig. 1E).

Plasma FFA and glycerol, the two main products resulted from lipolysis, were increased by twofold in CR rats relative to the controls ($P<.001$ and $P<.05$, respectively; Fig. 1F, G). To develop a better understanding of the lipolytic process under CR conditions, gene expression of adipose triglyceride lipase (ATGL), a novel lipase, in adipose tissue and in isolated adipocytes was determined. CR induced a 2.4-fold increase in ATGL mRNA levels in adipose tissue ($P<.01$; Fig. 1H). Consistently, ATGL gene expression in adipocytes was 3.1-fold higher in CR rats compared with *ad libitum* fed control animals ($P<.05$; Fig. 1I).

3.2. Effects of CR on gene expressions of adiponectin and its receptors in adipose tissue

Since CR has been shown to reverse aging-related insulin resistance [7,9,28], it is possible that CR improves insulin sensitivity through the modulation of adiponectin. The relative adiponectin

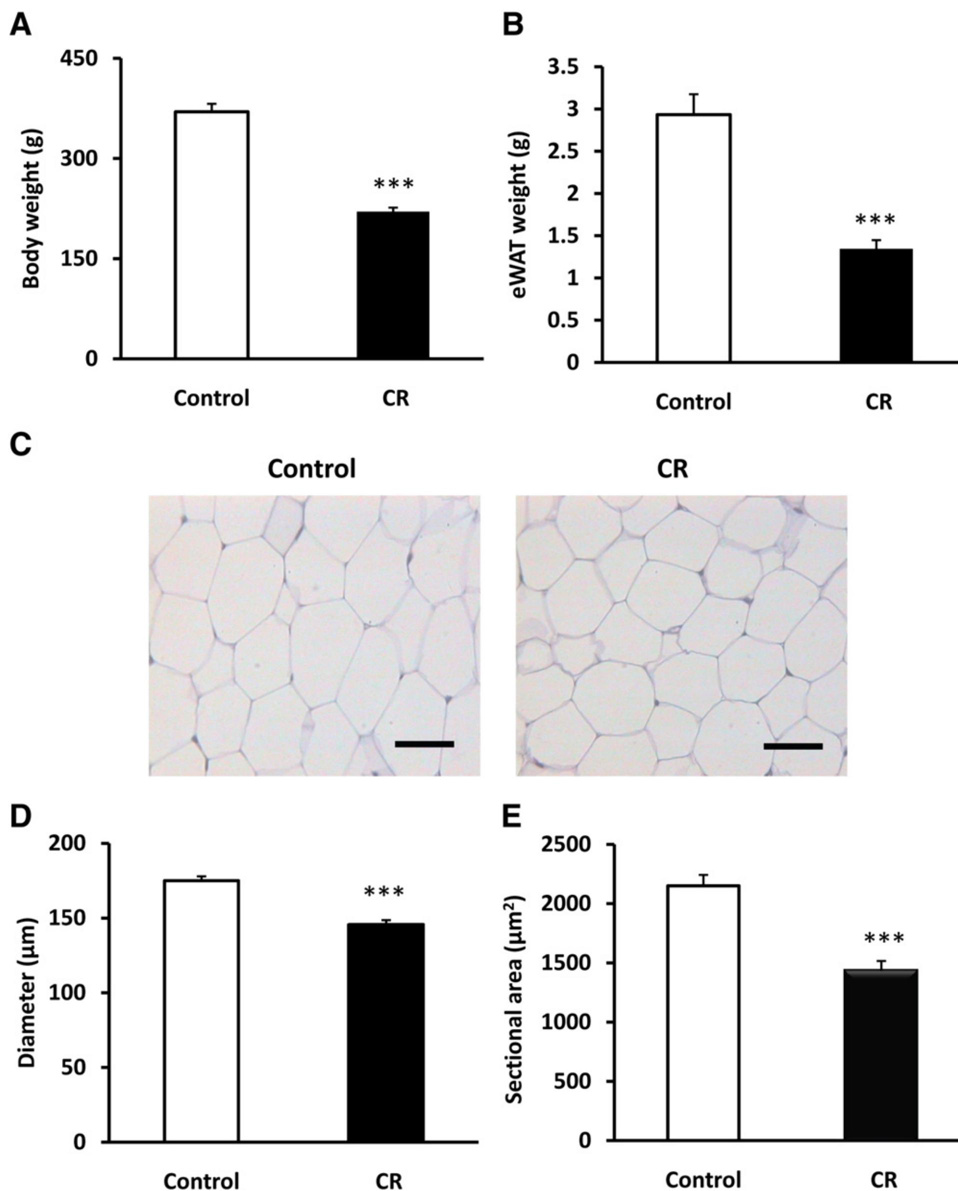

Fig. 1. Effects of CR on body weight (A) and epididymal adipose tissue mass (B) of Brown Norway rats. (C) Hematoxylin–eosin-stained sections of epididymal adipose tissue (bar=50 μm). Morphometric analysis of diameter (D) and sectional area (E) of adipocytes of epididymal adipose tissue. Plasma levels of FFA (F) and glycerol (G) in control and caloric-restricted rats. ATGL mRNA levels in epididymal adipose tissue (H) and isolated adipocytes (I) in control and CR rats. mRNA levels were measured using real-time PCR and normalized to β-actin. Data are means±S.E.M. for groups of eight. *$P<.05$, **$P<.01$, ***$P<.001$ versus controls.

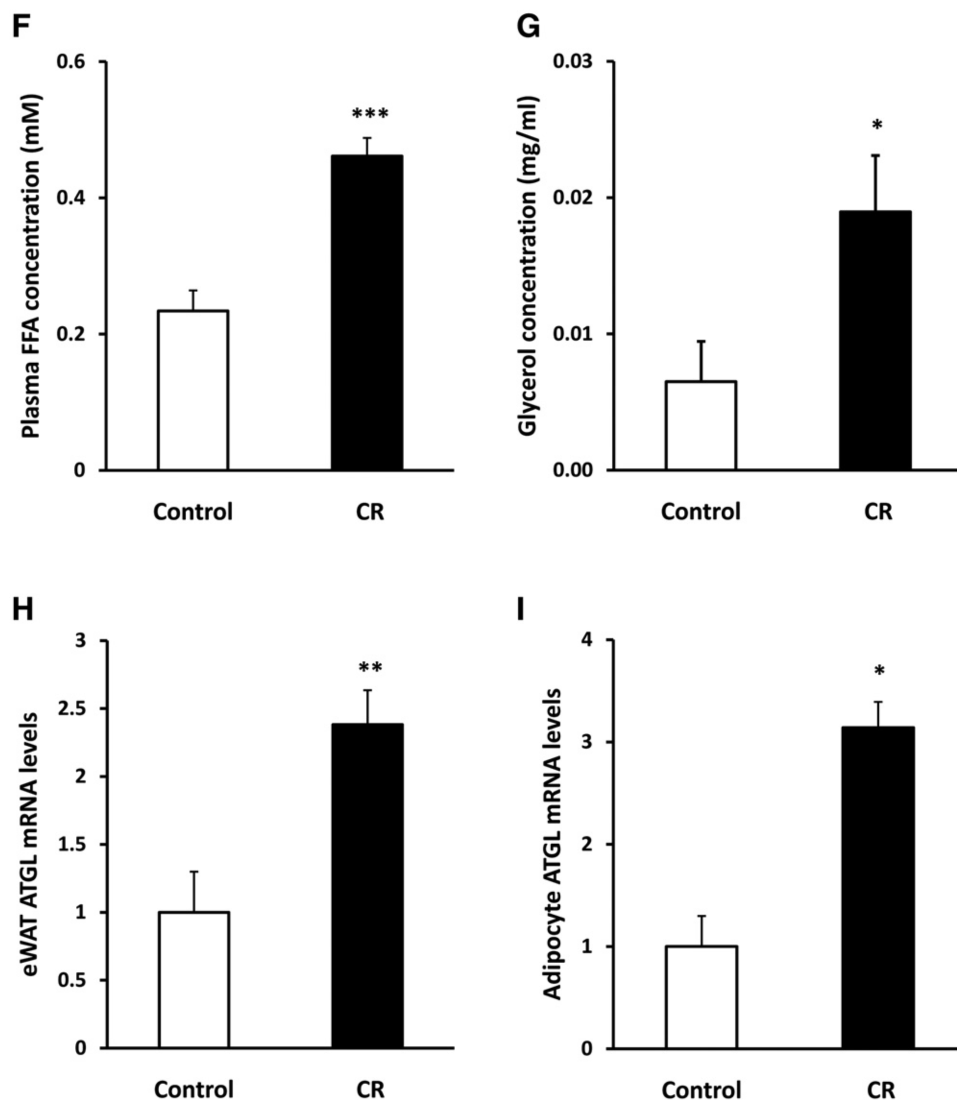

Fig. 1. (*continued*).

mRNA levels were determined by real-time PCR, and CR induced a 2.4-fold ($P<.05$) increase in adiponectin mRNA levels in epididymal adipose tissue (Fig. 2A).

However, there was no significant difference in AdipoR1 mRNA levels between the control and CR group (Fig. 2B). Similarly to AdipoR1, AdipoR2 mRNA levels were not affected by CR feeding (Fig. 2C).

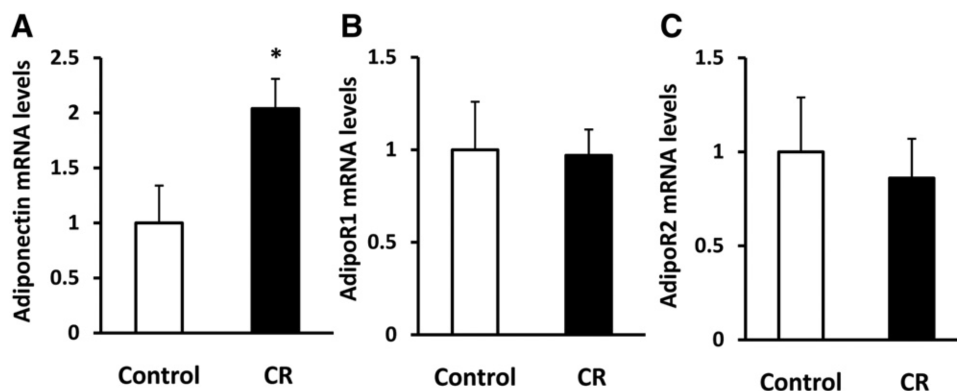

Fig. 2. Effects of CR on gene expression of adiponectin and adiponectin receptors in epididymal adipose tissue of rats. (A) Adiponectin mRNA levels in epididymal fat depot of control and CR rats. (B) AdipoR1 mRNA levels in epididymal fat depot of control and CR rats. (C) AdipoR2 mRNA levels in epididymal fat depot of control and CR rats. mRNA levels were measured using real-time PCR and normalized to β-actin . Data are means±S.E.M. for groups of eight, presented as changes relative to controls. *$P<.05$ versus controls.

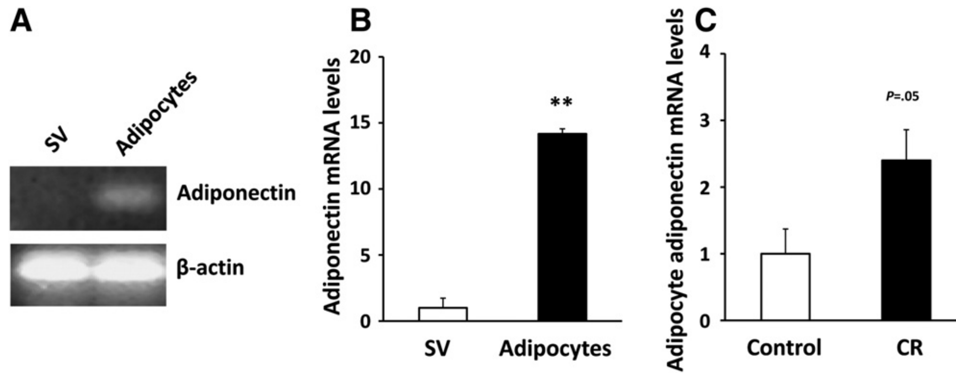

Fig. 3. Effects of CR on gene expression of adiponectin in isolated adipocytes and SV fraction of epididymal adipose tissue of rats. (A) RT-PCR analysis of adiponectin gene expression in adipocytes and SV fraction. (B) Adiponectin mRNA levels in isolated adipocytes and SV fraction by real-time PCR. (C) Adiponectin mRNA levels in isolated adipocytes in control and CR rats by real-time PCR. RNA was isolated from adipocytes and SV fraction, obtained by collagenase digestion of epididymal adipose tissue. Data are means±S.E.M. for groups of eight, presented as changes relative to controls. ***P*<.01 versus controls.

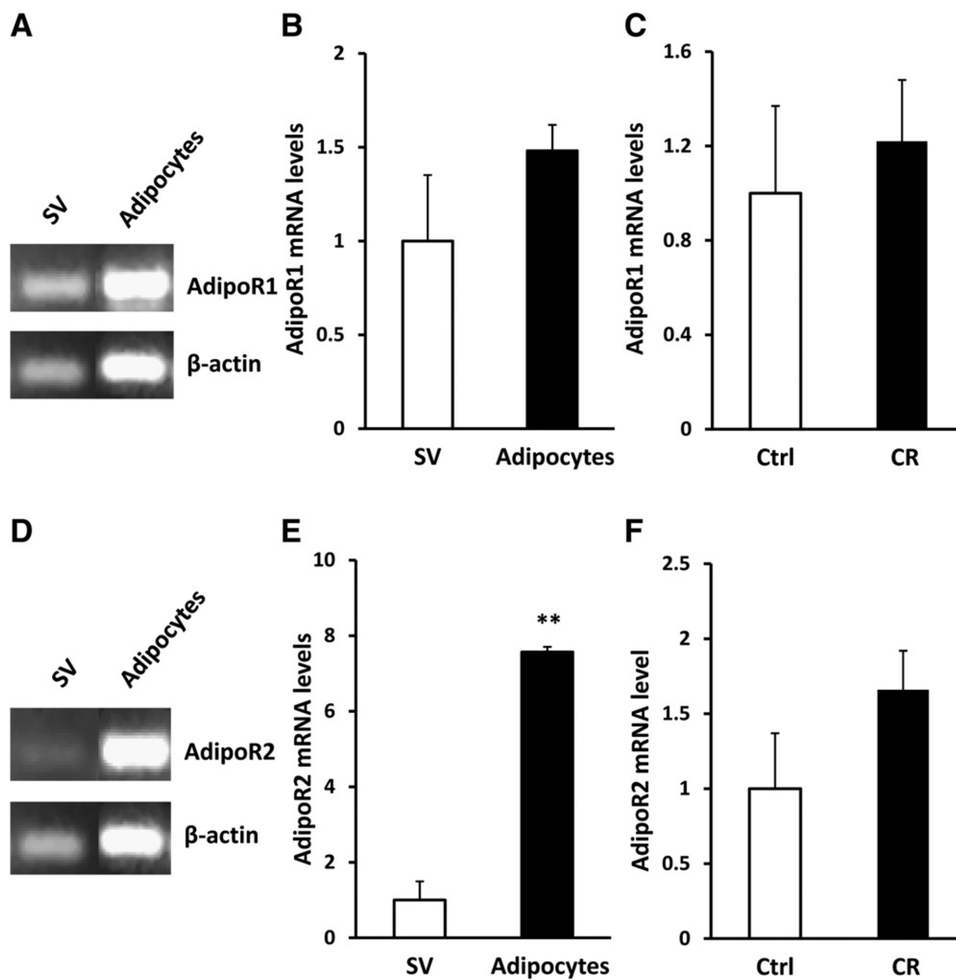

Fig. 4. Effects of CR on gene expression of adiponectin receptors in isolated adipocytes and SV fraction of epididymal adipose tissue of rats. (A) RT-PCR analysis of gene expression of AdipoR1 in isolated adipocytes and SV fraction of epididymal adipose tissue in rats. (B) AdipoR1 mRNA levels in isolated adipocytes and SV fraction by real-time PCR. (C) AdipoR1 mRNA levels in isolated adipocytes in control and CR rats by real-time PCR. (D) RT-PCR analysis of gene expression of AdipoR2 in isolated adipocytes and SV fraction of epididymal adipose tissue in rats. (E) AdipoR2 mRNA levels in isolated adipocytes and SV fraction by real-time PCR. (F) AdipoR2 mRNA levels in isolated adipocytes in control and CR rats by real-time PCR. RNA was isolated from adipocytes and SV fraction, obtained by collagenase digestion of epididymal adipose tissue. Data are means±S.E.M. for groups of eight, presented as changes relative to controls. ***P*<.01 versus controls.

3.3. Expression of adiponectin in isolated adipocytes and the SV fraction of adipose tissue

As shown in Fig. 3A, the adiponectin transcript was detected in adipocytes but not in the cells of the SV fraction by RT-PCR. The relative adiponectin mRNA levels in the two fractions were then determined by real-time PCR. A 14-fold higher expression level of adiponectin was seen in the isolated adipocytes than in the SV fraction (Fig. 3B).

Since adiponectin was predominantly expressed by adipocytes, the effect of CR feeding on adiponectin expression in isolated mature adipocytes was then examined. As shown in Fig. 3C, a 2.4-fold (P=.05) increase in adiponectin mRNA was observed in adipocytes from CR rats compared with controls (Fig. 3D).

3.4. Expression of adiponectin receptors in isolated adipocytes and the SV fraction of adipose tissue

As adiponectin receptors are expressed in adipose tissue, their expression patterns in the tissue fractions were determined. As shown in Fig. 4A and B, there was no significant difference in AdipoR1 mRNA levels between the SV and adipocyte fractions. Subsequently, the effect of CR on gene expression of AdipoR1 in adipocytes was examined. Similar to the finding in adipose tissue, CR feeding did not affect the expression of AdipoR1 in adipocytes (Fig. 4C).

In contrast to AdipoR1, gene expression of AdipoR2 was 7.6-fold higher in isolated adipocytes compared with the SV fraction (P<.01; Fig. 4D, E). However, no significant change in AdipoR2 mRNA levels in adipocytes was found between CR and control groups (Fig. 4F).

3.5. Effect of CR on circulating levels of adiponectin and insulin

Consistent with the gene expression results, CR significantly increased circulating adiponectin by 2.5-fold (P<.001; Fig. 5A). In contrast, plasma insulin levels were markedly reduced by sixfold (P<.01; Fig. 5B) in rats on CR.

3.6. Effect of insulin infusion on CR-induced changes in adiponectin expression and circulating adiponectin

Since CR significantly decreased plasma insulin levels, it was then assessed whether the CR-induced increase in adiponectin expression and protein release was due to low levels of insulin. Adiponectin expression in adipose tissue and circulating adiponectin concentrations were determined in CR rats that received insulin infusion for 7 days, in comparison with both controls and CR fed animals bearing pumps containing only vehicle. As shown in Fig. 6A, insulin treatment

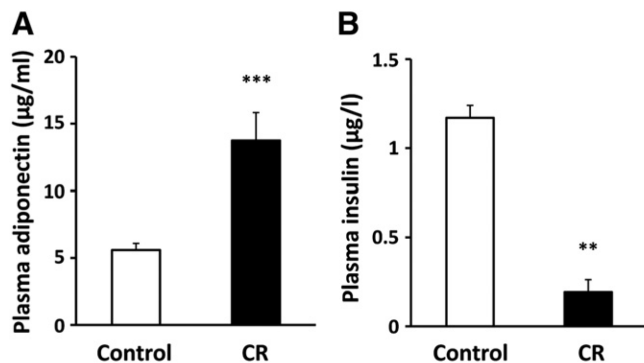

Fig. 5. Effect of CR on circulating adiponectin and insulin concentrations. (A) Plasma adiponectin concentration in control and CR rats, measured using ELISA. (B) Plasma insulin concentration in control and CR rats, measured using ELISA. Data are means± S.E.M. for groups of seven to eight, ***P<.001 versus controls.

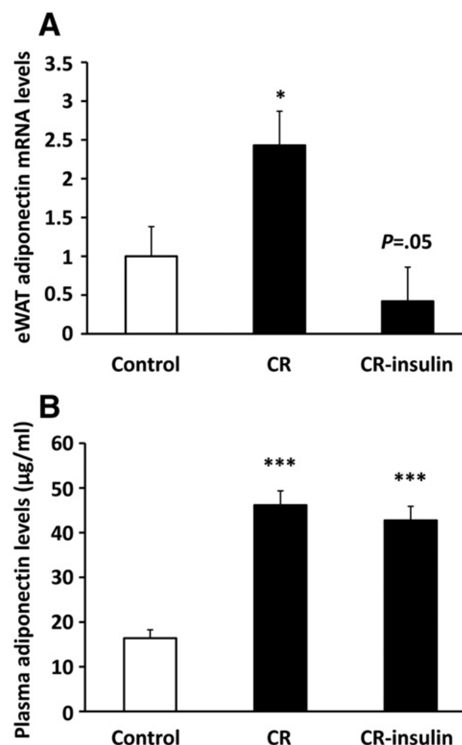

Fig. 6. Effect of insulin on CR-induced adiponectin expression and production. (A) Adiponectin mRNA levels in epididymal adipose tissue of control, CR and CR rats with insulin infusion for 1 week, using real-time PCR. (B) Plasma adiponectin levels in control, CR and CR rats with insulin infusion for 1 week, measured using ELISA. Data are means±S.E.M. for groups of four to six. CR-insulin, caloric-restricted rats treated with insulin. *P<.05, ***P<.001 versus controls.

significantly reduced adiponectin mRNA levels in adipose tissue. However, insulin had no effect on the CR-induced up-regulation of circulating adiponectin concentrations (Fig. 6B).

4. Discussion

This study demonstrated that rats maintained on a CR feeding regimen had significantly reduced body weight and fat mass, in agreement with previous studies in rodents [3,26]. In the present study, further morphometric analysis revealed that CR caused a significant reduction in adipocyte size. It has been shown that in comparison with small adipocytes, large adipocytes secrete higher levels of proinflammatory factors that promote insulin resistance, such as interleukin-6, interleukin-8 and monocyte chemoattractant protein-1 [29]. Furthermore, the reduction in adipocyte cell size indicates lowered cytoplasmic lipid content. In the present study, it was shown that a marked increase in lipid mobilization was induced by the CR feeding regimen. Plasma FFA and glycerol concentrations were doubled in CR rats. In agreement with this observation, a recent study has reported that alternate-day feeding and fasting (a different methodology of dietary restriction) increased plasma FFA concentrations and adipose triglyceride lipolysis [30]. ATGL, a novel triglyceride lipase, is expressed at a high level in adipose tissue and predominantly expressed by adipocytes, although it was found at lower activity in other tissues [31]. However, the expression and activity of ATGL under prolonged CR regimen in animal models are largely unknown, although it was reported that a short period of CR (2 weeks) increased ATGL expression in white fat of gilts [32]. In the present study, CR induced a significant rise in ATGL mRNA levels in adipose tissue as well as in isolated adipocytes. This result suggests

that ATGL activation could be involved in CR-promoted lipid mobilization in adipose tissue during restricted energy intake thereby. Taken together, these observations suggest that CR improves metabolic health, at least in part, through the reduction of adiposity via increased lipid mobilization.

Adiponectin as a major adipokine plays a key role in metabolic health, and the present study showed that CR induced a twofold increase in adiponectin mRNA levels in epididymal adipose tissue. Adipose tissue is known to comprise adipocytes and SV fraction, which contains several cell types, such as preadipocytes, fibroblasts, vascular endothelial cells and macrophages [33,34]. Data from the current study demonstrated that adiponectin was predominantly expressed by isolated adipocytes and that CR induced more than twofold increase in adiponectin mRNA in adipocytes. Therefore, enhanced adiponectin mRNA in adipose tissue reflects the up-regulation of its expression by adipocytes under the CR feeding.

Since adiponectin is an adipocyte-secreted protein, the expression of its receptors in adipose tissue might mediate the paracrine effects of adiponectin within the tissue. Unlike adiponectin, CR feeding did not alter mRNA levels of either AdipoR1 or AdipoR2 in adipose tissue. The effect of fasting on gene expression of the two adiponectin receptors in adipose tissue has been shown to be inconsistent in rodents, being unchanged in rats [23,35] while decreased in mice [36]. In the present study, further analyses revealed that AdipoR1 was expressed equally in isolated adipocytes and in the SV fraction, whereas AdipoR2 was expressed mainly in adipocytes. It has been reported previously that AdipoR2 gene expression was increased, whereas AdipoR1 mRNA levels were unchanged during human adipocyte differentiation [22]. Somewhat similar to adiponectin, AdipoR2 is mainly expressed by adipocytes and appears to be the main receptor for transmitting adiponectin signaling in adipose tissue. However, consistent with the results in adipose tissue, no effects of CR feeding on mRNA levels of AdipoR1 or AdipoR2 in adipocytes were found. This suggests that elevated adiponectin expression induced by CR feeding could be more important as an endocrine signal to other organs rather than acting locally within adipose tissue.

In addition to increased adiponectin expression in adipose tissue, the present study showed that plasma adiponectin concentrations were markedly increased by CR feeding. This is in contrast to plasma insulin levels, which were significantly decreased, suggesting an increase in insulin sensitivity. This result raises the question of whether insulin is involved in the modulation of adiponectin production under CR feeding regimens. The chronic effect of insulin on adiponectin production is unknown, although acute administration of insulin decreased circulating adiponectin concentrations in humans [37–39]. Data from the present study demonstrated that chronic insulin infusion for 7 days reduced adiponectin mRNA levels in adipose tissue of CR rats; however, insulin infusion failed to reverse the stimulatory effect of CR on adiponectin plasma concentrations. This suggests that insulin may override the induction of adiponectin transcription by CR but has limited effects on protein translation and/or release. Alternatively, insulin did not affect the degradation or clearance of circulating adiponectin. In addition, under CR feeding conditions, enhanced effect of glucagon [40] may contribute to the rise of adiponectin, and a recent study has shown that glucagon receptor and glucagon-like peptide 1 receptor agonists increased plasma adiponectin concentrations in diet-induced obese mice with reduced insulin plasma levels [41]. Collectively, these results provide evidence that a CR-induced rise in circulating adiponectin is sustainable even when challenged by 7 days of insulin infusion. Therefore, the stimulatory effects of CR on adiponectin plasma concentrations would be important under the pathophysiological conditions of insulin resistance, such as obesity and aging.

In conclusion, this study demonstrated that CR significantly reduced adiposity and stimulated lipid mobilization in rats. We also show that CR increased expression of adiponectin in adipose tissue and isolated adipocytes as well as elevated circulating adiponectin concentrations. Both adiponectin receptors, AdipoR1 and AdipoR2, are present in adipose tissue, but AdipoR2 is primarily expressed by adipocytes. However, CR had no effect on gene expression for adiponectin receptors. Finally, 7 days of insulin infusion reduced gene expression of adiponectin but did not reverse the CR-induced rise in circulating adiponectin concentrations in CR rats. Our data support a role for CR in protecting against insulin resistance, at least partially, through lowering adiposity and the up-regulation of adiponectin generation.

Acknowledgments

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