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The phytochemical, EGCG, extends lifespan by reducing liver and kidney function damage and improving age-associated inflammation and oxidative stress in healthy rats

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Summary

It is known that phytochemicals have many potential health benefits in humans. The aim of this study was to investigate the effects of long-term consumption of the phytochemical, epigallocatechin gallate (EGCG), on body growth, disease protection, and lifespan in healthy rats. 68 male weaning Wistar rats were randomly divided into the control and EGCG groups. Variables influencing lifespan such as blood pressure, serum glucose and lipids, inflammation, and oxidative stress were dynamically determined from weaning to death. The median lifespan of controls was 92.5 weeks. EGCG increased median lifespan to 105.0 weeks and delayed death by approximately 8–12 weeks. Blood pressure and serum glucose and lipids significantly increased with age in both groups compared with the levels at 0 week. However, there were no differences in these variables between the two groups during the whole lifespan. Inflammation and oxidative stress significantly increased with age in both groups compared with 0 week and were significantly lower in serum and liver and kidney tissues in the EGCG group. Damage to liver and kidney function was significantly alleviated in the EGCG group. In addition, EGCG decreased the mRNA and protein expressions of transcription factor NF-κB and increased the upstream protein expressions of silent mating type information regulation two homolog one (SIRT1) and forkhead box class O 3a (FOXO3a). In conclusion, EGCG extends lifespan in healthy rats by reducing liver and kidney damage and improving age-associated inflammation and oxidative stress through the inhibition of NF-κB signaling by activating the longevity factors FoxO3a and SIRT1.

Key words: EGCG; inflammation and oxidative stress; lifespan; liver and kidney; Phytochemicals.

Introduction

The associations between phytochemicals and health have involved heated discussions in recent years. Numerous clinical trials and animal studies have demonstrated that biologically active phytochemicals provide numerous health benefits including anti-inflammation, antioxidative stress, lowering body weight, blood pressure, serum glucose and lipids, strengthening immunity, and increasing lifespan (Holst & Williamson, 2008; Manach *et al.*, 2009; Krzyzanowska *et al.*, 2010; Scalbert *et al.*, 2011). However, these studies mainly focused on the effects of phytochemicals on human disease status and animal disease models (Valenzano *et al.*, 2006; Sun *et al.*, 2010). Few studies have investigated the effects of phytochemicals during the lifespan of healthy individuals. It is well known that nutrients are essential for normal body function and maintenance of health (Saito 2007); however, it is unknown whether phytochemicals are also essential during the course of lifespan. In addition, the effects of phytochemicals on body growth, health maintenance, illness or disease protection, and human lifespan remain to be clarified.

Epigallocatechin gallate (EGCG), the main and the most important polyphenol in green tea, is a common phytochemical that is claimed to have many potential health benefits (Nagle *et al.*, 2006; Suzuki *et al.*, 2012). In this study, we selected EGCG as a representative phytochemical and investigated its potential effects on health outcomes, including lifespan, body weight, blood pressure, serum glucose and lipids, inflammation and oxidative stress levels, and both liver and kidney function variables, during the whole lifespan of healthy rats.

Results

Lifespan, body weight, and food consumption

Figure 1A shows that the survival curves of the control and EGCG groups began to diverge at 55 weeks and remained separated. When the experiment ended at 108 weeks, 70.6% of the control animals had died (median lifespan 92.5 weeks), compared with 55.9% of the EGCG group (median lifespan 105 weeks), and the cumulative survival reached statistical significance ($P = 0.023$) (Fig. 1A). Although we did not observe the time of death in all animals, deaths in the EGCG group were delayed by approximately 8–12 weeks compared with the control group, and Cox proportional hazards regression showed that EGCG reduced the risk of death by 44% compared with the controls (hazard ratio = 0.56, $\chi^2 = 4.412$, $P = 0.036$).

The body weight of all rats in the two groups increased steadily up to 70 weeks, and then slowly declined (Fig. 1B). Although rats in the EGCG group were slightly lighter than the controls after 40 weeks, there was no significant difference in body weight between the two groups. There was also no difference in food consumption between the groups (Fig. 1C). These results suggest that increased lifespan following EGCG administration may not be due to changes in body weight or food intake.

Blood pressure, heart rate, serum glucose and lipids

We dynamically determined blood pressure, heart rate, serum glucose and lipids at 0, 18, 36, 54, 72, 90, and 108 weeks. Two-way analysis of variance showed that blood pressure, heart rate, serum glucose and

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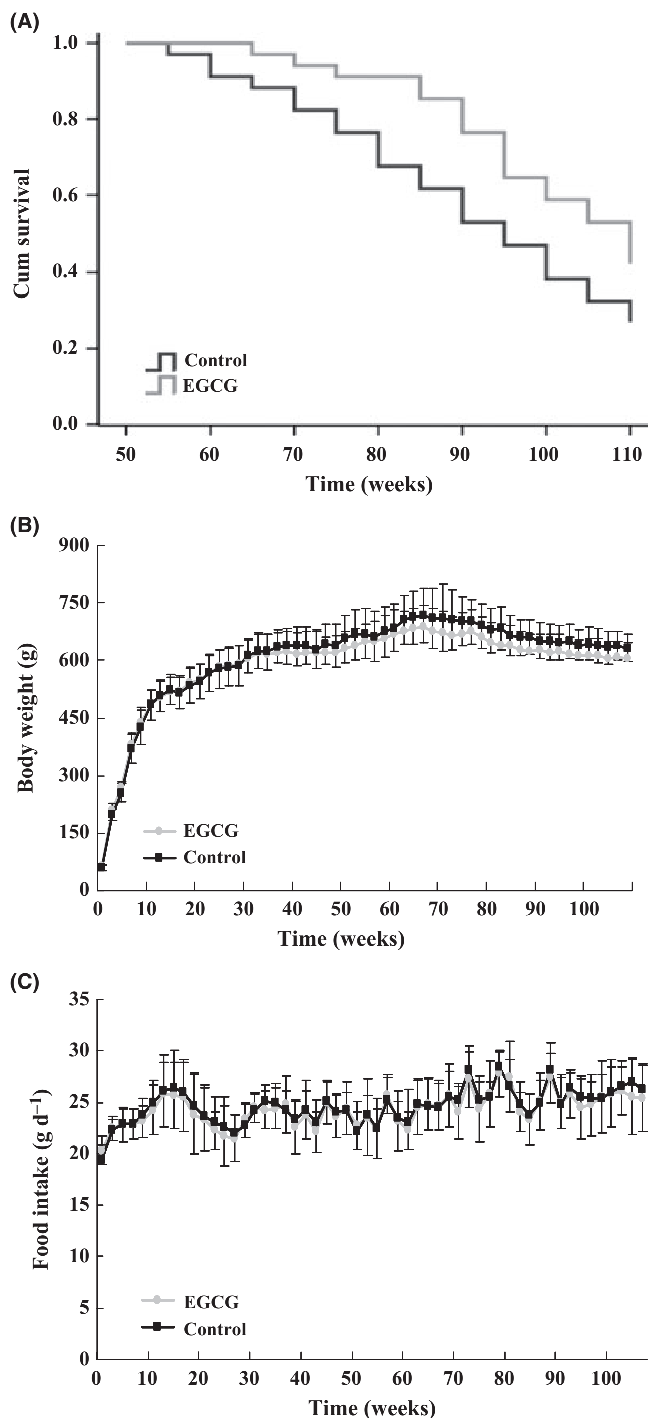

Fig. 1 Effects of EGCG on lifespan, body weight, and food intake in healthy rats. 68 male Wistar rats were randomly divided into the control group (n = 34) and EGCG group (25 mg kg^{-1}day^{-1}, n = 34). The experiment lasted for 108 weeks. (A) Kaplan–Meier survival curves. Hazard ratio for the EGCG group was 0.56 (χ^2 = 4.412, P = 0.036). (B) Body weight. (C) Food intake. Data are presented as means ± SD, $P < 0.05$ was considered significant.

lipids, angiotensin-converting enzyme (ACE), and nitric oxide (NO) levels yielded a significant main effect for time ($P < 0.05$) (Table 1). EGCG tended to decrease systolic blood pressure (SBP) and ACE, total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), and serum glucose (GLU) levels and increased the levels of NO and high-density lipoprotein cholesterol (HDL-C), especially in the late phase of the experiment. However, there were no differences in these variables at all time points compared with the control group (Table 1). Therefore, these data suggest that long-term consumption of EGCG may not influence blood pressure, serum glucose and lipids in healthy rats.

Blood biochemistry variables related to liver and kidney function

The levels of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), creatinine (CRE), and blood urea nitrogen (BUN) significantly increased with age, which suggested that damage to liver and kidney function was aggravated (Table 2). Compared with the controls, most of these age-associated variables were lower in the EGCG group. Compared with the control group, variables in the EGCG group were statistically different with duration of treatment: serum ALT (from 54 weeks), AST (from 72 weeks), ALP (108 weeks), serum CRE (from 72 weeks), and BUN (from 90 weeks) ($P < 0.05$). These results indicate that EGCG alleviated liver and kidney function damage during the whole lifespan.

Necropsy of dead rats and liver and kidney pathology

On necropsy, we found that all rats had one or more lesions, and most of the dead rats had pale, swollen, and obvious local necrosis in the liver, and the kidneys had slight atrophy and hyperemia in both groups. In the controls, the renal tubules showed epithelial swelling and glomerular swelling with a reduction in Bowman's capsular space, infiltration of numerous inflammatory cells, and fibrous tissue proliferation in the renal interstitium. However, only mesangial proliferation was observed in the EGCG group (Fig. 2A–C). In addition, EGCG significantly decreased the tubular injury score of kidney pathology. Infiltration of inflammatory cells, small focal necrosis, and cell pyknosis and apoptosis were found in the liver histology of controls. However, only liver cell edema occurred in the EGCG group. EGCG significantly decreased the HAI score in liver pathology (Fig. 2D–F). These results also indicate that EGCG alleviated liver and kidney function damage, especially infiltration of inflammatory cells.

Inflammation and oxidative stress levels in serum, liver, and kidney tissues

With increasing age, the levels of reactive oxygen species (ROS), malondialdehyde (MDA), TNF-α, and IL-6 significantly increased ($P < 0.05$), while the levels of serum superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) significantly decreased in both the control and experimental groups ($P < 0.05$) (Table 2). EGCG significantly decreased the levels of serum TNF-α, IL-6 (from 72 weeks), ROS (from 54 weeks), and MDA (from 90 weeks), and increased the levels of SOD and GSH-Px (from 72 weeks) compared with the control group ($P < 0.05$) (Table 2). Similar effects on these variables were also found in the liver and kidney tissues of rats treated with EGCG (Fig. 3). These data indicate that EGCG improved inflammation and oxidative stress compared with the controls.

mRNA levels of inflammatory and oxidative stress markers in liver and kidney tissues

To further validate the effects of EGCG on improving oxidative damage and chronic inflammation, the mRNA levels of CuZnSOD, MnSOD,

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Table 1 Effects of EGCG on blood pressure, heart rate, serum glucose and lipids in healthy rats

Variable	Group (n = 10)	Weeks 0	18	36	54	72	90	108	*P* value (time)
SBP (mmHg)	Control	99.8 ± 10.9	104.4 ± 12.2	106.6 ± 9.3	109.3 ± 12.9	117.9 ± 13.5$^{\#}$	123.8 ± 15.7$^{\#\#}$	132.3 ± 19.9$^{\#\#}$	0.004
	EGCG	100.7 ± 13.1	106.2 ± 11.7	108.8 ± 15.6	116.2 ± 14.4	110.7 ± 12.9	119.1 ± 14.4	124.5 ± 16.2	
HR (beats min^{-1})	Control	335.4 ± 28.2	328.1 ± 27.8	337.2 ± 24.3	339.6 ± 30.1	345.7 ± 39.7	357.6 ± 33.4	370.6 ± 38.3$^{\#}$	0.003
	EGCG	332.3 ± 27.6	333.2 ± 29.1	343.5 ± 41.6	342.2 ± 27.5	341.6 ± 35.2	352.5 ± 36.1	359.2 ± 40.7	
ACE (pg mL^{-1})	Control	93.7 ± 10.8	95.9 ± 9.6	104.2 ± 14.7	106.8 ± 15.8	101.8 ± 17.0	100.4 ± 16.1	107.9 ± 16.2$^{\#}$	0.036
	EGCG	92.5 ± 10.1	91.6 ± 10.9	95.4 ± 16.5	99.5 ± 15.4	96.9 ± 15.1	100.5 ± 16.2	102.2 ± 15.3	
NO (μmol L^{-1})	Control	175.2 ± 16.2	166.3 ± 15.3	162.1 ± 15.5	157.3 ± 13.8$^{\#}$	148.3 ± 15.0$^{\#\#}$	140.1 ± 14.4$^{\#\#}$	136.3 ± 12.8$^{\#\#}$	<0.001
	EGCG	176.4 ± 15.9	169.9 ± 14.2	165.6 ± 13.8	160.1 ± 15.6	156.8 ± 15.1	147.8 ± 13.5	141.7 ± 16.4	
GLU (mmol L^{-1})	Control	4.09 ± 0.78	4.43 ± 0.85	4.68 ± 0.74	4.76 ± 0.77	4.89 ± 0.76	5.01 ± 0.95$^{\#}$	5.16 ± 1.02$^{\#}$	<0.001
	EGCG	4.03 ± 0.76	4.23 ± 0.90	4.47 ± 0.88	4.62 ± 0.95	4.85 ± 0.84	4.92 ± 1.04	4.99 ± 0.98	
TC (mmol L^{-1})	Control	2.10 ± 0.31	2.22 ± 0.42	2.44 ± 0.56	2.75 ± 0.66$^{\#}$	3.30 ± 1.04$^{\#\#}$	3.76 ± 1.06$^{\#\#}$	4.13 ± 1.15$^{\#\#}$	<0.001
	EGCG	2.05 ± 0.36	2.16 ± 0.35	2.59 ± 0.47	2.61 ± 0.77	2.97 ± 0.71	3.72 ± 0.97	4.04 ± 1.03	
TG (mmol L^{-1})	Control	0.98 ± 0.21	1.06 ± 0.17	1.04 ± 0.21	1.18 ± 0.29	1.25 ± 0.37	1.41 ± 0.49$^{\#}$	1.65 ± 0.63$^{\#\#}$	<0.001
	EGCG	1.00 ± 0.18	1.02 ± 0.24	1.08 ± 0.38	1.17 ± 0.30	1.27 ± 0.41	1.36 ± 0.58	1.58 ± 0.46	
HDL-C (mmol L^{-1})	Control	2.29 ± 0.47	2.23 ± 0.63	2.18 ± 0.60	1.93 ± 0.33	1.96 ± 0.52	1.79 ± 0.34$^{\#}$	1.64 ± 0.44$^{\#\#}$	<0.001
	EGCG	2.31 ± 0.42	2.20 ± 0.53	2.29 ± 0.67	2.02 ± 0.48	2.04 ± 0.62	1.85 ± 0.39	1.79 ± 0.37	
LDL-C (mmol L^{-1})	Control	0.87 ± 0.10	0.84 ± 0.15	0.98 ± 0.16	1.00 ± 0.22	1.06 ± 0.31	1.17 ± 0.25$^{\#\#}$	1.28 ± 0.46$^{\#\#}$	<0.001
	EGCG	0.83 ± 0.12	0.88 ± 0.19	1.02 ± 0.21	0.97 ± 0.25	0.99 ± 0.29	1.12 ± 0.34	1.15 ± 0.29	

SBP, systolic blood pressure; HR, heart rates; ACE, angiotensin-converting enzyme; NO, nitric oxide; TC, total cholesterol; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; GLU, serum glucose.

All values are mean ± SD.

$^{\#}P < 0.05$ or $^{\#\#}P < 0.01$ vs. 0 week. *P* value in the last column is the statistical results of two-factor repeated measures ANOVA over time.

Table 2 Effects of EGCG on blood biochemistry characteristics in healthy rats

Variable	Group (n = 10)	Weeks 0	18	36	54	72	90	108	*P* value (time)
ALT (U L^{-1})	Control	34.2 ± 4.7	38.5 ± 5.3	45.3 ± 6.3$^{\#}$	59.2 ± 8.9$^{\#\#}$	81.8 ± 11.2$^{\#\#}$	88.9 ± 13.1$^{\#\#}$	106.3 ± 16.3$^{\#\#}$	0.016
	EGCG	33.1 ± 4.8	35.3 ± 5.0	39.9 ± 5.9	49.7 ± 7.5*	59.3 ± 9.6**	66.2 ± 10.3**	72.4 ± 11.2**	
AST (U L^{-1})	Control	138.5 ± 18.3	149.9 ± 21.6	154.5 ± 23.6	166.5 ± 26.4$^{\#}$	180.1 ± 29.9$^{\#\#}$	195.3 ± 27.2$^{\#\#}$	214.8 ± 31.7$^{\#\#}$	0.040
	EGCG	137.4 ± 16.9	142.6 ± 16.2	145.4 ± 19.4	149.0 ± 25.6	151.9 ± 26.2*	157.1 ± 24.3**	164.3 ± 24.2**	
ALP (U L^{-1})	Control	72.1 ± 12.2	71.9 ± 13.6	74.8 ± 15.3	78.50 ± 13.6	80.3 ± 18.4	86.9 ± 13.3$^{\#}$	91.3 ± 12.4$^{\#\#}$	0.047
	EGCG	72.9 ± 13.5	74.4 ± 12.4	76.9 ± 13.7	74.7 ± 16.9	74.9 ± 13.8	79.9 ± 12.9	79.5 ± 11.2*	
BUN (mmol L^{-1})	Control	5.12 ± 1.40	5.44 ± 1.61	5.46 ± 1.43	5.97 ± 1.29	7.12 ± 1.83$^{\#}$	8.69 ± 1.69$^{\#\#}$	9.92 ± 1.87$^{\#\#}$	0.008
	EGCG	5.01 ± 1.33	5.03 ± 1.42	5.76 ± 1.57	5.63 ± 1.64	6.58 ± 1.35	6.97 ± 1.57*	7.63 ± 1.71**	
CRE (μmol L^{-1})	Control	25.21 ± 4.64	25.50 ± 4.86	28.61 ± 4.86	30.87 ± 5.25$^{\#}$	34.25 ± 5.76$^{\#\#}$	38.87 ± 5.98$^{\#\#}$	43.75 ± 5.88$^{\#\#}$	0.020
	EGCG	25.97 ± 4.05	26.12 ± 4.72	26.42 ± 4.72	30.12 ± 5.09	29.63 ± 5.37*	31.05 ± 5.19**	34.62 ± 5.27**	
SOD (U mL^{-1})	Control	53.75 ± 7.69	49.25 ± 7.94	51.75 ± 5.31	44.13 ± 7.03	39.62 ± 6.65	34.75 ± 6.46$^{\#\#}$	33.76 ± 6.74$^{\#\#}$	0.024
	EGCG	52.87 ± 7.23	51.50 ± 8.81	51.87 ± 7.39	47.23 ± 6.33	48.03 ± 6.56*	44.85 ± 6.20**	44.01 ± 6.93**	
MDA (nmol mL^{-1})	Control	5.34 ± 0.96	5.41 ± 0.94	6.97 ± 2.29	7.35 ± 2.29$^{\#}$	9.82 ± 2.02$^{\#\#}$	13.37 ± 2.66$^{\#\#}$	12.35 ± 2.42$^{\#\#}$	0.001
	EGCG	5.29 ± 0.99	5.68 ± 1.08	6.64 ± 1.20	6.35 ± 1.94	8.74 ± 1.99	10.82 ± 2.36*	9.53 ± 1.82**	
GSH-Px (nmol mL^{-1})	Control	164.8 ± 18.3	152.7 ± 19.9	145.2 ± 17.5	141.2 ± 20.2	137.3 ± 19.1$^{\#}$	132.4 ± 23.1$^{\#}$	128.2 ± 20.6$^{\#\#}$	0.039
	EGCG	169.3 ± 23.4	164.9 ± 24.4	158.7 ± 18.6	153.6 ± 18.8	156.2 ± 17.6	155.5 ± 24.6*	158.4 ± 25.4**	
ROS (U mL^{-1})	Control	453.5 ± 72.6	484.2 ± 72.3	548.7 ± 95.4$^{\#}$	667.1 ± 109.5$^{\#\#}$	734.6 ± 123.9$^{\#\#}$	823.6 ± 147.3$^{\#\#}$	880.9 ± 153.4$^{\#\#}$	0.007
	EGCG	444.6 ± 67.9	466.7 ± 81.4	523.8 ± 110.1	567.4 ± 97.8*	601.3 ± 108.4*	639.3 ± 126.7**	678.1 ± 127.9**	
TNF-α (pg mL^{-1})	Control	9.14 ± 3.16	9.92 ± 3.52	12.46 ± 4.19	14.98 ± 4.29$^{\#}$	18.77 ± 5.26$^{\#\#}$	24.99 ± 5.43$^{\#\#}$	28.58 ± 6.07$^{\#\#}$	0.025
	EGCG	9.00 ± 3.29	9.01 ± 3.54	11.18 ± 4.11	13.22 ± 4.54	13.38 ± 4.75*	15.15 ± 5.54**	17.89 ± 6.11**	
IL-6 (pg mL^{-1})	Control	13.22 ± 4.81	14.67 ± 4.23	16.74 ± 5.87	18.77 ± 6.86	24.88 ± 6.61$^{\#\#}$	27.70 ± 7.17$^{\#\#}$	29.87 ± 9.32$^{\#\#}$	0.031
	EGCG	13.04 ± 3.87	14.83 ± 4.66	15.98 ± 5.33	17.05 ± 5.99	18.22 ± 7.08*	18.91 ± 6.67**	19.76 ± 8.97**	

AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; BUN, blood urea nitrogen; CRE, creatinine; SOD, superoxide dismutase; MDA, malondialdehyde; GSH-Px, glutathione peroxidase; ROS, reactive oxygen species; TNF-α, tumor necrosis factor-α; IL-6, interleukin-6.

All values are mean ± SD.

$^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs. 0 week. $*P < 0.05$, $**P < 0.01$ vs. control group. *P* value in the last column is the statistical results of two-factor repeated measures ANOVA over time.

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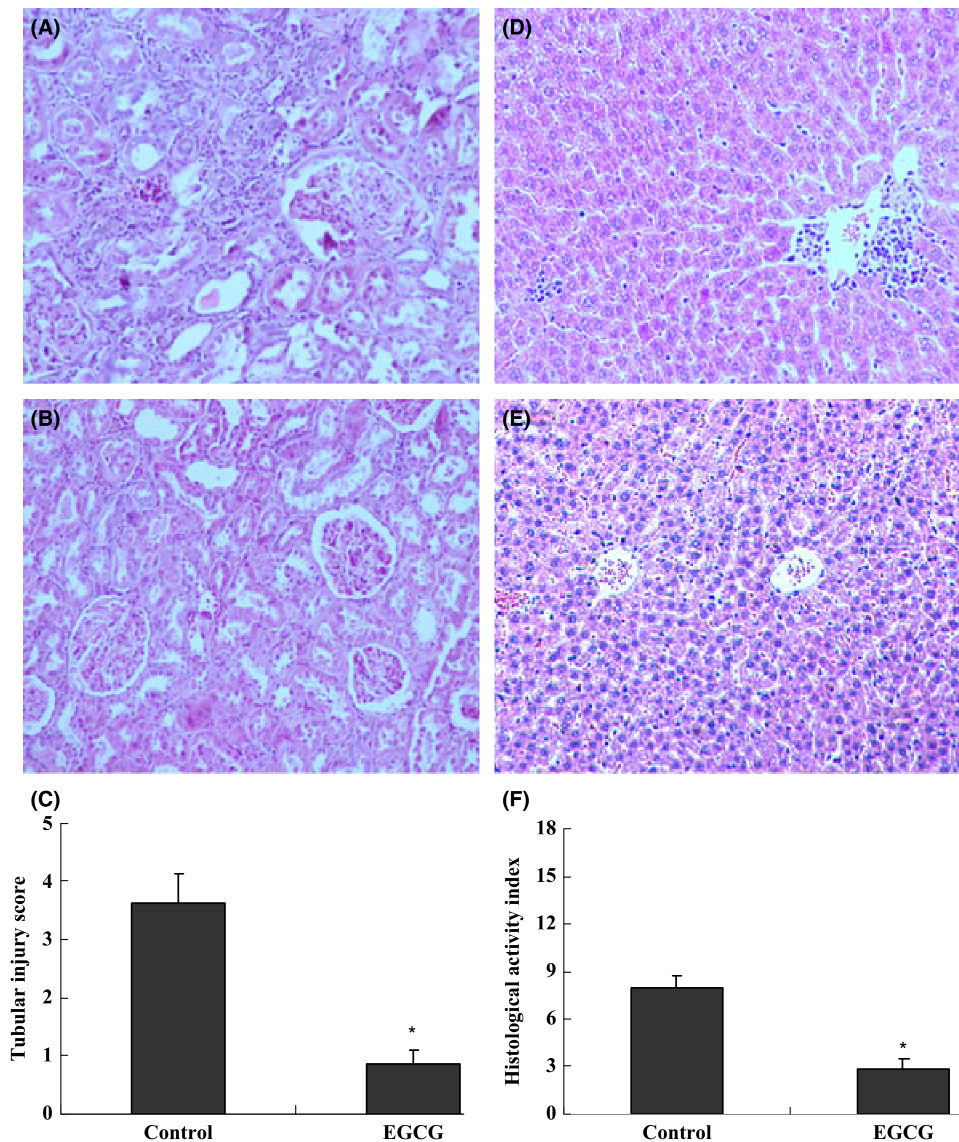

Fig. 2 Hepatic and renal histological analysis stained with hematoxylin and eosin (10×). (A) The kidney section of control rats. (B) The kidney section of EGCG rats. (C) The tubular injury score of kidney histology. (D) The liver section of control rats. (E) The liver section of EGCG rats. (F) The histological activity index (HAI) scoring of liver specimens. *$P < 0.05$ compared with the control group.

GSH-Px, TNF-α, and IL-6 were measured in the liver and kidney tissues of healthy rats. The results indicated that the mRNA levels of CuZnSOD, MnSOD, and GSH-Px showed a significantly higher expression level than the control group ($P < 0.01$, Fig. 4A–C) and that TNF-α and IL-6 mRNA expressions were inhibited in rats treated with EGCG ($P < 0.05$, Fig. 4D,E).

Protein expressions of NF-κB, silent mating type information regulation 2 homolog 1 (SIRT1) and forkhead box class O 3a (FOXO3a)

Epigallocatechin gallate significantly decreased the expression levels of NF-κB mRNA and nuclear proteins ($P < 0.01$, Fig. 5A,B) in the liver and kidney tissues of rats. SIRT1 and FOXO3a, two longevity factors that may inhibit the NF-kB-dependent pro-aging processes of inflammation and oxidative stress, were significantly increased in liver and kidney tissues of healthy rats treated with EGCG (Fig. 5C,D). These results indicate that EGCG could improve oxidative damage and inflammation possibly through down-regulating NF-κB signaling by increasing SIRT1 and FOXO3a expression.

Discussion

In this study, we found that EGCG increased the median lifespan, protected against liver and kidney function damage, and alleviated inflammation and oxidative stress in healthy rats.

As EGCG has not been supplemented for such a long period of time before, the first factor we considered was the dosage. To exclude any potential effects of the phytochemical in the chow, all rats were fed with synthetic diet according to the AIN-93 formula without phytochemicals. The dosage of EGCG was determined based on a recent human dietary survey, which showed that the average daily catechin (EGCG: 25%) intake was 50 mg day^{-1} following an investigation of 6200 people in Dutch (Arts *et al.*, 2001). This dose is about 5 mg $kg^{-1}day^{-1}$ when it is extrapolated to rats using the method of specific surface area according to the average human weighing 60 kg. We initially used

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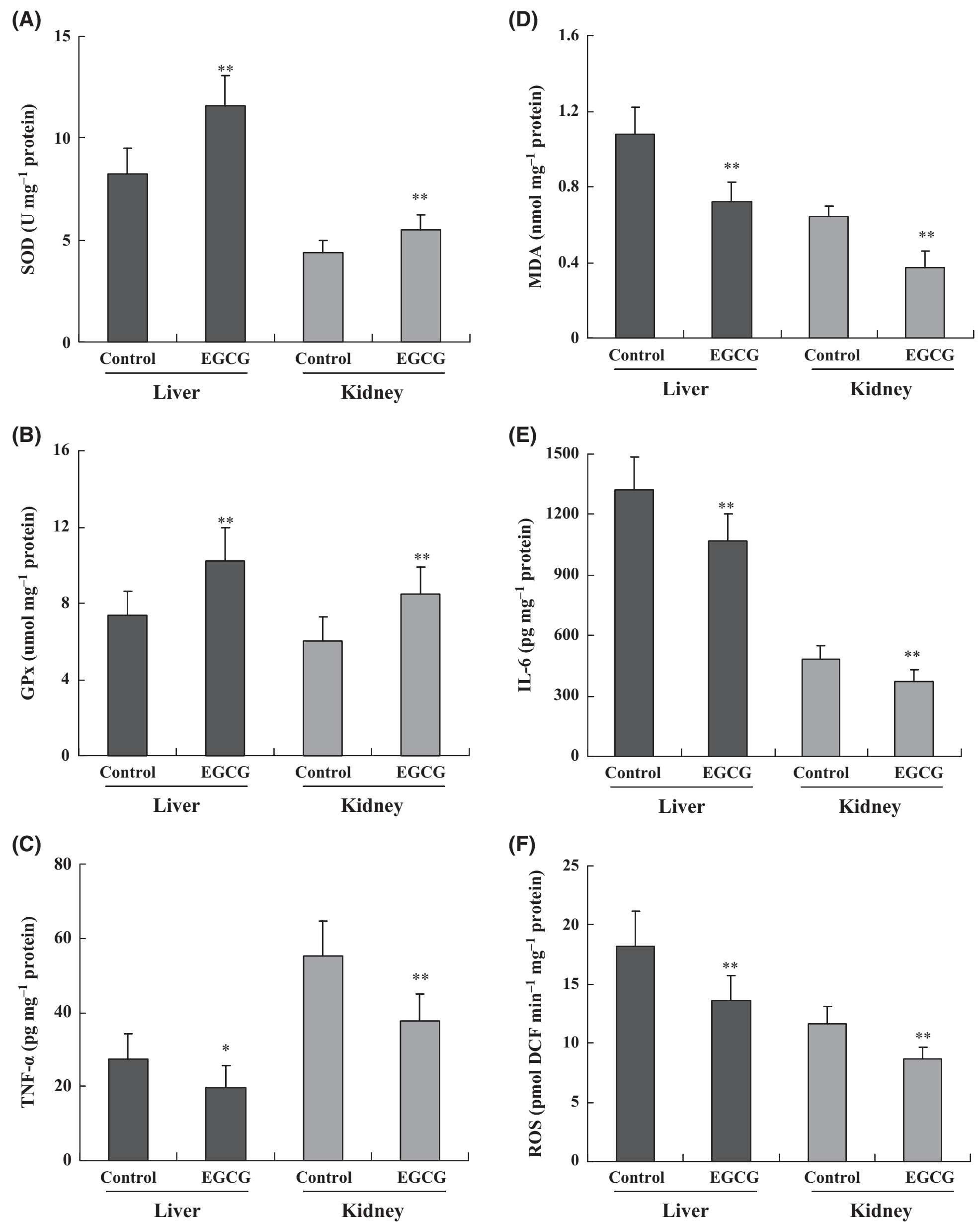

Fig. 3 Effects of EGCG on SOD, MDA, GSH-Px, ROS, TNF-α, and IL-6 levels in liver and kidney homogenates of healthy rats. 68 male Wistar rats were randomly divided into the control group (n = 34) and EGCG group (25 mg kg^{-1}day^{-1}, n = 34). The experiment lasted for 108 weeks. (A) SOD, (B) MDA, (C) GSH-Px, (D) TNF-α, and (E) IL-6 were measured by ELISA methods using commercial kits. (F) ROS were measured by a spectrofluorimeter with a commercial kit. All values are means ± SD of 10 animals in each group. *$P < 0.05$ or **$P < 0.01$ compared with the control group.

5 mg kg^{-1}day^{-1} of EGCG to treat rats in the low-dose group and 25 mg kg^{-1}day^{-1} of EGCG by increasing this dose five times in the high-dose group in our experiment. However, 5 mg kg^{-1}day^{-1} of EGCG did not yield any significant differences in most of the variables evaluated in our study. Thus, we did not provide the results of the treatment with 5 mg kg^{-1}day^{-1} EGCG in order to save editing space.

As the median lifespan could completely reflect the whole lifespan and which has been widely applied as a standard index for lifespan investigation in many studies (Aihie Sayer *et al.*, 2001; Bernardes de Jesus *et al.*, 2012), we ended the experiment that when over half of the rats in each group died. Lifespan was found to be prolonged in the EGCG supplemented (25 mg kg^{-1}day^{-1}) group, and the median lifespan in the EGCG group was increased to 105 weeks compared with 92.5 weeks of the controls in healthy rats, which is consistent with the record of our laboratory. However, this value of median lifespan in healthy rats vary significantly in different studies, which range from 72 to 147 weeks (Knoll, 1988; Aihie Sayer *et al.*, 2001), indicating that the lifespan of animals might be influenced by the conditions of the laboratory. Two epidemiological surveys of Japanese populations showed that daily consumption of green tea can prolong life by avoiding cardiovascular disease and cancer (Nakachi *et al.*, 2003; Kuriyama *et al.*, 2006). Other studies have reported that EGCG increased the lifespan of *Caenorhabditis elegans* or animal models induced by external factors (Li *et al.*, 2007; Abbas & Wink, 2009). However, a very recent study by Strong *et al.* reported that only green tea extract might diminish the risk of midlife deaths in females, but green tea extract, curcumin, oxaloacetic acid, medium-chain triglyceride oil, and resveratrol have no effect on the whole lifespan of male and female genetically heterogeneous mice. (Strong *et al.*, 2013). As for the reason of nonsignificant findings in the male mice, there might be several explanations for this discrepancy. First, EGCG concentration in the green tea extract is unknown in their study. In addition, different constituents in green tea might also lead to an

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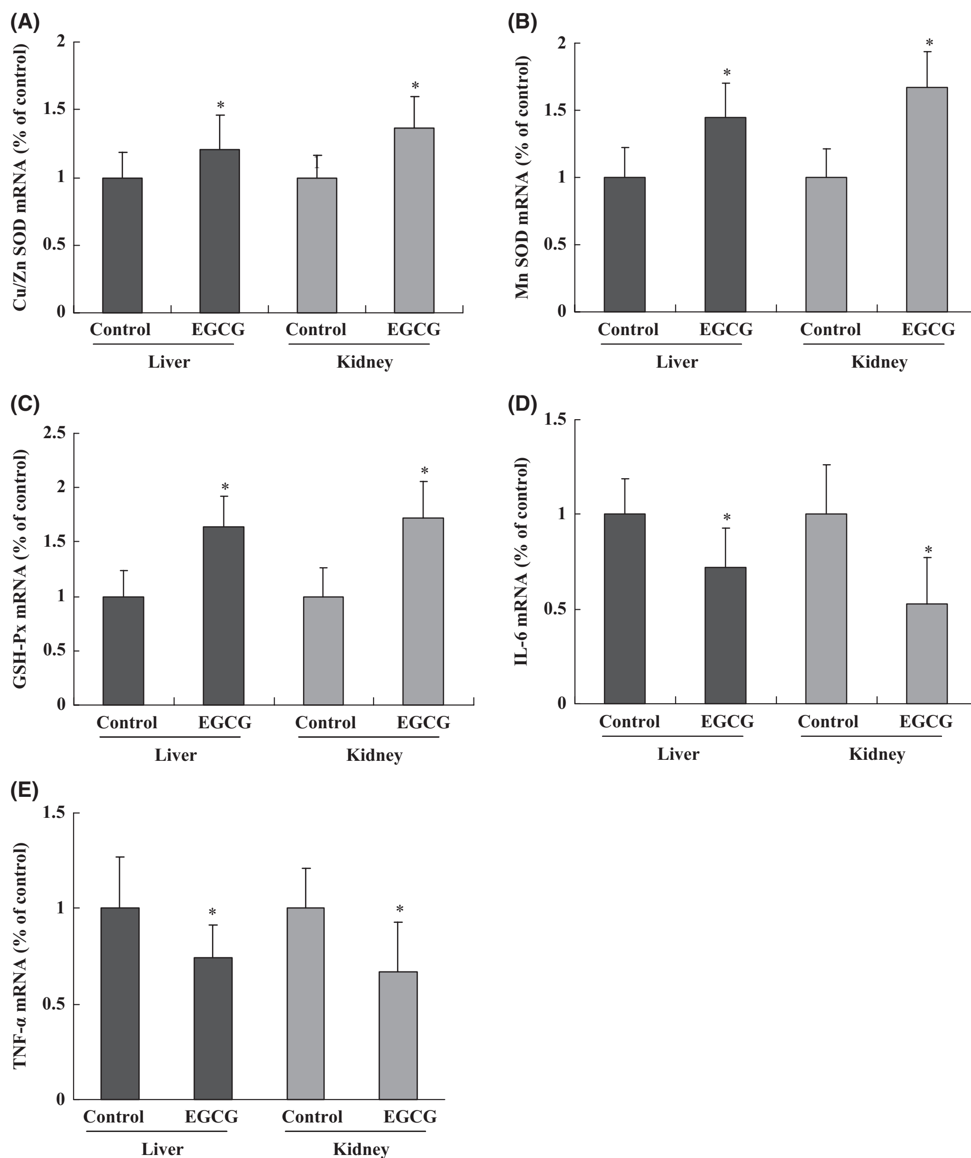

Fig. 4 Effects of EGCG on CuZnSOD, MnSOD, GSH-Px, TNF-α, and IL-6 mRNA levels in liver and kidney tissues of healthy rats. The total RNA was isolated from the liver and kidney tissues, and CuZnSOD, MnSOD, GSH-Px, TNF-α, and IL-6 mRNA levels were examined by quantitative real-time RT-PCR, using specific primers. The expression levels of these mRNAs were normalized to the level of β-actin mRNA. The experiments were repeated three times, and all values are the means ± SD. *$P < 0.05$ compared with the control group.

unpersuasive result. Second, we started the treatment at 4 weeks of the age, while, Strong *et al.* started at 16 weeks. Third, peculiarities of animals are different to obtain a more stable data, we selected Wistar rats in our study. Strong *et al.* might obtain a different result using mice in their study.

Lifespan is associated with chronic diseases such as hypertension, hyperlipidemia, and diabetes (Harrap *et al.*, 1994; Maahs *et al.*, 2011). In our study, blood pressure, serum glucose and lipids levels consistently increased significantly with increasing age. Some studies reported that EGCG may reduce blood pressure, lower serum glucose and lipids and improve insulin resistance in disease models (Potenza *et al.*, 2007; Han *et al.*, 2011); however, there are no such reports in healthy rats. In our study, we observed that EGCG had only a slight decreasing effect on the levels of blood pressure, serum glucose and lipids, which did not reach statistical significance. Our findings indicated that EGCG may not influence the increases in blood pressure, serum glucose and lipids in healthy rats, which did not contribute to the increased lifespan in healthy rats in the present study.

Inflammation and oxidative stress are considered to play a major role in the whole lifespan and are closely associated with aging and age-related diseases (Golden *et al.*, 2002; Finch, 2010). In the present study, inflammation and oxidative stress were aggravated in serum and tissues with age. Compared with the controls, EGCG significantly decreased the levels of ROS and MDA, and increased the enzyme activities and mRNA levels of SOD (Mn SOD, Cu/Zn SOD) and GSH-Px in serum, liver and kidney tissues in healthy rats. In addition, EGCG decreased the levels of inflammatory factors and mRNA expression of TNF-α and IL-6 in serum, liver, and kidney tissues. Data in our study are supported by other reports that EGCG or green tea consumption can increase antioxidative ability, offer protection against oxidative damage induced by external factors, and decrease the levels of TNF-α and IL-6 in *in vitro* and *in vivo* (Ames *et al.*, 1993; Chung *et al.*, 2002; Tsubota, 2007). Therefore, it is suggested that EGCG increases lifespan possibly by improving inflammation and oxidative stress.

Liver and kidney tissues are sensitive to oxidative stress and vulnerable to various pro-inflammatory insults during the lifespan of rats (Hoek &

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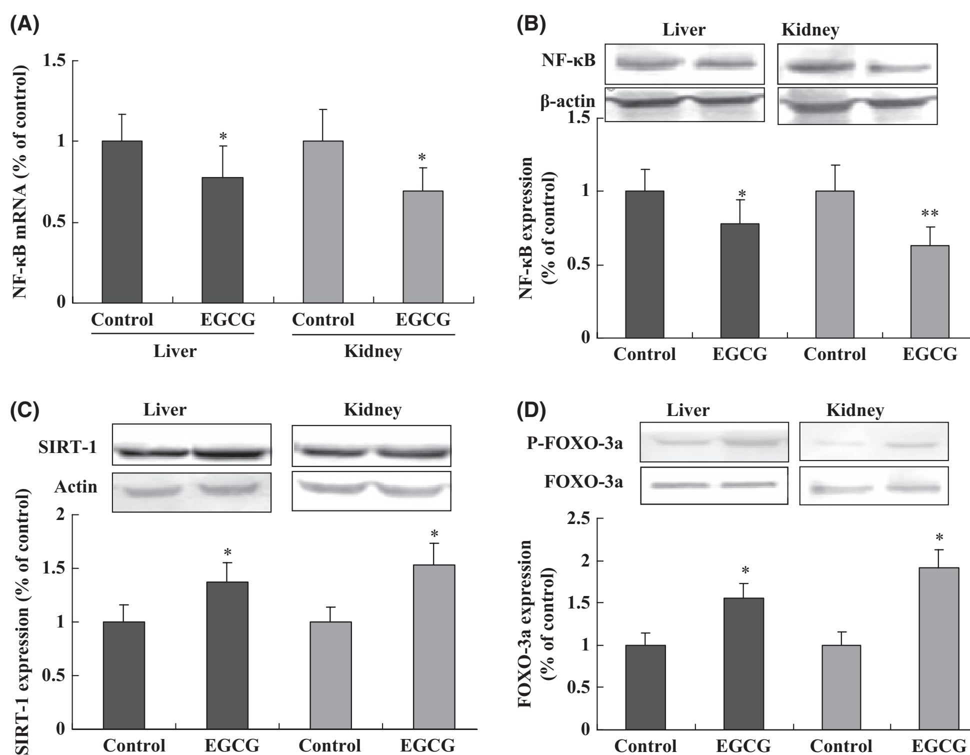

Fig. 5 Effects of EGCG on NF-κB mRNA, the protein expressions of nuclear NF-κB, SIRT1, and FOXO3a in liver and kidney tissues. The total RNA was isolated from the liver and kidney tissues, and the expression level of NF-κB mRNA (A) was examined by quantitative real-time RT-PCR. The protein expressions of nuclear NF-κB (B), SIRT1 (C), and FOXO3a (D) in liver and kidney tissues were examined by Western blot methods, results are the representative of three similar experiments. All values are the means ± SD. *$P < 0.05$ or **$P < 0.01$ compared with the control group.

Pastorino, 2002), and liver and kidney damage was significantly aggravated with increasing age in the present study. Compared with the controls, EGCG significantly decreased the levels of ALT, AST, ALP, BUN, and CRE in the latter part of the present experiment, which reflected the degree of damage to liver and kidney function. In addition, the histology of liver and kidney tissues showed more infiltration of inflammatory cells and serious structural damage in the control group, and EGCG significantly decreased the quantitative injury scores of liver and kidney histopathology. Therefore, these findings suggest that EGCG may increase lifespan by reducing liver and kidney function damage and improving inflammation and oxidative stress.

The activated NF-κB pathway is concerned with both oxidative stress and inflammation (Campo *et al.*, 2008; Srinivasan *et al.*, 2010). Oxidative stress and its consequent lipid peroxidation can aggravate free radical chain reactions, disrupt the functions of some organs including liver and kidney, activate NF-κB signaling and promote the production of inflammatory mediators (Chung *et al.*, 2011; Morgan & Liu, 2011). In our study, EGCG inhibited the mRNA and nuclear protein levels of NF-κB in the liver and kidney tissues. The inhibition of NF-κB activity by EGCG has been widely shown in many cell and animal experiments (Giakoustidis *et al.*, 2010; Jiang *et al.*, 2012). SIRT1 and FOXO3a are two longevity genes that are associated with inflammation and oxidative stress, which probably directly regulate the transcriptional activity of NF-κB (Salminen *et al.*, 2008). However, it is still unknown whether EGCG can regulate these factors to influence NF-κ B. This study firstly proved that EGCG increased the protein levels of SIRT1 and FOXO3a in liver and kidney tissues, which suggest that EGCG may inhibit the activity of NF-κB by activating SIRT1 and FOXO3a.

In conclusion, this study showed that the phytochemical, EGCG, significantly extended lifespan by reducing liver and kidney function damage, which may have been achieved by the suppression of inflammation and oxidative stress through the inhibition of NF-κB signaling and activation of the longevity factors FOXO3a and SIRT1. Therefore, our study suggests that long-term consumption of phytochemicals with antioxidant and anti-inflammatory activities could be beneficial in promoting health and extending lifespan.

Experimental procedures

Animals

Sixty-eight weaning male Wistar rats, 4 weeks old, and weighting 40–60 g were obtained from Vital River Laboratory Animal Technology Company LTD (Beijing, China). The animals were individually housed in stainless steel cages in a room at 22°C ± 2°C on a 12-h light/dark cycle with free access to food and water. After an acclimatization period of 1 week, the animals were randomly divided into two groups: the control group (n = 34) and EGCG group (25 mg $kg^{-1}day^{-1}$, n = 34). EGCG (>90%, HPLC) was obtained from Medherb Biotechnology Co., Ltd (Shengzhen, China). In order to simulate the method of a human drinking tea, an amount of EGCG was added to the drinking water according to the weight of the rats. A little water containing EGCG before the addition of more water was fed to rats first to ensure the dosage of EGCG was the same in each group. Throughout their lifespan, the rats were observed once daily by the veterinary staff and animal care staff. Frequent communication regarding animal health issues occurred among all personnel involved in daily care, and the precise date of death of each rat was recorded. All experiments were approved by the Institutional Animal Care and Use Committee of Harbin Medical University and were conducted in compliance with the animal-use guidelines (SYXK (Hei) 2006-010).

All rats received regular AIN-93 diet in this experiment. Food consumption and body weights were assessed by routine measurements once a week. Blood pressure, heart rate, serum glucose and lipids,

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inflammation, oxidative stress, and biochemistry variables were determined at 0, 18, 36, 54, 72, 90, and 108 weeks. Ten rats were randomly evaluated at the different time points.

SBP and HR

Systolic blood pressure and heart rate were measured by the tail-cuff method with a BP-6 Animal NonInvasive Blood Pressure Measuring System (Chengdu Taimeng Science and Technology Ltd, China) in awake rats. Three consecutive readings of systolic blood pressure and heart rate were collected with an interval of 2 min. Each value was the average of three readings.

Blood chemistry

TC, TG, HDL, LDL, GLU, AST, ALT, ALP, BUN, CRE, and ACE (Co-Health Laboratories Co. Ltd, Beijing, China) were measured using a ROCHE Modular P800 Automatic Biochemical Analyzer (Roche Diagnostics, Mannheim, Germany). SOD, GSH-Px, MDA, NO, and ROS were measured with commercial kits using enzymatic methods (Jiancheng Technology, Nanjing, China). TNF-α and IL-6 (R&D Systems Europe, Abingdon, UK) were assayed using an enzyme-linked immunosorbent assay (ELISA) with commercial kits, according to the manufacturers' instructions.

Inflammatory and oxidative stress levels in tissues

Liver and kidney tissues were removed, cleaned, and washed in ice-cold normal saline. The tissues were homogenized in 1.15% (w/v) potassium chloride containing 1 mmol/L phenylmethylsulfonyl fluoride, centrifuged at 3000 rpm for 15 min, the supernatant was filtered through a 0.2-mm membrane filter, and the filtrate was analyzed (Finamor *et al.*, 2012). SOD, GSH-Px, MDA, TNF-α, IL-6, and ROS were measured with commercial kits using previously published methods.

Liver and kidney pathology

Formaldehyde-fixed paraffin sections of liver and kidney were stained with hematoxylin and eosin (H&E). Histological examinations were performed by pathologists blinded to the conditions. At least 5–10 fields were reviewed for each slide. The histological changes in kidney were scored by counting the percentage of tubules that displayed cell necrosis, loss of brush border, cast formation, and tubule dilatation as follows: 0, none; 1, <10%; 2, 11%−25%; 3, 26%−45%; 4, 46%−75%; and 5, >76% (Oh *et al.*, 2008). The histological diagnosis of liver was based on accepted criteria and the histological activity index (HAI) (Knodell *et al.*, 1981). Briefly, HAI represents the sum of the scores attributed to the necro-inflammatory lesions, that is, periportal and bridging necrosis (0–10), intralobular degeneration and focal necrosis (0–4), portal inflammation (0–4), and fibrosis (0–4).

RNA isolation and real-time PCR

Total mRNAs from rat liver and kidney were extracted using Trizol (Invitrogen, Carlsbad, CA, USA) and treated with RNase-free DNase to remove any residual genomic DNA. Single-stranded cDNAs were synthesized from 1 μg of total RNA using High-Capacity cDNA Reverse Transcription Kits (PE Applied Biosystems, Stockholm, Sweden), according to the manufacturer's instructions. The real-time PCR amplification reactions were conducted in a volume of 20 μL containing 10 μL of SYBR PCR Green master mix (Qiagen), 2 μL of forward and reverse primers each at a final concentration of 10 pmol μL^{-1} and 1 μL of cDNA. The primer sequences used in real-time PCR were as follows: TNF-α, forward 5′-TGT CTC AGC CTC TTC TCA TT-3′, reverse 5′-AGA TGA TCT GAG TGT GAG GG-3′; IL-6, forward 5′-GCC ACT GCC TTC CCT ACT TCA-3′, reverse 5′-GAC AGT GCA TCA TCG CTG TTC A-3′; Cu-Zn SOD, forward 5′-GTT CCG AGG CCG CGC GT-3′, reverse 5′-GTC CCC ATA TTG ATG GAC-3′; Mn SOD, forward 5′-GGC CAA GGG AGA TGT TAC AA-3′, reverse 5′-GCT TGA TAG CCT CCA GCA AC-3′; NF-κBp65, forward 5′- ACG ATC TGT TTC CCC TCA TCT-3′, reverse 5′-TGC TTC TCT CCC CAG GAA TA-3′; β-ACTIN, forward 5′-AGG GAA ATC GTG CGT GAC-3′, reverse 5′-CGC TCA TTG CCG ATA GTG-3′. Thermal cycling and fluorescence detection were performed on an ABI Prism 7500HT Sequence Detection System (Applied Biosystems, Foster City, CA, USA). Thermal cycling was carried out at 95°C for 10 min, followed by 40 cycles of denaturing at 95°C (15 s), annealing at 60°C (30 s), and extension at 72°C (30 s). Data were analyzed using the comparative $2^{-\Delta\Delta Ct}$ method, taking β-actin as the normalizer.

Protein measurement and western blotting

The protein expressions of NF-kB, SIRT1, and FOXO3a were determined by Western blot analysis. Antibodies against NF-kB, FOXO3a, and p-FOXO3a were purchased from Cell Signaling (Beverly, MA, USA), and antibodies against SIRT1 and β-actin were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Protein from tissues was extracted with a RIPA lysis buffer, and the nuclear protein was extracted with the Nuclear and Cytoplasmic Protein Extraction Kit (Beyotime, China). Protein concentrations were determined by the Bradford method (Kruger, 1994). Equal amounts of protein were separated by SDS-PAGE, and electrotransferred onto polyvinylidene difluoride (PVDF) membranes. The signal was amplified by color development using the ProtoBlot II AP System with a stabilized substrate (Promega Corporation, Madison WI, USA). For each study, Western blot analysis was performed three times and representative blots are shown.

Statistical analysis

Statistical analyses were carried out using SPSS 17.0 (SPSS Inc., Chicago, IL, USA). Data are presented as mean ± SD. Two-factor repeated measures ANOVA was used to analyze variables over time and with EGCG. Paired samples *t*-test was used for all comparisons of sample averages. All P values were 2-tailed, and a P value < 0.05 was considered significant for all statistical analyses in this study.

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Author contributions

YN, YL, CS designed and performed the experiments, analyzed the data, and wrote the manuscript; LN, LG, YZ, QL performed the experiments and analyzed the data; YN, LN, RF analyzed the data and wrote the manuscript.

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References

Abbas S, Wink M (2009) Epigallocatechin gallate from green tea (*Camellia sinensis*) increases lifespan and stress resistance in *Caenorhabditis elegans*. *Planta Med.* **75**, 216–221.

Aihie Sayer A, Dunn R, Langley-Evans S, Cooper C (2001) Prenatal exposure to a maternal low protein diet shortens life span in rats. *Gerontology* **47**, 9–14.

Ames BN, Shigenaga MK, Hagen TM (1993) Oxidants, antioxidants, and the degenerative diseases of aging. *Proc. Natl Acad. Sci. USA* **90**, 7915–7922.

Arts IC, Hollman PC, Feskens EJ, Bueno de Mesquita HB, Kromhout D (2001) Catechin intake and associated dietary and lifestyle factors in a representative sample of Dutch men and women. *Eur. J. Clin. Nutr.* **55**, 76–81.

Bernardes de Jesus B, Vera E, Schneeberger K, Tejera AM, Ayuso E, Bosch F, Blasco MA (2012) Telomerase gene therapy in adult and old mice delays aging and increases longevity without increasing cancer. *EMBO Mol. Med.* **4**, 691–704.

Campo GM, Avenoso A, Campo S, D'Ascola A, Traina P, Sama D, Calatroni A (2008) NF-kB and caspases are involved in the hyaluronan and chondroitin-4-sulphate-exerted antioxidant effect in fibroblast cultures exposed to oxidative stress. *J. Appl. Toxicol.* **28**, 509–517.

Chung HY, Kim HJ, Kim KW, Choi JS, Yu BP (2002) Molecular inflammation hypothesis of aging based on the anti-aging mechanism of calorie restriction. *Microsc. Res. Tech.* **59**, 264–272.

Chung HY, Lee EK, Choi YJ, Kim JM, Kim DH, Zou Y, Kim CH, Lee J, Kim HS, Kim ND, Jung JH, Yu BP (2011) Molecular inflammation as an underlying mechanism of the aging process and age-related diseases. *J. Dent. Res.* **90**, 830–840.

Finamor IA, Saccol EM, Gabriel D, Ourique GM, Riffel AP, Konrad SP, Bello-Klein A, Partata W, Baldisserotto B, Llesuy SF, Pavanato MA (2012) Effects of parboiled rice diet on oxidative stress parameters in kidney of rats with streptozotocin-induced diabetes. *J. Med. Food* **15**, 598–604.

Finch CE (2010) Evolution in health and medicine Sackler colloquium: Evolution of the human lifespan and diseases of aging: roles of infection, inflammation, and nutrition. *Proc. Natl Acad. Sci. USA* **107**(Suppl 1), 1718–1724.

Giakoustidis DE, Giakoustidis AE, Iliadis S, Koliakou K, Antoniadis N, Kontos N, Papanikolaou V, Papageorgiou G, Kaldrimidou E, Takoudas D (2010) Attenuation of liver ischemia/reperfusion induced apoptosis by epigallocatechin-3-gallate via down-regulation of NF-kappaB and c-Jun expression. *J. Surg. Res.* **159**, 720–728.

Golden TR, Hinerfeld DA, Melov S (2002) Oxidative stress and aging: beyond correlation. *Aging Cell* **1**, 117–123.

Han JY, Kim CS, Lim KH, Kim JH, Kim S, Yun YP, Hong JT, Oh KW (2011) Increases in blood pressure and heart rate induced by caffeine are inhibited by (-)-epigallocatechin-3-O-gallate: involvement of catecholamines. *J. Cardiovasc. Pharmacol.* **58**, 446–449.

Harrap SB, Mirakian C, Datodi SR, Lever AF (1994) Blood pressure and lifespan following brief ACE inhibitor treatment in young spontaneously hypertensive rats. *Clin. Exp. Pharmacol. Physiol.* **21**, 125–127.

Hoek JB, Pastorino JG (2002) Ethanol, oxidative stress, and cytokine-induced liver cell injury. *Alcohol.* **27**, 63–68.

Holst B, Williamson G (2008) Nutrients and phytochemicals: from bioavailability to bioefficacy beyond antioxidants. *Curr. Opin. Biotechnol.* **19**, 73–82.

Jiang J, Mo ZC, Yin K, Zhao GJ, Lv YC, Ouyang XP, Jiang ZS, Fu Y, Tang CK (2012) Epigallocatechin-3-gallate prevents TNF-alpha-induced NF-kappaB activation thereby upregulating ABCA1 via the Nrf2/Keap1 pathway in macrophage foam cells. *Int. J. Mol. Med.* **29**, 946–956.

Knodell RG, Ishak KG, Black WC, Chen TS, Craig R, Kaplowitz N, Kiernan TW, Wollman J (1981) Formulation and application of a numerical scoring system for assessing histological activity in asymptomatic chronic active hepatitis. *Hepatology* **1**, 431–435.

Knoll J (1988) The striatal dopamine dependency of life span in male rats. Longevity study with (-)deprenyl. *Mech. Ageing Dev.* **46**, 237–262.

Kruger NJ (1994) The Bradford method for protein quantitation. *Methods Mol. Biol.* **32**, 9–15.

Krzyzanowska J, Czubacka A, Oleszek W (2010) Dietary phytochemicals and human health. *Adv. Exp. Med. Biol.* **698**, 74–98.

Kuriyama S, Shimazu T, Ohmori K, Kikuchi N, Nakaya N, Nishino Y, Tsubono Y, Tsuji I (2006) Green tea consumption and mortality due to cardiovascular disease, cancer, and all causes in Japan: the Ohsaki study. *JAMA* **296**, 1255–1265.

Li YM, Chan HY, Huang Y, Chen ZY (2007) Green tea catechins upregulate superoxide dismutase and catalase in fruit flies. *Mol. Nutr. Food Res.* **51**, 546–554.

Maahs DM, Nadeau K, Snell-Bergeon JK, Schauer I, Bergman B, West NA, Rewers M, Daniels SR, Ogden LG, Hamman RF, Dabelea D (2011) Association of insulin sensitivity to lipids across the lifespan in people with Type 1 diabetes. *Diabet. Med.* **28**, 148–155.

Manach C, Hubert J, Llorach R, Scalbert A (2009) The complex links between dietary phytochemicals and human health deciphered by metabolomics. *Mol. Nutr. Food Res.* **53**, 1303–1315.

Morgan MJ, Liu ZG (2011) Crosstalk of reactive oxygen species and NF-kappaB signaling. *Cell Res.* **21**, 103–115.

Nagle DG, Ferreira D, Zhou YD (2006) Epigallocatechin-3-gallate (EGCG): chemical and biomedical perspectives. *Phytochemistry* **67**, 1849–1855.

Nakachi K, Eguchi H, Imai K (2003) Can teatime increase one's lifetime? *Ageing Res. Rev.* **2**, 1–10.

Oh DJ, Dursun B, He Z, Lu L, Hoke TS, Ljubanovic D, Faubel S, Edelstein CL (2008) Fractalkine receptor (CX3CR1) inhibition is protective against ischemic acute renal failure in mice. *Am. J. Physiol. Renal Physiol.* **294**, F264–F271.

Potenza MA, Marasciulo FL, Tarquinio M, Tiravanti E, Colantuono G, Federici A, Kim JA, Quon MJ, Montagnani M (2007) EGCG, a green tea polyphenol, improves endothelial function and insulin sensitivity, reduces blood pressure, and protects against myocardial I/R injury in SHR. *Am. J. Physiol. Endocrinol. Metab.* **292**, E1378–E1387.

Saito M (2007) Role of FOSHU (food for specified health uses) for healthier life. *Yakugaku zasshi* **127**, 407–416.

Salminen A, Ojala J, Huuskonen J, Kauppinen A, Suuronen T, Kaarniranta K (2008) Interaction of aging-associated signaling cascades: inhibition of NF-kappaB signaling by longevity factors FoxOs and SIRT1. *Cell. Mol. Life Sci.* **65**, 1049–1058.

Scalbert A, Andres-Lacueva C, Arita M, Kroon P, Manach C, Urpi-Sarda M, Wishart D (2011) Databases on food phytochemicals and their health-promoting effects. *J. Agric. Food Chem.* **59**, 4331–4348.

Srinivasan V, Kriete A, Sacan A, Jazwinski SM (2010) Comparing the yeast retrograde response and NF-kappaB stress responses: implications for aging. *Aging Cell* **9**, 933–941.

Strong R, Miller RA, Astle CM, Baur JA, de Cabo R, Fernandez E, Guo W, Javors M, Kirkland JL, Nelson JF, Sinclair DA, Teter B, Williams D, Zaveri N, Nadon NL, Harrison DE (2013) Evaluation of resveratrol, green tea extract, curcumin, oxaloacetic acid, and medium-chain triglyceride oil on life span of genetically heterogeneous mice. *J. Gerontol. A Biol. Sci. Med. Sci.* **68**, 6–16.

Sun X, Seeberger J, Alberico T, Wang C, Wheeler CT, Schauss AG, Zou S (2010) Acai palm fruit (*Euterpe oleracea* Mart.) pulp improves survival of flies on a high fat diet. *Exp. Gerontol.* **45**, 243–251.

Suzuki Y, Miyoshi N, Isemura M (2012) Health-promoting effects of green tea. *Proc. Jpn. Acad. Ser. B. Phys. Biol. Sci.* **88**, 88–101.

Tsubota K (2007) [Oxidative stress and inflammation: hypothesis for the mechanism of aging]. *Nippon Ganka Gakkai zasshi.* **111**, 193–205; discussion 206.

Valenzano DR, Terzibasi E, Genade T, Cattaneo A, Domenici L, Cellerino A (2006) Resveratrol prolongs lifespan and retards the onset of age-related markers in a short-lived vertebrate. *Curr. Biol.* **16**, 296–300.

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