Hyperadiponectinemia protects against premature death in metabolic syndrome model mice by inhibiting AKT signaling and chronic inflammation

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Abstract

We previously reported that transgenic (Tg) expression of adiponectin significantly prolonged the lifespan of normal mice. The aim of this study was to elucidate the mechanism involved in the longevity effects of adiponectin using KK/Ta mice, a murine model of metabolic syndrome. We established a Tg line of KK/Ta (Tg-KK/Ta) mice expressing human adiponectin in the liver, and assessed their lifespan. The cause of death was determined by macroscopic and microscopic examinations immediately after death. The expressions of SIRT1, C-reactive protein (CRP), inflammatory cytokines, AMPK, and AKT were measured by quantitative real-time PCR, ELISAs, and/or western blotting. KK/Ta mice had lower serum adiponectin levels and shorter lifespan (57.6 ± 13.9 vs 106.5 ± 18.3 weeks, P < 0.0001) than C57BL/6N mice. Tg adiponectin expression significantly extended the lifespan of KK/Ta mice (73.6 ± 16.6 weeks, P < 0.001) without affecting body weight, daily food consumption, or plasma glucose levels. Neoplasms were observed in only three of 22 KK/Ta mice that died spontaneously because of tumors. Atherosclerotic lesions were not detected in any mice. SIRT1 levels were not significantly different between KK/Ta and Tg-KK/Ta mice. Gene expressions of Crp, Tnfa, Il6, and Nfkb were increased in KK/Ta mice, but they were significantly attenuated in Tg-KK/Ta mice. Phosphorylated AMPK levels were increased and phosphorylated AKT levels were decreased in Tg-KK/Ta mice. The anti-inflammatory effects of adiponectin, achieved by inhibiting the AKT signaling pathway, may explain how adiponectin slows the accelerated aging process associated with the metabolic syndrome.

Introduction

Adiponectin, the most abundant adipokine in human plasma, has insulin-sensitizing and antiatherogenic effects (Ouchi et al. 1999, Okamoto et al. 2000, Goldstein & Scalia 2004). Unlike other adipokines, adiponectin secretion and its circulating levels are inversely proportional to body fat content (Arita et al. 1999). Thus, hypoadiponectinemia is closely associated with metabolic and cardiovascular disorders in metabolic syndrome (Hotta et al. 2000, Okamoto et al. 2000, Lindsay et al. 2002). We previously reported that transgenic (Tg) expression of human adiponectin in mice blocked excessive accumulation of subcutaneous and visceral fat, and prevented premature death, particularly that was induced by a high-fat/high-sugar diet (Otabe et al. 2007). Based on urinary 8-hydroxydeoxyguanosine (8-OHdG) levels, hyperadiponectinemia attenuated oxidative DNA damage induced by high-calorie diets, suggesting that the suppression of oxidative stress is involved in the longevity effect of adiponectin. However, many questions remain concerning the mechanism involved in the lifespan–extending effects of adiponectin, including the causes of death inhibited by adiponectin, the role of its anti-obesity effects, the involvement of chronic inflammation, and the possible effects of adiponectin on sirtuin levels. To answer these questions, we established KK/Ta mice expressing human adiponectin in the liver. The KK/Ta mouse is an animal model of metabolic syndrome exhibiting moderate obesity with excessive visceral fat accumulation, hyperinsulinemia, dyslipidemia and hyperglycemia (Liao et al. 2003, Tanimoto et al. 2004, Akagiri et al. 2008, Murakoshi et al. 2010). Here, we showed that hyperadiponectinemia prolonged the lifespan of this murine model of metabolic syndrome without affecting body weight by attenuating AKT signaling and chronic low-grade inflammation.
Materials and Methods

Animals

KK/Ta mice and C57BL/6N mice as a control were purchased from CLEA Japan (Tokyo, Japan). Tg mice bearing human adiponectin were generated as previously described (Otabe et al. 2007). For this study, we transferred the adiponectin transgene (line 11) to KK/Ta mice with repeated backcrosses for over 15 generations, and established a Tg line of KK/Ta (Tg-KK/Ta) mice. Male Tg-KK/Ta mice heterozygous for the transgene and non-Tg male KK/Ta mice were used in this study. All animals were maintained on regular mouse chow (CE-2; CLEA Japan; 352 kcal/100 g; 5% fat (fish oil and soy bean oil), 52% carbohydrates and 25% protein) and housed at 23 °C with a 12 h light:12 h darkness cycle. Body weight was measured twice weekly at 1100 h. At 10 weeks of age, each mouse (n=12 mice/group) was housed in an individual cage for 3 days, and food intake was measured during this time. All mice were treated in accordance with the guidelines for the care and use of laboratory animals of Kurume University School of Medicine based on the National Institutes of Health Guidelines.

Liver and fat weights and liver histology

Five male C57BL/6N, KK/Ta, and Tg-KK/Ta mice were killed under anesthesia at the age of 20 weeks, and the liver and epididymal fat were removed and weighed after obtaining blood samples. The livers were histologically examined with hematoxylin/eosin.

Cause of death analysis

Twenty male C57BL/6N mice, 22 male KK/Ta mice, and 22 male Tg-KK/Ta mice were monitored daily until they died spontaneously. Mice that stopped eating and were deemed likely to die were killed under anesthesia. The carcasses were covered with plastic sheets and placed on ice. After macroscopic observation, all organs, including the brain, were fixed in paraformaldehyde, embedded in paraffin, and sections were stained with hematoxylin/eosin. The cause of death for each mouse was determined by experienced pathologists following a standard protocol. A male KK/Ta mouse was killed under anesthesia at the age of 40 weeks, and the cerebrum, cerebellum, heart, thoracic aorta, liver, kidney and spleen were removed. The organs were histologically examined with hematoxylin/eosin, Elastica van Gieson and Congo-red staining to detect microscopic pathological changes, arteriosclerotic lesions, and amyloid depositions respectively.

Measurement of glucose, lipids and peptides

Glucose levels were measured by the glucose dehydrogenase method using a FreeStyle Meter (Nipro, Osaka, Japan). Total cholesterol and triglyceride levels were determined with DRI-CHEM 5500 (Fujifilm, Tokyo, Japan). Insulin, IGF1, and C-reactive protein (CRP) levels were measured using ELISA kits from Shibayagi (Gunma, Japan), R&D (Minneapolis, MN, USA) and Kamiya (Seattle, WA, USA).

Figure 1 (a) Mean body weight of male C57BL/6N (n=20, triangles), KK/Ta (n=22, open circles), and Tg-KK/Ta (n=22, closed circles) mice. Values are means ±S.D. *P<0.0001 vs C57BL/6N mice. There were no significant differences in body weight between KK/Ta and Tg-KK/Ta mice at any time. (b) Mean food intake in male C57BL/6N (n=12, closed column), KK/Ta (n=12, open column), and Tg-KK/Ta (n=12, shaded column) mice. Values are means ±S.D. NS, not significant. (c) Serum mouse adiponectin levels in male C57BL/6N (n=8, black column), KK/Ta (n=8, white column), and Tg-KK/Ta (n=8, dotted column) mice at the age of 10 weeks. Values are means ±S.D. The serum adiponectin level in male Tg-KK/Ta mice (n=8, grey column) at the age of 10 weeks was 53.5±26.8 μg/ml, which included 14.1±9.4 μg/ml HMW human adiponectin.
respectively. The serum levels of human and mouse adiponectin were measured using specific ELISAs for human adiponectin (Otsuka, Tokushima, Japan) and mouse adiponectin (AdipoGen, Seoul, Korea) respectively (Otabe et al. 2007). The high-, middle- and low-molecular-weight isoforms of circulating human adiponectin were analyzed using an enzyme immunoassay (EIA) kit (Adiponectin Multimetric EIA; Daiichi, Tokyo, Japan).

Insulin sensitivity and glucose tolerance

Insulin sensitivity and glucose tolerance were evaluated at the ages of 15 and 20 weeks respectively as previously described (Shimomura et al. 1998, Yamauchi et al. 2001). Insulin sensitivity was determined based on the decrease in plasma glucose after an i.p. injection of human insulin (0.75 U/kg body weight; Eli Lilly). Glucose tolerance was assessed using an intraperitoneal glucose tolerance test by injecting overnight-fasted mice with 2 g/kg glucose (10% glucose solution). Blood samples were obtained from the tail veins at 0, 30, 60, and 120 min after the glucose injection. To determine the areas under the curves, we calculated the areas defining the mean value at 0 min in C57BL/6N mice as zero.

Quantitative real-time PCR

Total RNA was obtained from isolated mouse liver, quadriceps muscle and epididymal fat using an RNA isolation kit (RNA-Bee, TEL-TEST, Inc., Friendswood, TX, USA) according to the manufacturer’s protocol. We used 1 μg of total RNA for reverse transcription using SuperScript III (Invitrogen). Quantitative real-time PCR was performed with 5 ng of cDNA and SYBR Green Master mix (Applied Biosystems, Tokyo, Japan) in a StepOne Plus Real Time PCR System (Applied Biosystems). The SYBR Green I Dye assay (Applied Biosystems) was used for specific gene amplification. The primer sequences for Ccr2 were 5'-GACTCG-TATGCCTGACTT-3' and 5'-AAACATTGCTGCTTGGCTTCAG-3'. The primers for Sirt1 (Ramadori et al. 2008) and Tnfα (Rinella et al. 2011) were as previously described. The primers for Il6 were 5'-CCCCATGGCCATTGCCACA-CAAC-3'. The primers for nuclear factor kappa b (Miyake) were 5'-CACCTAGCTGCCAAGGAAGG-3' and 5'-GCAGGGCTTTCATCGACTAC-3'. The primers for Cd68 antigen were 5'-CCAATTCAGGGTGGAA-3' and 5'-CTCGGGCTCTGATGTAGGTC-3'. The primers for Mcp1 were 5'-CCCCATGAG- TAGGCTTGAGA-3' and 5'-TCTGGACC- CATTTCTCTTGG-3'. Each sample was assayed in duplicate and negative controls were included in each experiment. Automatic relative quantification was performed using StepOne software version 2.1 (Applied Biosystems) and normalized for Gapdh expression.

Western blot analysis

Liver, muscle, and adipose tissues were lysed in ice-cold 0.5 mmol/l Tris–HCl buffer (pH 6.8) containing 2% SDS, 5% glycerol, and 0.05% bromophenol blue. Proteins were separated on 10% sodium dodecyl sulfate polyacrylamide gels and transferred to nitrocellulose membranes. After blocking for 1 h, the membranes were incubated with primary antibodies overnight at 4°C. The following primary antibodies were used: anti-Grp (1:1000, Abcam, Cambridge, UK), anti-Sirt1 (1:1000, Cell Signaling Technology, Danvers, MA, USA), anti-Tnfα (1:1000, Abcam), anti-Ill6 (1:1000, Abcam), anti-NFκB (1:1000, Cell Signaling Technology), anti-C68 (1:500, Abcam), anti-Mcp1 (1:1000, Abcam), and anti-Gapdh (1:1000, Santa Cruz Biotechnology, Dallas, TX, USA). Following a further incubation with secondary antibodies (1:20 000, Amersham, Buckinghamshire, UK) for 1 h, the membranes were developed using the enhanced chemiluminescence method (Amersham).

Figure 2 Kaplan–Meier survival analysis of male C57BL/6N (n=20, solid line), KK/Ta (n=22, dotted line), and Tg-KK/Ta (n=22, broken line) mice. The mean lifespan was significantly longer in Tg-KK/Ta mice than in KK/Ta mice (73.6±6.6 vs 57.6±13.9 weeks, P<0.001).

Figure 3 Elastica van Gieson staining of (a) the aortic valve and (b) the thoracic aorta from a 40-week-old male KK/Ta mouse. No atherosclerotic plaques were detected in the aortic valve or vessel wall.
6% β-mercaptoethanol, and 10% glycerol. The lysates were centrifuged at 20,000 g at 4°C for 30 min, and the supernatants were collected. After heating at 100°C for 5 min, the proteins were separated by 4–20% SDS–PAGE, and transferred onto nitrocellulose membranes. The membranes were incubated overnight at 4°C with antibodies (Cell Signaling, Danvers, MA, USA) specific for mouse Sirt1, total AMP-activated protein kinase α (AMPK), phosphorylated AMPK (p-AMPK) α (Thr172), total AKT, p-AKT (Ser473), GAPDH and/or β-actin. After washing, the membranes were incubated with peroxidase-conjugated goat anti-rabbit IgG (Wako, Osaka, Japan), and visualized using an ECL system (GE Healthcare Bio-Sciences, Piscataway, NJ, USA). Densitometry was performed using ImageJ software (National Institutes of Health, Bethesda, MD, USA).

**TNF-α, IL6 and 8-OHdG measurements**

Serum TNF-α and IL6 levels were measured using a Mouse TNF-α ELISA kit from Shibayagi and a Mouse IL6 Immunoassay kit from Biosource International (Camarillo, CA, USA) respectively. The urine levels of 8-OHdG were measured using a competitive ELISA kit from Shibayagi and a Mouse IL6 ELISA kit (Shibayagi). Serum IL-6 and 8-OHdG measurements were performed using a protease that selectively digests low-molecular-weight and middle-molecular-weight adiponectin. Key enzyme showed that the concentration of the high-molecular-weight isoform of human adiponectin was 141.1 ± 9.4 μg/ml.

**Statistical analysis**

Data are expressed as means and s.d. Comparisons between two groups were performed with the Wilcoxon–Kruskal–Wallis test, followed by the Bonferroni post hoc test when significance was observed in two or more groups were analyzed. Survival rates were compared between two groups (KK/Ta vs C57BL/6N and Tg-KK/Ta vs KK/Ta mice) using the Kaplan–Meier log-rank method. Values of *P* < 0.05 were considered statistically significant. All analyses were performed using StatView software (SAS Institute, Cary, NC, USA).

**Results**

The body weights of KK/Ta mice and Tg-KK/Ta mice were greater than those of normal C57BL/6N mice (Fig. 1a). However, no significant differences were observed in body weight between KK/Ta and Tg-KK/Ta mice up to the age of 45 weeks. We also measured liver and epididymal fat weights in each group at the age of 20 weeks. The liver and epididymal fat weights of KK/Ta mice (1.77 ± 0.28 and 1.38 ± 0.36 g, *n* = 6) and Tg-KK/Ka mice (1.75 ± 0.25 and 1.30 ± 0.45 g, *n* = 6) were both significantly greater than those of C57BL/6N mice (1.33 ± 0.22 and 0.87 ± 0.15 g, *n* = 6, *P* < 0.01). However, there were no significant differences in liver or epididymal fat weights between KK/Ta and Tg-KK/Ta mice. KK/Ka and Tg-KK/Ka mice consumed more food than C57BL/6N mice (Fig. 1b), but there was no difference in daily food intake between KK/Ta and Tg-KK/Ta mice.

Table adiponectin levels were significantly lower in KK/Ta mice than in C57BL/6N mice at the age of 10 weeks (29.4 ± 3.9 vs 37.0 ± 1.6 μg/ml, *n* = 8, Fig. 1c). However, Tg-KK/Ta mice had higher mouse adiponectin (57.3 ± 16.5 μg/ml) and human adiponectin levels (53.5 ± 26.8 μg/ml, *n* = 8). An EIA using a protease that selectively digests low-molecular-weight and middle-molecular-weight adiponectin showed that the concentration of the high-molecular-weight isoform of human adiponectin was 141.1 ± 9.4 μg/ml.

Survival rate analysis revealed that the lifespan was significantly shorter in KK/Ta mice than in C57BL/6N mice (57.6 ± 13.9 vs 106.5 ± 18.3 weeks, *P* < 0.0001, Fig. 2). However, Tg adiponectin expression protected Tg-KK/Ta from premature death by significantly increasing their lifespan (73.6 ± 16.6 weeks) compared with KK/Ta mice (*P* < 0.001). Neoplastic lesions were detected in only three of 22 KK/Ta mice: two of these mice had malignant lymphomas; and one mouse had a hepatic hemangiomatous tumor. Other than mild fatty liver, no pathological lesions were observed in the heart, liver, pancreas, kidney, lung or brain in the remaining 19 mice. Elastica van Gieson staining showed no atherosclerotic plaques in the aortic valves or vessel wall of the thoracic aorta (Fig. 3a and b). Congo-red staining showed no amyloid deposition in any organ. The livers obtained from KK/Ta and Tg-KK/Ta mice at the age of 20 weeks showed no histological changes consistent with inflammation. KK/Ta mice had significantly higher levels of insulin and IGF1 than C57BL/6N mice. The elevated levels of serum insulin and IGF1 in KK/Ta mice were attenuated in Tg-KK/Ta mice, although the differences were not statistically significant (Table 1). No significant differences were observed

| Table 1 Effects of Tg adiponectin expression on fasting blood glucose, insulin, IGF1, triglyceride, and total cholesterol levels |
|---------------------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
| Fasting blood glucose (mg/dl)  |
| C57BL/6N                      | 78.4 ± 7.6        | 268.3 ± 153.6     | 218.5 ± 11.7      | 85.0 ± 20.1       | 84.7 ± 16.0       |
| KK/Ta                         | 83.5 ± 14.2       | 1528.3 ± 2028.5*  | 274.1 ± 16.0*     | 95.7 ± 3.1        | 145.0 ± 100.4     |
| Tg-KK/Ta                      | 70.3 ± 11.5       | 744.2 ± 776.5     | 238.9 ± 20.2      | 84.7 ± 14.6       | 102.7 ± 9.8       |

*P* < 0.05 (KK/Ta vs C57BL/6N).
in glucose, triglyceride or total cholesterol concentrations among KK/Ta, Tg-KK/Ta and C57BL/6N mice. The basal blood glucose levels were not significantly different among KK/Ta, Tg-KK/Ta and C57BL/6N mice at the age of 20 weeks. However, blood glucose levels at 60 and 120 min after the i.p. glucose load were significantly higher in KK/Ta and Tg-KK/Ta mice than in C57BL/6N mice (Fig. 4a). Furthermore, the serum levels of CRP in KK/Ta mice (1.37 ± 0.75 ng/ml, n = 8) tended to be higher than those in age-matched male Tg-KK/Ta mice (1.02 ± 0.38 ng/ml, n = 8) and C57BL/6N mice (1.03 ± 0.48 ng/ml, n = 8).

Moreover, intraperitoneal insulin tolerance tests showed no significant differences in insulin sensitivity among C57BL/6N, KK/Ta and Tg-KK/Ta mice at the age of 15 weeks (Fig. 4b). Adipose tissue Tnfa, Il6 and Nfkb mRNA expression levels in KK/Ta mice at the age of 20 weeks (n = 5) were significantly greater than those in age-matched male Tg-KK/Ta mice (n = 5) and C57BL/6N mice (n = 5) (Fig. 7 a, b and c). To investigate whether macrophages were significantly elevated in KK/Ta and Tg-KK/Ta mice compared with wild-type C57BL/6N mice at the age of 20 weeks (Fig. 5a and b). However, there were no significant differences in Sirt1 mRNA or SIRT1 protein levels between KK/Ta and Tg-KK/Ta mice. In contrast, the upregulated hepatic Crp mRNA expression in KK/Ta mice was significantly lower in Tg-KK/Ta mice than in age-matched male C57BL/6N mice (Fig. 6). Furthermore, the serum levels of Sirt1 mRNA expression in the liver, quadriceps muscle and white adipose tissue, and SIRT1 protein levels in the liver and muscle were significantly elevated in KK/Ta and Tg-KK/Ta mice compared with wild-type C57BL/6N mice at the age of 20 weeks. However, there were no significant differences in Sirt1 mRNA or SIRT1 protein levels between KK/Ta and Tg-KK/Ta mice. In contrast, the upregulated hepatic Crp mRNA expression in KK/Ta mice was significantly lower in Tg-KK/Ta mice than in age-matched male C57BL/6N mice (Fig. 6). Furthermore, the serum levels of CRP in KK/Ta mice (1.37 ± 0.75 ng/ml, n = 8) tended to be higher than those in age-matched male Tg-KK/Ta mice (1.02 ± 0.38 ng/ml, n = 8) and C57BL/6N mice (1.03 ± 0.48 ng/ml, n = 8).

Adipose tissue Tnfa, Il6 and Nfkb mRNA expression levels in KK/Ta mice at the age of 20 weeks (n = 5) were significantly greater than those in age-matched male Tg-KK/Ta mice (n = 5) and C57BL/6N mice (n = 5) (Fig. 7 a, b and c). To investigate whether macrophages
infiltrating into fat tissue are responsible for the high plasma adiponectin levels, we determined the mRNA expression levels of \( Cd68 \) antigen and \( Mcp1 \) in visceral fat tissue as macrophage markers. \( Cd68 \) antigen mRNA expression was significantly increased in KK/Ta \((n=5)\) and Tg-KK/Ta \((n=5)\) mice at the age of 20 weeks compared with those in age-matched male C57BL/6N mice \((n=5)\). However, there was no difference in \( Cd68 \) antigen mRNA expression between KK/Ta and Tg-KK/Ta mice (Fig. 7d). \( Mcp1 \) mRNA expression in visceral fat tissue was not significantly different among the C57BL/6N, KK/Ta, and Tg-KK/Ta mice at the age of 20 weeks \((n=5)\) (Fig. 7e).

Serum levels of TNF-\( \alpha \) and IL6, and urine levels of 8-OHdG tended to be higher in KK/Ta mice at the age of 20 weeks \((n=5)\) than in C57BL/6N mice at the same age \((n=5)\). By contrast, their levels were lower in Tg-KK/Ta mice \((n=5)\) than in KK/Ta mice (Fig. 8a, b and c).

The male C57BL/6N and KK/Ta mice at the age of 20 weeks had similar p-AMPK/total AMPK protein level ratios in the liver and heart. However, p-AMPK/total AMPK protein level ratios in the liver and heart were upregulated in age-matched male Tg-KK/Ta mice compared with the other two groups (Fig. 9a). In contrast, p-AKT/total AKT protein level ratios in these organs were lower in Tg-KK/Ta mice than in C57BL/6N and KK/Ta mice (Fig. 9b).

**Discussion**

The inbred KK/Ta mouse strain spontaneously develops abdominal obesity, glucose intolerance, hyperinsulinemia, dyslipidemia and hypoadiponectinemia (Nakamura & Yamada 1967, Ikeda 1994). Furthermore, we have found that the lifespan of KK/Ta mice was much shorter than that of control mice. These phenotypes prompted us to use the KK/Ta mouse as a model for the metabolic syndrome. We

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**Figure 6** Crp mRNA expression in the liver of male C57BL/6N \((n=8, \text{closed column})\), KK/Ta \((n=8, \text{open column})\) and Tg-KK/Ta \((n=8, \text{shaded column})\) mice. Values are means ± s.d.

**Figure 7** mRNA expression of (a) Tnf-\( \alpha \), (b) Il6, (c) Nfkb, (d) Cd68 antigen and (e) Mcp1 in adipose tissues of male C57BL/6N \((n=5, \text{closed column})\), KK/Ta \((n=5, \text{open column})\) and Tg-KK/Ta \((n=5, \text{shaded column})\) mice. Values are means ± s.d.
previously established Tg mice expressing full-length human adiponectin in the liver on a C57BL/6N genetic background (Otabe et al. 2007). For this study, we repeatedly backcrossed the adiponectin Tg mice with KK/Ta mice for over 15 generations and generated a Tg line on a KK/Ta genetic background (i.e. Tg-KK/Ta mice). Despite abdominal obesity, the Tg-KK/Ta mice exhibited hyperadiponectinemia, including the high-molecular-weight form, which binds most strongly to its receptors and stimulates AMPK (Waki et al. 2003).

Here, we showed that Tg-KK/Ta mice had a significantly longer lifespan than KK/Ta mice. In our previous study (Otabe et al. 2007), hepatic Tg adiponectin expression was associated with reduced weight gain. However, we found no difference in body weight between the Tg-KK/Ka and KK/Ta mice. In fact, the Tg-KK/Ta mice were slightly heavier, although this difference was not statistically significant. In our previous study (Otabe et al. 2007), C57BL/6N mice overexpressing adiponectin showed reduced body weight under a high-calorie diet condition, whereas the body weight of mice in the current study was not affected by adiponectin overexpression. In the former study, the body weight reduction in C57BL/6N mice was possibly due to an increase in physical activity. In the current study, we used KK/Ka mice, which have different genetic background from C57BL/6N, and are spontaneously obese and innately inactive (Matsumoto & Shino 1972, Taketomi et al. 1982, 1988, Ikeda 1994). Therefore, Tg-KK/Ka mice did not show a significant reduction in body weight, unlike the previous study in C57BL/6N mice. Moreover, the finding that body weight was not significantly different between KK/Ta and Tg-KK/Ka mice suggests that the longevity effect of adiponectin is not mediated by a reduction in body weight. Similarly, the beneficial effect of adiponectin was not attributable to reduced food intake, even though calorie restriction is the most widely recognized lifespan-extending intervention in a variety of organisms (Guarente & Picard 2005, Kenyon 2005).

Neoplasms and cardiovascular disease are the two main causes of death associated with the metabolic syndrome in humans. To test the hypothesis that adiponectin inhibits the development of neoplasms, and thereby prevents premature death in KK/Ta mice, we autopsied 22 male KK/Ta mice. Unexpectedly, neoplasms were found in only three of 22 KK/Ta mice that spontaneously died. Although malignant neoplasms are common causes of spontaneous death in old normal mice (Cameron et al. 1985), the longevity effect of adiponectin in the metabolic syndrome mice was unlikely to be mediated by the inhibition of tumorigenesis. Notably, there were no marked pathological changes that may have caused death in any of the organs, including the heart, liver, pancreas, kidney, lung and brain, in the remaining mice. Similarly, no atherosclerotic changes were observed in the aortic wall or in the cardiac valves. Thus, unlike human metabolic syndrome, malignant neoplasms and cardio- and cerebrovascular disorders were not the major causes of death in these KK/Ta mice. Furthermore, we found no atherosclerotic plaques and no amyloid deposition in any of the organs examined in the male KK/Ta mouse killed at the age of 40 weeks. Therefore, these findings suggest that there are

Figure 8 (a) Serum TNF-α, (b) serum IL6 and (c) urinary 8-OHdG levels in male C57BL/6N (n=5, closed column), KK/Ta (n=5, open column) and Tg-KK/Ta (n=5, shaded column) mice. Values are means ± S.D.

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still unknown causes of death, other than histochemically detectable pathological abnormalities, that might be responsible for the accelerated aging process in KK/Ta mice.

Genes related to yeast silent information regulator 2 (Sir2) encode NAD-dependent deacetylases and promote longevity in yeast, worms and flies (Chen & Guarente 2007). The mammalian Sir2 ortholog Sirt1 targets numerous regulatory factors that affect stress management and metabolism (Chen & Guarente 2007). Sirt1 levels are increased in response to calorie restriction, the most consistent nonpharmacological intervention to increase lifespan and protect against deteriorations in biological functions in many key metabolic tissues (Cohen et al. 2004, Nisoli et al. 2005, Rodgers et al. 2005, Gerhart-Hines et al. 2007, Chen et al. 2008). Hence, we determined the mRNA expression of Sirt1 in the major organs. We found that Sirt1 expression was elevated in KK/Ta mice, and there were no significant differences in its expression between KK/Ta and Tg-KK/Ta mice. These observations suggest that the beneficial effects of adiponectin are not mediated by upregulation of Sirt1 expression, although sirtuin activity was not measured in this study.

Next, we compared the hepatic expression of Cyp among the three groups of mice because several recent studies have shown that chronic low-grade inflammation is a major risk factor for age-related diseases. CRP is the most frequently measured marker for systemic inflammation and is independently associated with total body fat, central fat and insulin resistance (Festa et al. 2001, Pannacciulli et al. 2001, Lapice et al. 2009). We found that hepatic Cyp expression was increased in KK/Ta mice, and this was attenuated by adiponectin. Thus, the anti-inflammatory effects of adiponectin may be associated with reduced DNA damage and slowing of the aging process in adiponectin Tg mice.

Although the gene expression levels of Tnfα, Il6 and Njkβ, which encode major inflammatory cytokines, were increased in adipose tissues in KK/Ta mice, these levels were attenuated in Tg-KK/Ka mice. As hyperadiponectinemia did not influence the gene expression of Cdx68 antigen or Mip1, two macrophage makers, adiponectin might exert anti-inflammatory effects in a pathway independent of macrophages. In addition, serum TNF-α, serum IL6, and urinary 8-OHdG levels tended to be lower in Tg-KK/Ka mice than in KK/Ta mice. This might be because hyperadiponectinemia causes a sustained, but subtle decline in the serum levels of major inflammatory cytokines and oxidative stress. Taken together, the significant reduction in histological inflammatory factors and/or chronic low-grade inflammation in terms of serum cytokines might facilitate lifetime extension in Tg-KK/Ka mice.

The anti-inflammatory effects of adiponectin may be attributable to the reduced phosphorylation of AKT, because AKT is involved in the activation of NF-κB by TNF-α, following the activation of PI3K (Ozes et al. 1999). AKT phosphorylation may be decreased by p-AMPK, probably via inhibition of PI3K. Furthermore, the PI3K/AKT signaling pathway was implicated in the initiation and maintenance of several cancers (Yuan & Cantley 2008). Thus, the reduced phosphorylation of AKT may prevent cancer-related death, although we could not determine the possible anticancer effects of adiponectin using these model mice, which died before cancer could develop.

In conclusion, the lifespan of a murine model of metabolic syndrome was shorter than that of control mice. Adiponectin prevented premature death in these mice without affecting body weight or daily food consumption. The longevity effect of adiponectin was not attributable to the prevention of neoplasms or cardiovascular disease, nor to upregulated Sirt1 expression. The observations in this study suggest that adiponectin may slow the accelerated aging process associated with the metabolic syndrome by inhibiting AKT signaling, thereby attenuating chronic low-grade inflammation.
Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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References


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