

Collaborative Effects of Caloric Restriction and Quercetin on Age-related Oxidative Stress Reduction through NQO1/Sirt1 Gene Regulation

Abstract

Background: Aging is caused by the progressive accumulation of various changes in the body, which is associated with an increase in free radicals and oxidative stress (OS). The aim of this study was to investigate the potential of caloric restriction (CR) and quercetin (QUER) in alleviating OS in aging and the involvement of the NAD (P) H quinone oxidoreductase 1 (NQO1)/SIRT1 signaling pathway in these effects. **Methods:** Two age groups of male Wistar rats (eight and 20 weeks of age) were included in the study and subdivided into normal diet (ND), ND with QUER (15 mg Kg⁻¹, IP), ND with CR, and ND with QUER and CR groups. The activities of catalase (CAT), paraoxonase (PON1), liver enzymes and lipid profiles, and the expression of SIRT1 and NQO1 genes were analyzed using the desired methods. **Results:** We showed higher liver enzymes (aspartate aminotransferase [AST], alanine transaminase [ALT], and alkaline phosphatase [ALP]), increased atherogenic lipids, and reduced PON1 activity in 20-week-old rats compared with eight-week-old rats, and the administration of QUER and CR restored these values to the normal range. The expression of NQO1 and SIRT1 is also affected by CR and QUER. CR alone and in combination with QUER significantly raised the expression of the NQO1 and SIRT1 genes. This effect was notable in SIRT1. **Conclusions:** QUER and CR together improved the detrimental effects of aging by modulating antioxidant signaling pathways, suggesting this combination is a complementary therapeutic regime for aging and age-related diseases.

Keywords: Aging, caloric restriction, catalase, NQO1, paraoxonase, quercetin

Introduction

Aging is a general term that can be defined from physiological, behavioral, social, and time-related perspectives. The aging process is the accumulation of various harmful alterations in cells and tissues, which increases the risk of disease and death. Aging-related changes may be due to genetic and developmental defects, the environment, and various diseases. They can also be the result of a natural process. The sum of harmful free radical reactions, which are constantly flowing throughout cells and tissues, adds up to the aging process.^[1] One of the most prominent free radicals in humans, are the reactive oxygen species (ROS) which are produced during various metabolic pathways and play a decisive role in the development of various diseases.^[2,3] To eliminate the harmful impacts of ROS, antioxidant defense systems such as catalase (CAT) and superoxide dismutase (SOD) play a crucial role in cell protection.^[3-5]

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Caloric restriction (CR) refers to a low-calorie diet without malnutrition. It was first mentioned in the 1930s that dietary restrictions significantly raised the lifespan of rodents. This increased lifespan can be attributed to the 25–60% total CR of carbohydrates, fats, and proteins.^[6]

CR increases the lifespan by delaying the onset of age-related illnesses (cancer, diabetes, cardiovascular, neurodegenerative, and autoimmune diseases) in mammals. In addition to extending lifespans, CR also postpones a wide range of age-related diseases.^[7,8] Quercetin (QUER) as a flavonoid has the ability to scavenge hydroxyl radicals and can chelate metal ions, which inhibit lipid oxidation.^[9-11]

One of the most important antioxidant enzymes, CAT, is an enzyme found in almost all living organisms. This enzyme activates the decomposition of hydrogen peroxide. It is also one of the enzymes

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Fereshte Ghorbani,
Arezou Biyabani,
Darya Ghadimi,
Keivan Nedaei¹,
Hadi
Khodabandehloo,
Mina Hemmati

Departments of Clinical
Biochemistry and ¹Medical
Biotechnology, School of
Medicine, Zanjan University of
Medical Sciences, Zanjan, Iran

Address for correspondence:

Dr. Mina Hemmati,
Biochemistry Department,
Faculty of Medicine, Zanjan
University of Medical Sciences,
Zanjan - 4513956111, Iran.
E-mail: mina1hemmati@yahoo.
com

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with great decomposition capacity which make it useful in reducing age-related oxidative stress (OS).^[12]

Paraoxonase (PON1) is a type of arylesterase and holds ester hydrolysis properties. Having peroxidase-like activity, PON1 reduces low-density lipoprotein (LDL)-cholesterol (LDL-C) peroxidation. PON1 also exhibits antioxidant and anti-inflammatory functions. The activity of PON1 decreases with age and age-associated disorders.^[13,14]

NAD (P) H quinone oxidoreductase 1 (NQO1) is a cytosolic antioxidant enzyme and an obligate two-electron reductase that plays an influential role in chemical protection and activates certain anti-tumor quinones. It is also involved in antioxidant defense by producing the antioxidant forms of ubiquinone and vitamin E.^[15,16]

Sirtuins are a family of antiaging proteins that regulate many vital biological processes, including a variety of age-related pathophysiologicals, in mammals. The SIRT1 protein is a nicotinamide-dependent deacetylase (NAD) and reveals the association between aging and metabolism.^[17]

Given the role of aging and age-related OS in the development of various diseases, this study set out to evaluate OS in elderly and young rats and to probe the impact of QUER and CR.

Materials and Methods

Chemicals and reagents

QUER (3,3',4',5,7-pentahydroxyflavone) and hydrogen peroxide were purchased from Sigma Aldrich, USA. Ammonium heptamolybdate tetrahydrate, tris (hydroxymethyl) aminomethane, and calcium chloride (CaCl₂) were purchased from Merck, Germany. Phenylacetate (Merck, Germany) was obtained from the University of Zanjan, chemistry department. Aspartate aminotransferase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), high-density lipoprotein (HDL)-cholesterol (HDL-C), and total cholesterol (TC) colorimetric assay kits were purchased from Pars Azmoun Co., Iran.

Experimental animal groups

This study was performed on 40 male Wistar rats aged 8–20 weeks. These rats were randomly divided into eight groups. They were maintained under standard conditions of light (12 hours' light/12 hours' dark cycle), temperature, and humidity with free access to water and a standard chow diet. All experiments were conducted in accordance with the ethics community guidelines for the use of animals in research and approved by the local ethics committee (IR. ZUMS.REC.1400.262). The groups used in this study are summarized in Table 1.

Sample preparation

At the end of the experiment (four weeks), rats were anesthetized using diethyl ether-impregnated cotton. Blood

Table 1: Different experimental groups used in the study

	Standard normal diet (ND)
Eight-week-old male rats	ND with QUER (15 mg/kg, I.P injection) ^[18]
	ND with two CR
	ND with QUER (15 mg/kg, I.P. injection) and CR
20-week-old male rats	ND
	ND with QUER (15 mg/kg, I.P. injection)
	ND with CR
	ND with QUER (15 mg/kg, I.P. injection) and CR

¹I.P. injection: intraperitoneal injection. ²CR groups: ND with two fasting days with an interval of two days and five ad libitum feeding days in a week

samples were taken from the heart. The serum was isolated and stored at -70°C for further analysis.

In this study, 500 mg of liver tissue was homogenized using Ultra Turrax T25 homogenization with buffer (Tris-Hcl 50 mM, CaCl₂ 2N PH = 8), and the 1:10 diluted supernatant was used to measure CAT and PON1 enzyme activity and malondialdehyde (MDA).

Evaluation of OS status

The activity of CAT in serum/liver homogenate was measured according to the method proposed by Samimi *et al.*^[19] For this, the test involved combining serum, hydrogen peroxide (20 mM), and distilled water. After incubating the samples at 37°C for three minutes, ammonium molybdate was added to all samples to stop the reactions. Absorbance was read at a wavelength of 374 nm for all samples, and CAT activity has been measured according to the samimi *et al.*, study, and KU refers to kilo unit/liter.

The measurements of PON1 activity in serum/liver homogenate were performed based on the method introduced by Rezaei *et al.*^[20] To determine the arylesterase activity of PON1, the test contained serum and a reaction buffer (20 mM Tris-HCL, 1 mM CaCl₂, and 1 mM phenylacetate). The initial absorption was read at a wavelength of 270 nm. After the incubation of the samples at 37°C for 90 seconds, absorption was read again. The difference in the absorption was multiplied by 1310, and the enzyme activity was obtained based on U/L.

MDA as a lipid peroxidation marker was measured with the thiobarbituric acid reactive substance (TBARS) method.^[21]

Measurement of liver enzyme and lipid profile

Triacylglycerol (TAG), TC, HDL-C, LDL-C, and the liver enzymes (AST, ALT, and ALP) in serum were computed using the relevant kits and the autoanalyzer (Mindray BS 200, made in China).

Total ribonucleic acid (RNA) extraction and real-time polymerase chain reaction (PCR)

Total RNA was extracted from the liver tissues using TRIzol (Bioneer, South Korea). Real-time PCR was performed

using single-stranded copy deoxyribonucleic acid (cDNA). For cDNA synthesis, the Yekta Tajhiz Azma kit (Iran) was used, and the recommended protocols of the kit were meticulously considered. The expression levels of the Sirt1 and NQO1 genes were normalized against GAPDH. PCR primers for target cDNAs were as follows: NQO1: 5'GAA AGG ACA TCA CAG3' (forward) and 5'CTG GAA TAT CAC AA3' (reverse); Sirt1: 5'CAT CTT GCC TGA TTT GTA AA3' (forward) and 5'AAC TTC ATC TTT GTC ATA CTT C3' (reverse). The $\Delta\Delta\text{CT}$ method was employed to compare the relative gene expression of different groups. Amplification of the target gene cDNA was normalized to GAPDH expression.

Data analysis

Statistical Package for the Social Sciences (SPSS) version 19 was used to analyze the data. The data was reported as mean \pm standard deviation. The one-way analysis of variance (ANOVA) and Tukey's test (for *post hoc* analysis) were run to compare the means of different groups. A *P* value less than 0.05 was considered statistically significant.

Results

Effect of CR and QUER on CAT activity in young and old rats

Figure 1 in our study depicts the serum activity of the CAT enzyme in both age groups. The results did not display any significant difference between the CAT activities of the two age control groups. CR, QUER, and their combination increased CAT activity in both age groups, but the effect patterns were not the same. CR and the combination of CR and QUER significantly increased CAT activity in both age groups ($P < 0.05$). QUER alone significantly increased CAT

activity in young rats, while this effect was not observed in older rats. As observed in Figure 1, CR significantly boosted the activity of the CAT enzyme in young and older rats. The combination of QUER and CR increased CAT activity more than QUER alone [Figure 1a and b]. In eight-week-old rats, all treatments had a significant effect on the enzyme activity, while QUER exhibited no significant impact in 20-week-old rats [Figure 1a and b]. This shows that CR played a remarkable role in this change.

Effect of CR and QUER on PON1 activity in young and old rats

The results in Figure 2 illustrate that PON1 activity is higher in the eight-week group than in the 20-week age group with no significant difference. In the young group [Figure 2a], CR and the combination of CR and QUER significantly augmented the activity of PON1, which was higher than QUER alone. A similar pattern is seen in older rats [Figure 2b]. QUER, CR, and their combination led to a greater increase in PON1 activity in the younger rats (100 U/L for young rats versus an increase to 40 U/L in the case of older rats).

Effect of CR and QUER on biochemical parameters in liver tissue and serum

Evaluation of hepatic MDA as a marker of macromolecular OS in the liver showed that older rats had higher levels of MDA compared to the young group [Table 2]. CR and QUER declined lipid peroxidation in both age groups; however, this effect was noticeable in the case of young rats.

TC and TAG were higher in older rats compared with young group; while in the case of HDL-C this was

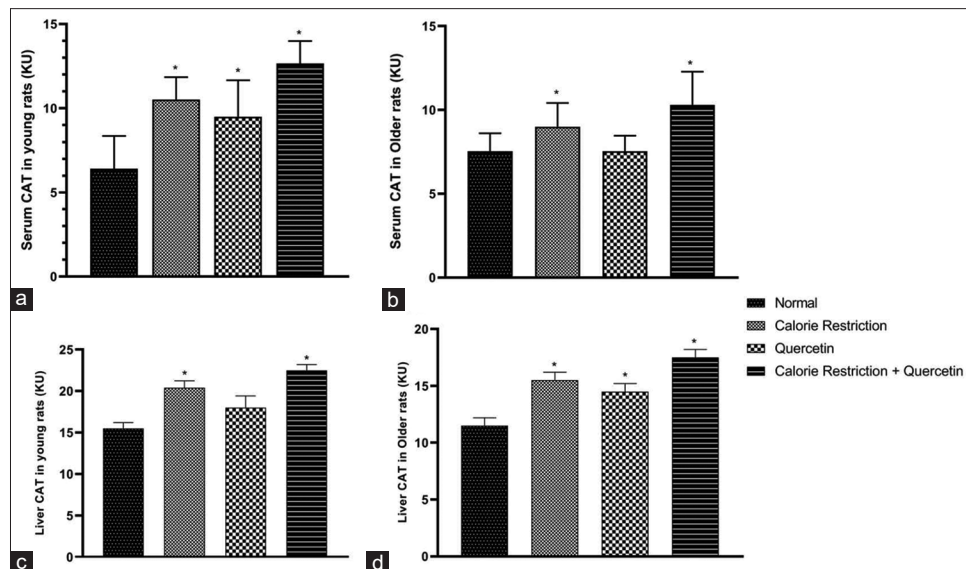


Figure 1: a) Comparison of serum CAT activity in different groups of young rats; b) serum CAT activity in elderly rats; c) liver homogenate CAT activity in different groups of young rats; d) liver homogenate CAT activity in different groups of elderly rats. In the group of young rats, CR, QUER, and their combination significantly boosted the CAT activity. In the group of elderly rats, CR alone and its combination with QUER significantly increased CAT activity. Values are expressed as the mean \pm standard deviation of five rats in each group. *Indicates a significant difference at $P < 0.05$ as compared to the control group (one-way ANOVA and Tukey's test)

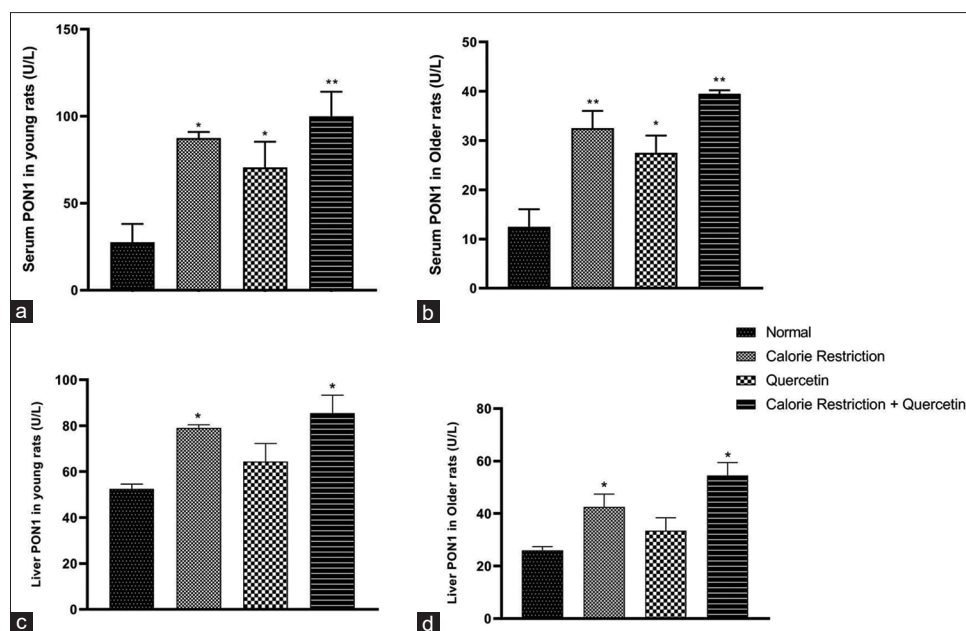


Figure 2: a) Serum PON1 activity in different groups of young rats; b) serum PON1 activity in different groups of elderly rats. c) liver hemogenate PON1 activity in young rats; d) liver hemogenate PON1 activity in elderly rats. In young rats, CR, QUER, and their combination significantly boosted the PON1 activity (the PON1 activity in these groups exceeds 50 U/L). In the elderly groups, CR alone and its combination with QUER elevated the PON1 activity. Values are expressed as the mean \pm standard deviation of five rats in each group. *Indicates a significant difference at $P < 0.05$, ** indicates a significant difference at $P < 0.01$ as compared to the control group (one-way ANOVA and Tukey's test)

Table 2: Effect of QUER and CR on biochemical parameters in eight-week-old and 20-week-old rats

Experimental groups ¹	Eight-week-old rats				20-week-old rats			
	Control	+ QUER	+ CR	+ QUER/CR	Control	+ QUER	+ CR	+ QUER/CR
LDL-C (mg/dl)	31 \pm 2 ^{2a}	30 \pm 4 ^a	30 \pm 6 ^a	29 \pm 6 ^a	41 \pm 6 ^b	40 \pm 5 ^b	39 \pm 6 ^b	28 \pm 4 ^c
HDL-C (mg/dl)	38 \pm 3 ^a	36 \pm 3 ^a	34 \pm 4 ^a	46 \pm 8 ^b	28 \pm 4 ^c	28 \pm 3 ^c	30 \pm 4 ^c	44 \pm 7 ^b
TAG (mg/dl)	80 \pm 7 ^a	75 \pm 6 ^a	71 \pm 3 ^a	61 \pm 6 ^b	108 \pm 8 ^c	98 \pm 8 ^d	93 \pm 5 ^d	92 \pm 8 ^d
TC (mg/dl)	67 \pm 5 ^a	67 \pm 7 ^a	64 \pm 7 ^a	65 \pm 8 ^a	88 \pm 3 ^b	84 \pm 9 ^b	80 \pm 4 ^b	72 \pm 5 ^c
Hepatic MDA (mM/L)	3 \pm 0.4 ^a	2.5 \pm 0.1 ^a	2 \pm 0.4 ^b	1.5 \pm 0.1 ^b	6.8 \pm 0.5 ^c	5.2 \pm 0.6 ^c	4.7 \pm 0.7 ^d	4.1 \pm 0.5 ^d
AST (U/L)	110 \pm 9 ^a	108 \pm 9 ^a	98 \pm 7 ^a	81 \pm 9 ^b	300 \pm 21 ^c	295 \pm 15 ^c	252 \pm 14 ^d	139 \pm 8 ^c
ALT (U/L)	28 \pm 4 ^a	25 \pm 4 ^a	25 \pm 4 ^a	20 \pm 4 ^a	49 \pm 4 ^b	45 \pm 7 ^b	39 \pm 9 ^c	35 \pm 4 ^c
ALP (U/L)	310 \pm 19 ^a	298 \pm 11 ^a	280 \pm 15 ^b	260 \pm 21 ^c	520 \pm 18 ^d	498 \pm 12 ^c	485 \pm 21 ^c	410 \pm 26 ^f

¹For details of the experimental conditions, see the text. Treatment with QUER, CR, and a combination of these for eight-week-old and 20-week-old rats were continued for weeks. ²Data are expressed as the mean \pm standard deviation of five rats in each group. In each row, figures bearing different superscripts are significantly different at $P < 0.05$ (one-way ANOVA and Tukey's test)

opposite. CR, QUER, and the simultaneous use of these improved lipid profiles in both age groups. These changes were notable for CR + QUER. The recent treatment resulted in a significant decrease in TAG and a significant increase in HDL-C in both age groups ($P < 0.05$).

Given the results in Table 2, liver enzymes AST, ALT, and ALP were significantly higher in the 20-week group than in the eight-week rats ($P < 0.05$). In young rats, QUER and CR decreased the activity of AST, ALT, and ALP; this decrease was not significant in the case of ALT. In older rats' concomitant ingestion of CR and QUER decreased liver enzymes in the same pattern of young rats except that the level of enzymes in older rats was still higher than the young group.

Effect of CR and QUER on NQO1 and SIRT1 gene expression in the liver

Figure 3 show that CR alone and in combination with QUER significantly raised the NQO1 gene expression in the young rats group (3a). CR and QUER had a similar impact on the NQO1 expression of young and old rats, except that the ratio of changes was higher in the young rats than old group (3b).

In our study as depicted in Figure 4, in the group of young rats CR, QUER, and their combination significantly enhanced the SIRT1 gene expression [Figure 4a]. In this age group effect of concomitant ingestion of QUER and CR was notable. In old rats CR plus QUER significantly increased the SIRT1 gene expression [Figure 4b], while in this age group, CR alone and QUER alone had no significant effect.

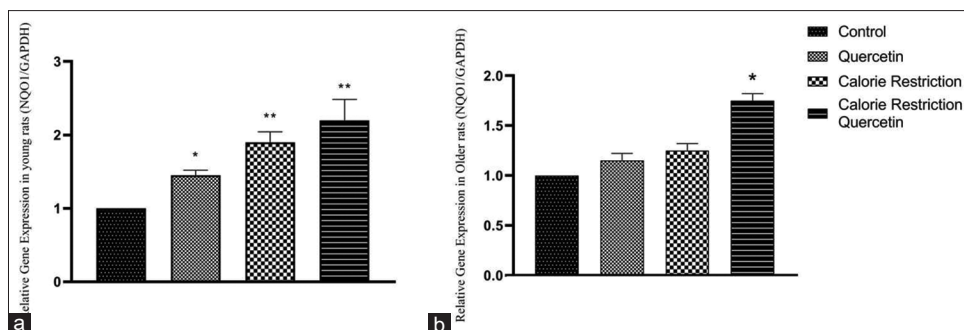


Figure 3: a) NQO1 gene expression in young rats; b) NQO1 gene expression in old rats. In young rats, CR and QUER alone and together could significantly increase NQO1 gene expression. In the elderly group, CR and QUER alone had no effect on NQO1 gene expression, but the combination of CR with QUER could significantly increase NQO1 gene expression. Values have been shown as the mean \pm standard deviation of triplicate experiments. *Indicates a significant difference at the level of $P < 0.05$, **indicates a significant difference at the level of $P < 0.01$ compared to the control group (one-way ANOVA and Tukey's test)

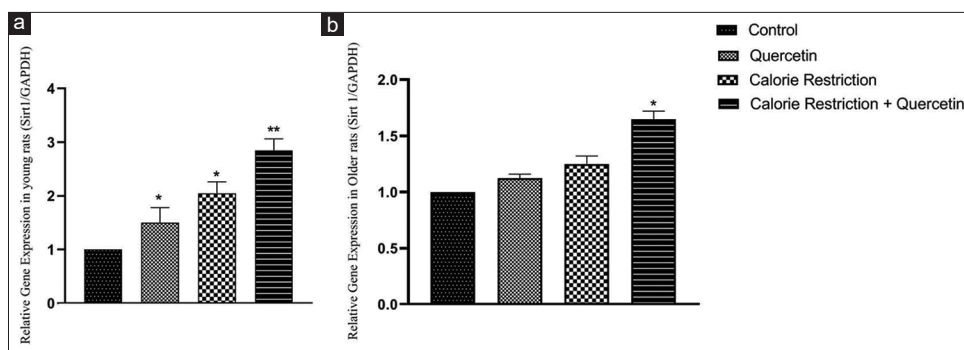


Figure 4: a) SIRT1 gene expression in young rats, b) SIRT1 gene expression in older rats. In the young group, CR, QUER, and its combination could significantly increase SIRT1 gene expression. In the elderly group, CR and QUER alone had no effect on SIRT1 gene expression, but the combination of CR with QUER could significantly increase SIRT1 gene expression. Values have been shown as the mean \pm standard deviation of triplicate experiments. *Indicates a significant difference at the level of $P < 0.05$, **Indicates a significant difference at the level of $P < 0.01$ compared to the control group (one-way ANOVA and Tukey's test)

Discussion

The present study highlighted the impact of aging on antioxidant enzymes, especially PON1 activity. The data obtained in this study showed that QUER and CR by increasing NQO1 and SIRT1 gene expression and antioxidant enzymes activity (CAT and PON1) reduce the OS caused by aging.

In 1935, McCay *et al.* reported that CR prolonged mean and maximum lifespan and prevented or attenuated the severity of chronic disease in post-pubertal rats.^[22]

This was the first time that an influential environmental factor in increasing longevity had been discovered.

In recent decades, the attention of researchers has increased on the effect of CR on health and longevity. According to the available studies, an eight-week CR diet significantly improves the activity of SOD and CAT, which augments the lifespan.^[23,24]

The results of the present study revealed that CAT and PON1 activities decreased with aging and CR significantly increased the activity of these antioxidant enzymes. This result is consistent with the study of Alujoju *et al.*,^[25]

which showed that CR significantly improves age-related decrease in CAT activity.

CAT is at the forefront of defense against electrophilic toxins and OS.^[26] Therefore any factor that can increase CAT activity will have an effective role in reducing OS and improving antioxidant condition. According to our results, CR was able to achieve this effect.

Aging appears to significantly reduce PON1 activity, as we showed in our study. The activity of PON1 was measured in 2006 by Jaouad *et al.*^[27] for 54 healthy individuals in two age groups (young and old), and the results demonstrated a significant decrease in PON1 activity with aging. According to the literature, we can surmise that CR may improve antioxidant enzymes such as PON1, and since PON1 is an HDL-C-associated enzyme, increased activity of PON1 via CR may the anti-atherogenic potential of CR especially in elderly subjects.^[28] The present study also showed that CR increased HDL-C and decreased TAG and TC. These changes may reduce the age-related risk of atherosclerosis.

The effect of CR on MDA has been evaluated in many studies; however, the impact is not the same in all studies. Cicekdal *et al.*^[29] showed chronic CR notably decreased

MDA compared to intermittent CR. We showed a lower level of MDA in CR and QUER group, which may have the same effect as chronic CR. Regardless of age, it seems that CR can have a significant effect on reducing macromolecular OS.

Based on documentation, long-term CR without malnutrition significantly improved the risk factors for atherosclerosis and increased serum HDL-C in non-human mammals (rhesus monkeys, in this case) receiving a very low-fat diet.^[30] The results also confirmed that these diets lowered TC, TAG, and atherogenicity. A considerable volume of studies showed that aging is the dominant risk factor for atherosclerosis.^[31] Therefore, the use of factors that can postpone the risk factors related to aging is a useful strategy for reducing diseases associated with aging.

Holowko *et al.*^[32] concluded that CR helped reduce body mass through improvement of BMI and lipid profile (TC, LDL-C, and TAG). This effect was especially visible in subjects who had reduced their body weight by more than 3 kg. Reducing daily calorie consumption up to 30% is especially suitable for individuals with high body fat content as well as increased glucose, insulin, lipoprotein, and cholesterol.

Since aging leads to functional and structural impairments in the liver, it seems necessary to have useful strategies to decline age-related liver diseases. Overall, the studies disclose that utilizing dietary restriction as a useful strategy brings about a decrease in ALP and ALT. Dorling concluded that CR can protect liver tissue and reduce the risk of metabolic diseases in the elderly.^[33] The decline in liver enzymes such as AST is a sign of improvement in liver function, which was also shown in our study.

In addition to CR, studies have shown that flavonoids such as QUER may have a protective impact on the antioxidant enzyme PON1, lipid profile, and atherogenicity. Boesch-Saadatmandi *et al.*^[34] showed a significant induction of PON1 mRNA in mice fed QUER-enriched diets. The protective potential of QUER has been investigated in many studies. According to the literature, QUER modulates apoptosis-related genes in aged muscle cells.^[35] Furthermore, QUER can reduce the elevated level of liver enzymes that occur in various pathological states and improve these conditions.^[36]

Shanmugarajan *et al.*^[37] scrutinized the impact of QUER on azathioprine-induced oxidative liver diseases in Wistar rats and found that QUER had protective effects on hepatotoxicity by reducing ALT, AST, and ALP. This study also showed that QUER alleviates liver fat accumulation through lipid autophagy. The results of other studies have shown that administration of QUER to rats receiving methotrexate plays a protective role by increasing in the activity of antioxidant enzymes CAT and SOD.^[34] The molecular mechanism of this effect is through SIRT1/

AMPK pathway, which causes a decrease in TAG and increase in HDL-C.^[38]

According to the literature, CR and QUER extend lifespan by increasing efficient mitochondrial metabolism, reducing endogenous ROS production, and simultaneously boosting the secretion and activity of endogenous antioxidant enzymes.^[39,40]

Diaz-Ruiz *et al.* (2018) investigated the role of NQO1 in extending the lifespan. They observed that CR imposes a metabolic program that increases stress resistance and delays the onset of chronic diseases, including cancer. In rodents, CR promoted the regulation of NQO1, which provides electrons for energy metabolism. NQO1 is an NADH dehydrogenase that plays a crucial role in controlling metabolic homeostasis, which is a key indicator of longevity.^[41]

The NQO1 gene has antioxidant response elements (AREs) in its promoter that are essential for induction as well as repression in many cell systems.^[42] Based on the research reviewed by Venugopal *et al.*,^[43] AREs have been shown to mediate NQO1 gene regulation. ARE-mediated NQO1 gene expression is increased by various antioxidants, tumor promoters, and H₂O₂. They have also suggested that AP-1 and the transcription factors Nrf2 and Maf stimulate the induction of ARE-dependent genes such as NQO1. Accordingly, the induction of antioxidant and defensive genes such as NQO1 may protect cells against OS.

In a study performed by Peng *et al.*,^[44] hepatic expression of SIRT1 in the QUER-treated group significantly increased (threefold) compared to the diabetic control group. The results of immunohistochemistry and Western blotting showed that the protective effect of QUER may partially depend on the increased level of SIRT1 protein.

The study by Wei Yu *et al.*^[45] showed that the lifespan of the laboratory rodents subjected to the CR diet was extended. Numerous studies have indicated that CR can remarkably prolong the lifespan of a wide range of living organisms, from yeast to mammals. The Western blot analysis revealed that CR-induced SIRT1 protein expression was significantly boosted in cardiac tissues, and CR could activate the expression of SIRT2, SIRT4, and SIRT7 in cardiomyocytes *in vivo* and *in vitro*.^[46]

With age, the expression and activity of SIRT1 gradually decrease. Age-related loss of SIRT1 in human VSMC is associated with loss of vascular repair capacity, and increased senescence.^[47-49]

Endogenous mechanisms of longevity are stimulated by CR. This suggests that SIRT1 stimulation by CR may partially mediate the beneficial effect of preconditioning.^[50]

It is stated in the studies that CR protects the heart from ischemia/reperfusion injury. By failing to induce cardioprotection in SIRT1 knockout mice, the

cardioprotective effect of CR is partially mediated through activation of the Namp1-SIRT1 pathway, in which Namp1 plays an important role as a regulator of NAD⁺ synthesis in the cardiomyocyte. SIRT1 exerts cardioprotective and cytoprotective effects mainly by preventing apoptotic cell death. This action of SIRT1 can be induced by the impact of CR.^[51,52]

The beneficial effects of CR on increasing SIRT1 gene expression and delaying aging which we showed in our study are in line with the other studies.^[53] Considering the results obtained in this study and the other studies mentioned above, we can hope that CR can reduce the complications caused by aging. The use of QUER in various studies has shown that despite the antioxidant properties of QUER, it can become an oxidant in high doses. Miles *et al.* reviewed the current information about safety and consumption duration of QUER.^[54] Accordingly, QUER in low dose (1 μ M) shows antioxidant properties, while in high dose (100 μ M) becomes oxidant and can induce double-strand DNA breaks. A phase I clinical trial of QUER suggests 2.5 g for a 70-kg person at weekly intervals is not toxic.^[55] However, QUER in 50 mg/kg and higher doses shows renal toxicity. Undoubtedly, we will obtain more realistic results in animal studies, using older rats and conducting experiments over a longer duration, as well as examining the effect of QUER and CR in human studies.

Conclusions

The results of the current research, suggest the use of antioxidants (such as QUER) and the limitation of caloric intake (called CR) considerably contribute to reducing the effects of aging and the risk of aging-related diseases. Moreover, QUER consumption and CR promote the activity levels of CAT and PON1. Although the impacts of these treatments are age-dependent, the beneficial effects of CR and QUER on lipid profile, liver enzyme, and the activity of antioxidant enzymes are evident in both age groups under study. Given the effective role of PON1 enzyme in HDL-C metabolism and the inverse relationship between PON1 activity and atherogenic lipids, the use of CR along with antioxidants can reduce atherogenicity induced by aging. Therefore, the strategy of CR plus antioxidants is a promising solution to protect the human body against the destructive effects of aging. In the present study, rats aged 8 and 20 weeks were used. Using older rats and extending the duration of QUER treatment may provide accurate and more realistic results.

Ethics approval and consent to participate

The study was approved by the Deputy of Research and Technology and Ethics Committee of Zanjan University of Medical Sciences (ethic code: IR.ZUMS.REC.1400.262). Ethical considerations were considered at all stages of the research.

Author contributions

FGh, AB, and MH participated in study design and data analysis and helped in manuscript preparation. DGh, KN, HKh, and MH participated in data analysis. All authors read and approved the final manuscript.

Availability of data and materials

The data supporting the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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Conflicts of interest

There are no conflicts of interest.

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