Original Article

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

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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]

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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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 pathophysiologies, 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 ($CaCl_2$) 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.

Table 1: Different experimental groups used in the study

	Standard normal diet (ND)
Eight-week-old male rats	ND with QUER (15 mg/kg, [1]I.P injection)[18]
	ND with two CR
	ND with QUER (15 mg/kg, I.P. injection) and CR
	ND
20-week–old male rats	ND with QUER (15 mg/kg, I.P. injection)
	ND with CR
	ND with QUER (15 mg/kg, I.P. injection) and CR

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

Sample preparation

At the end of the experiment (four weeks), rats were anesthetized using diethyl ether-impregnated cotton. Blood 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, $CaCl_2$ 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 $CaCl_2$, 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 $^{\Delta\Delta}$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 ± 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

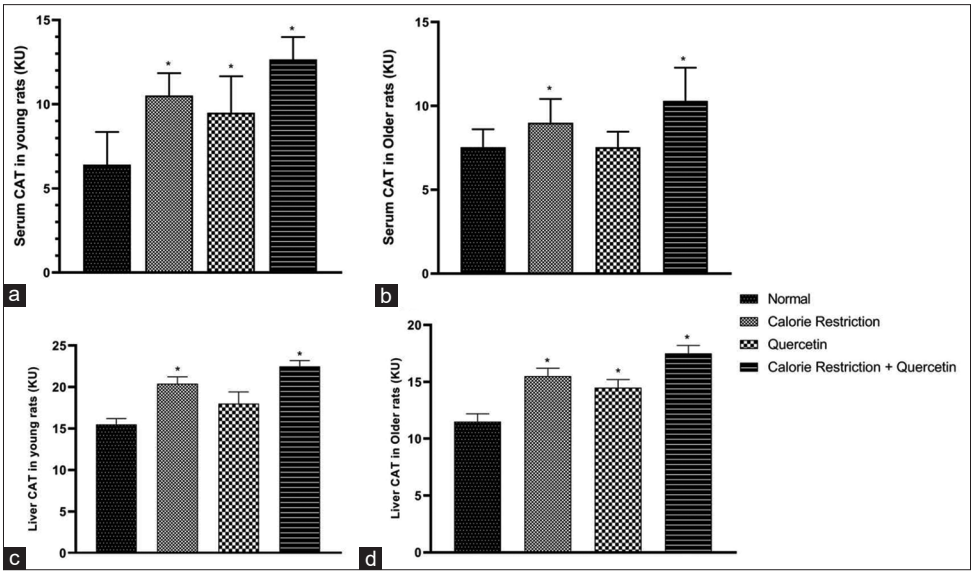

Figure 1: a) Comparison of serum CAT activity in different groups of young rats; b) serum CAT activity in elderly rats; c) liver homogenate CAT activity in different groups of young rats; d) liver homogenate CAT activity in different groups of elderly rats. In the group of young rats, CR, QUER, and their 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 ± 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)

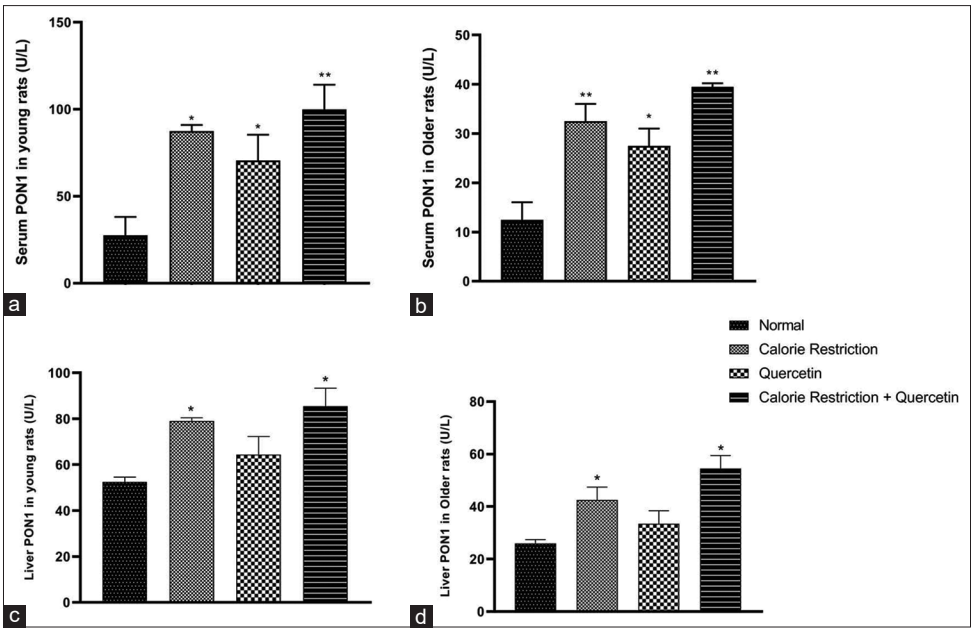

Figure 2: a) Serum PON1 activity in different groups of young rats; b) serum PON1 activity in different groups of elderly rats. c) liver hemogenate PON1 activity in young rats; d) liver hemogenate PON1 activity in elderly rats. In young rats. CR, QUER, and their combination significantly boosted the 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 ± 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			
Biochemical parameters	Control	+ QUER	+ CR	+ QUER/CR	Control	+ QUER	+ CR	+ QUER/CR
LDL-C (mg/dl)	31±2[2a]	30±4[a]	30±6[a]	29±6[a]	41±6[b]	40±5[b]	39±6[b]	28±4[c]
HDL-C (mg/dl)	38±3[a]	36±3[a]	34±4[a]	46±8[b]	28±4[c]	28±3[c]	30±4[c]	44±7[b]
TAG (mg/dl)	80±7[a]	75±6[a]	71±3[a]	61±6[b]	108±8[c]	98±8[d]	93±5[d]	92±8[d]
TC (mg/dl)	67±5[a]	67±7[a]	64±7[a]	65±8[a]	88±3[b]	84±9[b]	80±4[b]	72±5[c]
Hepatic MDA (mM/L)	3±0.4[a]	2.5±0.1[a]	2±0.4[b]	1.5±0.1[b]	6.8±0.5[c]	5.2±0.6[c]	4.7±0.7[d]	4.1±0.5[d]
AST (U/L)	110±9[a]	108±9[a]	98±7[a]	81±9[b]	300±21[c]	295±15[c]	252±14[d]	139±8[e]
ALT (U/L)	28±4[a]	25±4[a]	25±4[a]	20±4[a]	49±4[b]	45±7[b]	39±9[c]	35±4[c]
ALP (U/L)	310±19[a]	298±11[a]	280±15[b]	260±21[c]	520±18[d]	498±12[e]	485±21[e]	410±26[f]

[1]For 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. [2]Data are expressed as the mean±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' concomitance 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.

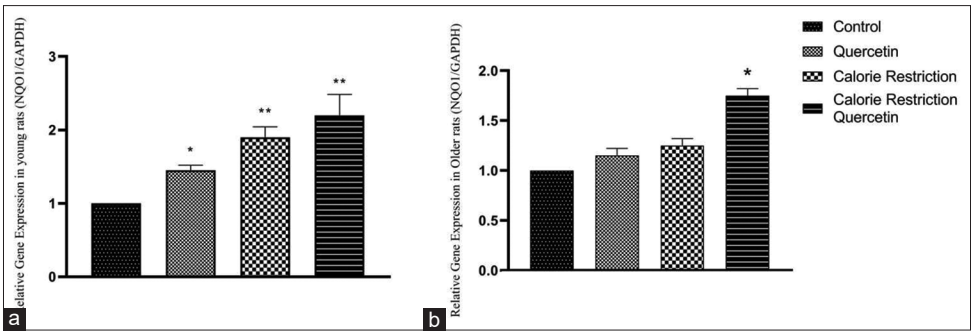

Figure 3: a) NQO1 gene expression in young rats; b) NQO1 gene expression in old rats. In young rats, CR and QUER alone and together could significantly increase NQO1 gene expression. In the elderly group, CR and QUER alone had no effect on NQO1 gene expression, but the combination of CR with QUER could significantly increase NQO1 gene expression. Values have been shown as the mean ± 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)

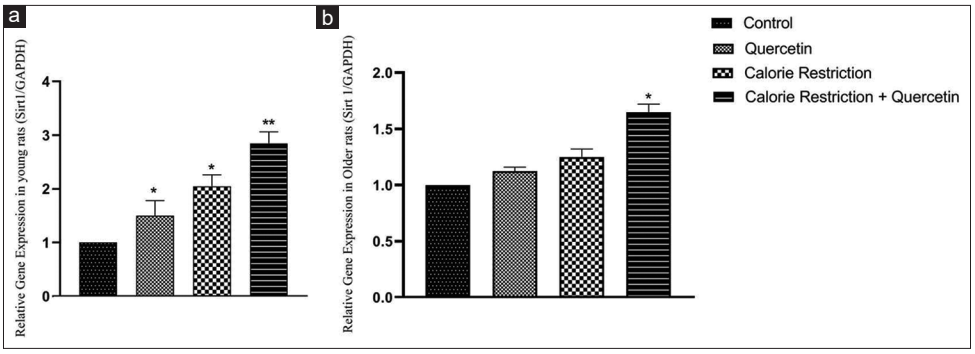

Figure 4: a) SIRT1 gene expression in young rats, b) SIRT1 gene expression in older rats. In the young group, CR, QUER, and its combination could significantly increase SIRT1 gene expression. In the elderly group, CR and QUER alone had no effect on SIRT1 gene expression, but the combination of CR with QUER could significantly increase SIRT1 gene expression. Values have been shown as the mean ± 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 NQO1and 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 Alugoju *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 H_2O_2. 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 Nampt-SIRT1 pathway, in which Nampt 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 μM) shows antioxidant properties, while in high dose (100 μM) 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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References

1. Harman D. Aging: Overview. Annals N Y Acad Sci 2001;928:1-21.
2. Rubio-Ruiz ME, Guarner-Lans V, Cano-Martínez A, Díaz-Díaz E, Manzano-Pech L, Gamas-Magaña A, *et al*. Resveratrol and quercetin administration improves antioxidant DEFENSES and reduces fatty liver in metabolic syndrome rats. Molecules 2019;24:1297.
3. Marchi S, Giorgi C, Suski JM, Agnoletto C, Bononi A, Bonora M, *et al*. Mitochondria-ros crosstalk in the control of cell death and aging. J Signal Transduct 2012;2012:329635.
4. Flekac M, Skrha J, Hilgertova J, Lacinova Z, Jarolimkova M. Gene polymorphisms of superoxide dismutases and catalase in diabetes mellitus. BMC Med Genet 2008;9:1-9.
5. Geng L, Liu Z, Zhang W, Li W, Wu Z, Wang W, *et al*. Chemical screen identifies a geroprotective role of quercetin in premature aging. Protein Cell 2019;10:417-35.
6. Koubova J, Guarente L. How does calorie restriction work? Genes Dev 2003;17:313-21.
7. Ramis MR, Esteban S, Miralles A, Tan D-X, Reiter RJ. Caloric restriction, resveratrol and melatonin: Role of SIRT1 and implications for aging and related-diseases. Mech Ageing Dev 2015;146:28-41.
8. López-LIuch G, Navas P. Calorie restriction as an intervention in ageing. J Physiol 2016;594:2043-60.
9. Rivera L, Morón R, Sánchez M, Zarzuelo A, Galisteo M. Quercetin ameliorates metabolic syndrome and improves the inflammatory status in obese Zucker rats. Obesity 2008;16:2081-7.
10. Uddin S, Ahmad S. Dietary antioxidants protection against oxidative stress. Biochem Educ 1995;23:2-7.

11. Jones E, Hughes R. Quercetin, flavonoids and the life-span of mice. Exp Gerontol 1982;17:213-7.
12. Rindler PM, Plafker SM, Szweda LI, Kinter M. High dietary fat selectively increases catalase expression within cardiac mitochondria. J Biol Chem 2013;288:1979-90.
13. Costa LG, Vitalone A, Cole TB, Furlong CE. Modulation of paraoxonase (PON1) activity. Biochem Pharmacol 2005;69:541-50.
14. Dantoine TF, Debord J, Merle L, Lacroixramiandrisoa H, Bourzeix L, Charmes JP. Paraoxonase 1 activity: A new vascular marker of dementia? Ann Acad Sci 2002;977:96-101.
15. Alrawaiq NS, Atia A, Abdullah A. Comparative study between NAD (P) H: Quinone Oxidoreductase 1 (NQO1) and Heme Oxygenase-1 (HO-1) enzymes induced by an equal dose of different classes of dietary chemicals in mice liver. J Pure Appl Sci 2019;18:477-84.
16. Ross D, Kepa JK, Winski SL, Beall HD, Anwar A, Siegel D. NAD (P) H: Quinone oxidoreductase 1 (NQO1): Chemoprotection, bioactivation, gene regulation and genetic polymorphisms. Chem Biol Interact 2000;129:77-97.
17. Azminah A, Erlina L, Radji M, Mun'im A, Syahdi RR, Yanuar A. In silico and *in vitro* identification of candidate SIRT1 activators from Indonesian medicinal plants compounds database. Comput Biol Chem 2019;83:107096.
18. Vessal M, Hemmati M, Vasei M. Antidiabetic effects of quercetin in streptozocin-induced diabetic rats. Comp Biochem Physiol Part C 2003;135: 357-64.
19. Samimi F, Baazm M, Eftekhar E, Rajabi S, Goodarzi MT, Mashayekhi FJ. Possible antioxidant mechanism of coenzyme Q10 in diabetes: Impact on Sirt1/Nrf2 signaling pathways. Res Pharm Sci 2019;14:524-33.
20. Rezaei N, Zaherijamil Z, Moradkhani S, Saidijam M, Oshaghi EA, Tavilani H. Kiwifruit supplementation increases the activity of the paraoxonase enzyme and decreases oxidized low-density lipoprotein in high-fat diet fed hamsters. Avicenna J Med Biochem 2020;8:58-63.
21. Yagi K. A simple fluorometric assay for lipoperoxide in blood plasma. Biochem Med 1976;15:212-6.
22. McCay CM, Crowell MF, Maynard LA. The effect of retarded growth upon the length of life span and upon the ultimate body size. 1935. Nutrition 1989;5:155-71.
23. Mesquita A, Weinberger M, Silva A, Sampaio-Marques B, Almeida B, Leão C, *et al*. Caloric restriction or catalase inactivation extends yeast chronological lifespan by inducing H2O2 and superoxide dismutase activity. Proc Natl Acad Sci 2010;107:15123-8.
24. Kanikowska D, Kanikowska A, Swora-Cwynar E, Grzymisławski M, Sato M, Bręborowicz A, *et al*. Moderate caloric restriction partially improved oxidative stress markers in obese humans. Antioxidants (Basel) 2021;10:1018.
25. Alugoju P, V K D KS, Periyasamy L. Effect of short-term quercetin, caloric restriction and combined treatment on age-related oxidative stress markers in the rat cerebral cortex. CNS Neurol Disord Drug Targets 2018;17:119-31.
26. Matés JM, Sánchez-Jiménez F. Antioxidant enzymes and their implications in pathophysiologic processes. Front Biosci 1999;4:339-45.
27. Jaouad L, de Guise C, Berrougui H, Cloutier M, Isabelle M, Fulop T, *et al*. Age related decrease in high-density lipoproteins antioxidant activity is due to an alteration in the PON1's free sulfhydylgroups. Atherosclerosis 2006;185:191-200.
28. Thomàs-Moyà E, Gianotti M, Lladó I, Proenza AM. Effects of caloric restriction and gender on rat serum paraoxonase 1 activity. J Nutr Biochem 2006;17:197-203.
29. Cicekdal MB, Tuna BG, Charehsaz M, Cleary MP, Aydin A, Dogan S. Effects of long-term intermittent versus chronic calorie restriction on oxidative stress in a mouse cancer model. IUBMB Life 2019;71:1973-85.
30. Verdery RB, Ingram DK, Roth GS, Lane MA. Caloric restriction increases HDL2 levels in rhesus monkeys (Macaca mulatta). Am J Physiol 1997;273:E714-9.
31. Head T, Daunert S, Goldschmidt-Clermont PJ. The aging risk and atherosclerosis: A Fresh look at arterial homeostasis. Front Genet 2017;8:216.
32. Holowko J, Michalczyk MM, Zając A, Czerwińska-Rogowska M, Ryterska K, Banaszczak M, *et al*. Six weeks of calorie restriction improves body composition and lipid profile in obese and overweight former athletes. Nutrients 2019;11:1461.
33. Dorling JL, Ravussin E, Redman LM, Bhapkar M, Huffman KM, Racette SB, *et al*. Effect of 2 years of calorie restriction on liver biomarkers: Results from the CALERIE phase 2 randomized controlled trial. Eur J Nutr 2021;60:1633-43.
34. Boesch-Saadatmandi C, Egert S, Schrader C, Coumoul X, Barouki R, Muller M, *et al*. Effect of quercetin on paraoxonase 1 activity—studies in cultured cells, mice and humans. J Physiol Pharmacol 2010;61:99-105.
35. Alm-Eldeen A, Khamis A, Elfiky N, Ahmad R. Quercetin modulates age-induced changes in the transcript levels of some apoptosis related genes in the skeletal muscles of male rats. Braz J Pharm Sci 2018;56:e18861.
36. Chen X. Protective effects of quercetin on liver injury induced by ethanol. Pharmacogn Mag 2010;6:135-41.
37. Shanmugarajan T, Prithwish N, Somasundaram I, Arunsundar M, Niladri M, Lavande J, *et al*. Mitigation of azathioprine-induced oxidative hepatic injury by the flavonoid quercetin in wistar rats. Toxicol Mech Methods 2008;18:653-60.
38. Zhao X, Wang J, Deng Y, Liao L, Zhou M, Peng C, *et al*. Quercetin as a protective agent for liver diseases: A comprehensive descriptive review of the molecular mechanism. Phytother Res 2021;35:4727-47.
39. Gargouri B, Mansour RB, Abdallah FB, Elfekih A, Lassoued S, Khaled H. Protective effect of quercetin against oxidative stress caused by dimethoate in human peripheral blood lymphocytes. Lipids Health Dis 2011;10:1-4.
40. Surmise Gomes IB, Porto ML, Santos MCL, Campagnaro BP, Pereira TM, Meyrelles SS, *et al*. Renoprotective, anti-oxidative and anti-apoptotic effects of oral low-dose quercetin in the C57BL/6J model of diabetic nephropathy. Lipids Health Dis 2014;13:184.
41. Diaz-Ruiz A, Lanasa M, Garcia J, Mora H, Fan F, Martin Montalvo A, *et al*. Overexpression of CYB 5R3 and NQO 1, two NAD+producing enzymes, mimics aspects of caloric restriction. Aging cell 2018;17:e12767.
42. Prestera T, Holtzclaw WD, Zhang Y, Talalay P. Chemical and molecular regulation of enzymes that detoxify carcinogens. Proc Natl Acad Sci 1993;90:2965-9.
43. Venugopal R, Jaiswal AK. Nrf1 and Nrf2 positively and c-Fos and Fra1 negatively regulate the human antioxidant response element-mediated expression of NAD (P) H: Quinone oxidoreductase1 gene. Proc Natl Acad Sci 1996;93:14960-5.
44. Peng J, Li Q, Li K, Zhu L, Lin X, Lin X, *et al*. Quercetin improves glucose and lipid metabolism of diabetic rats: Involvement of Akt signaling and SIRT1. J Diabetes Res2017;2017:3417306.
45. Yu W, Zhou HF, Lin RB, Fu YC, Wang W. Short term calorie restriction activates SIRT1, 4 and 7 in cardiomyocytes *in vivo* and *in vitro*. Mol Med Rep 2014;9:1218-24.

46. Lin S-J, Defossez P-A, Guarente L. Requirement of NAD and SIR2 for life-span extension by calorie restriction in Saccharomyces cerevisiae. Science 2000;289:2126-8.
47. Thompson AM, Wagner R, Rzucidlo EM. Age-related loss of SirT1 expression results in dysregulated human vascular smooth muscle cell function. Am J Physiol Heart Circ Physiol 2014;307:H533–41.
48. Sanchez-Fidalgo S, Villegas I, Sanchez-Hidalgo M, de la Lastra CA. Sirtuin modulators: Mechanisms and potential clinical implications. Curr Med Chem 2012;19:2414–41.
49. D'Onofrio N, Vitiello M, Casale R, Servillo L, Giovane A, Balestrieri ML. Sirtuins in vascular diseases: Emerging roles and therapeutic potential. Biochim Biophys Acta 2015;1852:1311–22.
50. Shinmura K, Tamaki K, Bolli R. Impact of 6-mo caloric restriction on myocardial ischemic tolerance: possible involvement of nitric oxide-dependent increase in nuclear Sirt1. Am J Physiol Heart Circ Physiol 2008;295:H2348–55.
51. Luo XY, Qu SL, Tang ZH, Zhang Y, Liu MH, Peng J, *et al*. SIRT1 in cardiovascular aging. Clin Chim Acta 2014;437:106–14.
52. Hsu CP, Zhai P, Yamamoto T, Maejima Y, Matsushima S, Hariharan N, *et al*. Silent information regulator 1 protects the heart from ischemia/reperfusion. Circulation 2010;122:2170–82.
53. Guarente L. Calorie restriction and sirtuins revisited. Genes Dev 2013;27:2072-85.
54. Miles S L, McFarland M, Niles RM. Molecular and physiological actions of quercetin: need for clinical trials to assess its benefits in human disease. Nutr Rev 2014;72:720-34.
55. Ferry DR, Smith A, Malkhandi J, Fyfe DW, deTakats PG, Anderson D, *et al*. Phase I clinical trial of the flavonoid quercetin: pharmacokinetics and evidence for in vivo tyrosine kinase inhibition. Clin Cancer Res 1996;2:659-68.