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Age-specific effects of short- and long-term caloric restriction on the expression of adiponectin and adiponectin receptors: Influence of intensity of food restriction

Bernd Niemann [a,b], Rolf-Edgar Silber [b], Susanne Rohrbach [a,*]

[a] Institute of Pathophysiology, Martin Luther University Halle-Wittenberg, Ernst-Grube-Strasse 40, 06097 Halle, Germany
[b] Department of Cardiothoracic Surgery, Martin Luther University Halle-Wittenberg, Germany

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ABSTRACT

Hormonal signals from adipose tissue regulate energy homeostasis and may be involved in anti-aging effects of caloric restriction (CR). The adipokine adiponectin is abundantly expressed in adipose tissue and directly sensitizes the body to insulin. The purpose of the current study was to investigate age-dependent effects of different levels of CR (16%, 2 months or 40%, 6 months) on adiponectin and on its receptors AdipoR1 and AdipoR2 in the left ventricle (LV).

In young and senescent rats, 2 months of moderate CR reduced serum leptin. The same diet was sufficient to enhance serum adiponectin, adiponectin expression (visceral fat) and left ventricular AdipoR1 expression in young but not in senescent rats. The higher degree of CR, however, resulted in a mild induction of adiponectin expression in adipose tissue and release into plasma together with increased LV AdipoR1 also in old rats, while these effects were more pronounced in young rats. These changes in adiponectin activation were associated with reduced LV triglyceride content, suggesting an adiponectin-mediated reduced ectopic lipid deposition in nonadipose tissues. Thus, aging is associated with a loss of adiponectin inducibility by moderate CR. This reduction can only partially be overcome by increasing the degree and duration of CR.

1. Introduction

Adiponectin (also termed acrp 30, adipoQ, APM-1, GBP28) is an adipocytokine that is specifically and abundantly expressed in adipose tissue and directly sensitizes the body to insulin. Adiponectin structurally belongs to the complement 1q family (review in Kishore et al., 2004) and it exists in oligomeric forms: a low-molecular weight (LMW) trimer, a middle-molecular weight (MMW) hexamer, and high-molecular weight (HMW) 12- to 18-mer adiponectin, influencing its biological activity in vivo (Pajvani et al., 2003, 2004). The effects of adiponectin seem to be mediated by two distinct receptors: the adiponectin receptors AdipoR1, ubiquitously expressed with highest abundance in skeletal muscle, and AdipoR2, most abundantly expressed in liver (Yamauchi et al., 2003). AdipoR1 and AdipoR2 are integral membrane proteins with an internal N-terminus and an external C-terminus (Yamauchi et al., 2003). A reduction in the gene expression of these receptors has been described to be associated with diabetes and obesity, while fasting results in renormalization (Tsuchida et al., 2004). In addition, T-cadherin has been suggested to act as an adiponectin-binding protein or coreceptor for an as-yet-unidentified signaling receptor (Hug et al., 2004), since it lacks an intracellular domain. AdipoR1 and AdipoR2 serve as receptors for adiponectin and mediate increased activation of the AMP-activated protein kinase (AMPK), PPARα activities, fatty-acid oxidation, and glucose uptake by adiponectin (Yamauchi et al., 2003). Both globular and full-length adiponectin activate AMPK, an enzyme that is switched on by increases in levels of AMP, via increased phosphorylation at Thr-172 of the α-subunit. AMPK functions to restore ATP concentration by stimulating energy-producing processes such as fatty acid oxidation. Activation of AMPK by adiponectin was shown to increase phosphorylation of acetyl-coenzyme A carboxylase (ACC), fatty acid oxidation and glucose uptake via Glut 4, resulting in a decreased triglyceride content and increased insulin sensitivity (Yamauchi et al., 2002). Blockade of AMPK activation by use of a dominant-negative mutant inhibited these effects of full-length or globular adiponectin, indicating that stimulation of glucose utilization and fatty-acid oxidation by adiponectin occurs through activation of AMPK (Yamauchi et al., 2002).

Unlike other adipokines such as TNF-α and resistin, which cause insulin resistance, the expression and circulating levels of adiponectin are reduced in diabetic and obese state in animals as well as in humans. Hypoadiponectinemia has also been demonstrated to be independently associated with the metabolic syndrome,

* Corresponding author. Tel.: +49 345 5571454; fax: +49 345 5571404.
E-mail address: susanne.rohrbach@medizin.uni-halle.de (S. Rohrbach).

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stronger than any other inflammatory marker (Matsushita et al., 2006). Exogenous administration of adiponectin on the other hand improved insulin sensitivity while lowering blood glucose levels in high fat obese mice (Yamauchi et al., 2001). It has been suggested from animal studies that caloric restriction may accomplish the improved insulin sensitivity through modulation of adiponectin levels. Short-term and long-term caloric restriction (−40%) were reported to result in an increase in adiponectin plasma levels compared to age-matched ad libitum fed rats (Zhu et al., 2004) as well as in mice (Combs et al., 2003). Similarly, some studies in the human show improved insulin sensitivity together with increased adiponectin plasma concentration following weight reduction by hypocaloric diet (Polak et al., 2007) or by bariatric surgery (Guldstrand et al., 2003). Furthermore, adiponectin has been suggested to act as a peripheral "starvation" signal promoting the storage of triglycerides preferentially in adipose tissue thereby reducing ectopic lipid deposition in nonadipose tissue (Kim et al., 2007). As a consequence, reduced triglyceride levels in liver, skeletal muscle and heart may convey improved systemic insulin sensitivity and improve metabolic syndrome.

Fat cell derived hormones such as adiponectin or leptin can be envisioned as putative mediators of some of the protective effects of caloric restriction. Although long-term caloric restriction is known to retard the aging process in many organisms including mammals, the basic mechanisms of its efficacy remain unclear and its actions in different organs are remarkably heterogenous (Masoro, 2005). CR results also in a metabolic and transcriptional reprogramming of the adipose tissue and may represent a key mechanism influencing a variety of peripheral organs. While there are many data describing the protective effects of CR on whole body insulin sensitivity mainly related to effects on skeletal muscle physiology, much less is known about changes in cardiac metabolism and cardiac function after application of CR. The duration and intensity of this energy intake restriction without essential nutrient deficiency may have a major impact on this process. Since it is very difficult to differentiate basic primary mechanisms from secondary compensations on the basis of long-term effects of caloric restriction, we applied the lowest degree of restriction (−16% energy intake) and the shortest interval (2 months), at which protective actions have been described (Merry, 2002) in the first group of animals, while the second group of animals was treated for 6 months with a food restriction amounting to −40%. Following this treatment, we investigated the age-dependent effects of different degrees and durations of CR on the expression and release of adiponectin from adipose tissue, the expression of its receptors AdipoR1 and AdipoR2 in left ventricular tissue and the storage of triglycerides in peripheral, adiponectin-dependent organs (LV, liver).

2. Methods

2.1. Animals and diet protocol

In order to differentiate basic primary mechanisms from secondary compensations on the basis of long-term effects of caloric restriction, we first applied the lowest degree of restriction (−16% energy intake) and the shortest interval (2 months), at which protective actions have ever been analyzed in the literature in mammals (Merry, 2002). Ad libitum-fed male young and senescent Sprague–Dawley (SD) rats were obtained from Charles River (Germany), caged individually with a light/dark cycle of 12 h and had tap water ad libitum. Prior to the application of the diet protocols, daily food intake of the normal, ad libitum offered diet of each rat was monitored for 14 days and averaged. In those young rats (4 months at the beginning of the study) on control diet (Altromin^R 1344/1800; 1800 cal/g, CD) the daily energy intake was 52.5 ± 3.8 kcal, in young rats on −16% caloric restriction (Altromin^R 1344/1500; 1500 cal/g, CR) this daily intake was 45.2 ± 0.7 kcal. In old rats (22–24 months at the beginning of the study) the daily energy intake amounted to 46.4 ± 5.0 kcal in control diet and to 39.9 ± 0.2 kcal in −16% caloric restricted rats.

In a second group of animals, rats were randomly assigned to either receive control diet or a calorically restricted diet for 6 months. During the diet protocol, rats on control diet (Altromin^R 1344/2500; 2505 cal/g, CD) received their individual prediet average, but not more, in order to avoid any degree of diet-induced obesity. Rats subjected to caloric restriction received also their prediet average, but of a caloric reduced, fibre-rich diet (Altromin^R 1344/1500; 1500 cal/g; CR). Thus, in young rats (4 months at the beginning of the study) on control diet for 6 months the daily energy intake was 64.8 ± 2.2 kcal and in young rats on −40% caloric restriction this daily intake was 37.8 ± 1.9 kcal. In old rats (22–24 months at the beginning of the study) the daily energy intake amounted to 57.9 ± 1.8 kcal in control diet and to 36.6 ± 0.1 kcal in −40% caloric restricted rats. All animals (groups 1 and 2) were fasted for 12 h before killing and blood was obtained by aortic puncture. All treatments of animals throughout this study received local institutional animal care and use committee approval.

2.2. RNA extraction

Total RNA was isolated from frozen visceral fat and from left ventricular (LV) tissue by guanidine thiocyanate/cesium chloride centrifugation. Integrity and quality of the RNA was confirmed by agarose gel electrophoresis and the concentration determined by measuring UV-absorption.

2.3. RT-PCR

Reverse transcription (RT) of RNA samples was carried out for 30 min at 42 °C. Real-time PCR and data analysis were performed using the Mx3000P Multiplex Quantitative PCR System (Stratagene). DNA amplification was performed as follows: initial denaturation at 95 °C for 10 min, 40 cycles of amplification (denaturation at 95 °C for 30 s, annealing at 60 °C for 60 s, and extension at 72 °C for 60 s), followed by a denaturation at 95 °C for 60 s and a melting curve over the range from 55 °C up to 95 °C. Fluorescence data were collected at the end of the annealing stage of amplification. We performed Real-time PCR of adiponectin (forward 5′-AATCCTGCCCAGTCATGAAG-3′; reverse 5′-CATCTCCT GGGTCACCCTTA-3′), AdipoR1 (forward 5′-CTTCTACTGCTCCCCAC AGC-3′; reverse 5′-GACAAAGCCCTCAGCGATAG-3′), AdipoR2 (forward 5′-TACACACAGAGACGGGCAAC-3′; reverse 5′-GCAGTACA CCGTGTGGAAGA-3′) and 18S rRNA (18S rRNA Control kit, Yakima Yellow®–Eclipse® Dark Quencher, Eurogentec) in samples derived from rat adipose tissue or left ventricles. Each assay was performed in duplicate and validation of PCR-runs was assessed by evaluation of the melting curve. All data of mRNA are given as relative units of 18S rRNA concentrations.

2.4. Western blotting

Frozen LV tissue was rapidly homogenized in a buffer containing 50 mM Tris–HCl, 150 mM NaCl, 5 mM EDTA, 0.1% SDS, 1% sodiumdeoxycholate and protease inhibitor cocktail (Sigma). Proteins were quantified using the BCA Protein Assay (Pierce). About 50 μg of protein were loaded on a 10% SDS–PAGE gel. After electrophoresis, proteins were transferred to a nitrocellulose membrane. The filters were blocked and then incubated with antibodies directed against adiponectin (BioVendor, diluted 1:1000), AdipoR1, AdipoR2 (both Abcam, 1:1000, Vimentin (Sigma, 1:2000) as well as