

Dietary quercetin supplementation increases serum antioxidant capacity and alters hepatic gene expression profile in rats

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Abstract

The aim of this study was to determine the effect of quercetin on hepatic gene expression profile in rats. Twenty male Wistar rats were divided into the control group and the quercetin-treated group, in which a diet containing 0.5% quercetin was provided. After two weeks of feeding, serum and liver samples were collected. Biomarkers of oxidative stress, including serum ferric reducing antioxidant power (FRAP) values and levels of ascorbic acid, vitamin E (VE), glutathione (GSH) and malondialdehyde (MDA) were measured. The hepatic gene expression profile was examined using a microarray technique. The results showed that serum FRAP value, levels of ascorbic acid and VE were increased significantly, whereas serum levels of GSH and MDA were not changed significantly after quercetin supplementation. The microarray analysis revealed that some hepatic genes involved in phase 2 reaction, metabolism of cholesterol and homocysteine, and energy production were expressed differentially in response to quercetin administration. These findings provide a molecular basis for the elucidation of the actions played by quercetin *in vivo*.

Keywords: quercetin, gene expression, liver, antioxidant capacity, rats

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Introduction

Quercetin, one of the major flavonoids present in the human diet, has been associated with a wide range of biological properties, including antioxidant, anti-inflammatory, antimicrobial and anticarcinogenic actions.^{1–5} Epidemiological studies have shown an inverse correlation between quercetin intake and some chronic diseases.^{6,7} The antioxidant action has been considered as the most likely mechanism for the beneficial effects of quercetin. However, there is increasing evidence suggesting that quercetin plays more than an antioxidant role *in vivo*. For example, quercetin may act as a signalling molecule in several pathways.⁸ Recently, An *et al.*⁹ reported that quercetin administration produced a significant impact on the urinary metabolic profile in rats based on ¹H nuclear magnetic resonance (NMR) analysis, indicating that some metabolic pathways were modulated by quercetin. Several studies have demonstrated that quercetin also displays activity on the molecular level. Boomgaarden *et al.*¹⁰ found that quercetin supplementation led to reproducible changes in human monocyte gene expression profiles. The genes expressed differentially were associated with those related to the immune system, nucleic acid metabolism, apoptosis and O-glycan biosynthesis.¹⁰ The microarray analysis conducted by Natsume *et al.*¹¹

showed that the effects of quercetin on the mouse intestinal epithelial cells were not simply dose-dependent but might be affected by multiple factors including the regulation of metabolic activity of the intestinal cells. Angeloni *et al.*¹² examined the gene expression profile of cultured rat primary cardiomyocytes treated with quercetin using DNA microarrays, and demonstrated a remarkable upregulation of gene expression related to phase 2 enzymes. These data reveal that the gene expression profile of different cells may respond to the quercetin treatment differentially.

The liver plays a fundamental role in the metabolism of endogenous and exogenous substances. It has been well demonstrated that quercetin is extensively transformed in the liver by conjugation with glucuronide, sulfate and/or methyl moieties.¹³ Bieger *et al.* found that after a long-term and high-dose dietary intake of quercetin, only organs involved in quercetin metabolism and excretion, including small intestine, liver and kidneys, contained significantly higher quercetin concentrations. They considered that these organs should be considered as primary targets of potential beneficial effects of quercetin.¹⁴ Previously, we showed that oral quercetin administration was effective in protecting against hepatic ischemia–reperfusion injury in

rats. The underlying mechanism was partially associated with improved hepatic antioxidant capacity.¹⁵ Molina *et al.*¹⁶ also noted that pretreatment of quercetin could protect against ethanol-induced oxidative stress in mouse liver. However, the molecular mechanisms of the hepatic protection provided by quercetin remain to be explored. To our knowledge, the hepatic gene expression profile in response to quercetin treatment has not yet been reported. We hypothesize that changes in the hepatic gene expression profile may be involved in the protection by quercetin against hepatic damage. The present study was, therefore, designed to examine the hepatic gene expression profile after quercetin supplementation in rats using a DNA microarray technique. The objective of this study is to provide evidence that quercetin may act *in vivo* by regulating hepatic gene expression. In addition, the changes of serum antioxidant status after quercetin supplementation were also measured in order to validate the antioxidant action of quercetin.

Materials and methods

Animals and diets

Twenty male Wistar rats, weighing 260–300 g, were obtained from the Laboratory Animal Center (Chinese Academy of Military Medical Sciences, Beijing, P R China) and housed individually in stainless-steel cages in a well-ventilated room. The room temperature was controlled between 18 and 24°C and relative humidity between 40% and 60%. The light/dark cycles were alternated every 12 h. Food and tap water were provided *ad libitum*. Dietary intake and body weight were recorded regularly. All procedures were performed in accordance with the current Chinese legislation on the care and use of laboratory animals.

After being acclimatized on a polyphenol-free semisynthetic diet (AIN-93 formula)¹⁷ for five days, the rats were divided randomly into the control group and the quercetin-treated group. The rats in the control group continued to be fed the AIN-93 diet, while those in the quercetin-treated group were switched to an AIN-93 diet containing 0.5% quercetin (minimal 98% in purity; Sigma-Aldrich, Inc, St Louis, MO, USA). We chose 0.5% as the supplemental dose in the present study, because Ameho *et al.*¹⁸ showed that it was effective in increasing plasma quercetin concentration in rats. After 14 d of feeding, all rats were fasted overnight and blood samples were collected from the orbital plexus under ether anesthetization. The serum was separated and stored at –20°C prior to antioxidant analysis. The liver tissues were immediately collected, cleaned up and frozen in liquid nitrogen before being subjected to microarray analysis.

Serum antioxidant capacity and levels of ascorbic acid, vitamin E, glutathione and malondialdehyde

The ferric reducing antioxidant power (FRAP) assay described by Benzie and Strain was followed to analyze serum antioxidant capacity.¹⁹ The serum level of ascorbic

acid was quantified spectrophotometrically based on the reaction with 2,4-dinitrophenylhydrazine.²⁰ An improved fluorometric method was used to determine serum vitamin E (VE) concentration.²¹ Serum glutathione (GSH) was assayed spectrophotometrically by the reaction of 5,5'-dithiobis-2-nitrobenzoic acid with thiols. The reagent kit was purchased from Jiancheng Bioengineering Institute (Nanjing, P R China). The serum level of malondialdehyde (MDA) was measured by thiobarbituric acid reactive species assay.²²

Microarray analysis

Total RNA was prepared from rat livers using TRIzol (Invitrogen, Carlsbad, CA, USA) and a RNeasy kit (Qiagen China, Shanghai, China) according to the manufacturer's instructions, including a DNase digestion step. The purity and integrity of extracted RNA was checked using the Nanodrop ND-1000 full-wavelength UV/visible scanning spectrophotometer (Infinigen Biotech, City of Industry, CA, USA) and denaturing gel electrophoresis. Equal amounts of the RNA from three rats of each group were mixed and used to generate Cy3-labeled complementary DNA according to the Schena method using an Agilent Quick Amp labeling kit (Agilent Technologies, Palo Alto, CA, USA). The hybridization was carried out on the Agilent 4 × 44K Whole Rat Genome Oligo microarrays (Agilent Technologies), which covers 41,000+ rat genes and transcripts annotated against NCBI genome Build 32. Scanning was performed with the Agilent G2505BA microarray scanner using settings recommended by Agilent Technologies. The microarray information was extracted and analyzed with Agilent Feature Extraction software (Agilent Technologies).

Statistical analysis

The data of serum FRAP values and levels of ascorbic acid, VE, GSH and MDA were expressed as mean ± standard deviation and analyzed with Student's *t*-test. Differences were considered significant at $P < 0.05$. In the microarray analysis, sequences with absolute fold change ≥ 2 between two groups were considered differentially expressed.

Results

Dietary intake and body weight gain during the experimental period

No significant difference in dietary intake was found between the control rats and the quercetin-treated rats (24.6 versus 24.8 g daily per rat on average). At the end of the experiment, the rats in the control group gained 80.7 g body weight, whereas those in the quercetin-treated group gained 86.3 g body weight on average, indicating that no detrimental effects on dietary intake or growth were noted for dietary supplementation of 0.5% quercetin.

Serum antioxidant capacity and levels of ascorbic acid, VE, GSH and MDA

As shown in Table 1, serum FRAP value was significantly increased after quercetin supplementation. Serum levels of ascorbic acid and VE were also elevated significantly in the quercetin-treated rats compared with the control rats. However, serum levels of GSH and MDA were not changed significantly in response to quercetin treatment.

Hepatic gene expression profile

Of the 41,000+ genes covered in the microarray analysis, 59 genes were found to be expressed differentially in rat livers after quercetin supplementation, in which 12 genes were identified as down-regulated and the rest up-regulated, with a magnitude of fold change ranging from -3.64 to 4.01. The top five genes that were up-regulated most in expression after quercetin treatment were *BF285957*, *Mug2*, *Hhex*, *Tloc1* and *Sult2a11*, whereas *XM_344411*, *Cdh17*, *Pnpla5*, *Blmt* and *MGC94251* were the top five genes that were down-regulated most. Based on gene functions, it was noted that 11.9% of the genes expressed differentially were associated with the metabolic process, 10.2% with the lipid metabolic process and 6.8% with the electron transport, regulation of progression through the cell cycle or amino acid biosynthetic process, respectively. However, 12 sequences without defined function were also found to be expressed differentially (Table 2).

Discussion

The bioavailability of quercetin has been well established in rats, pigs and humans.^{14,18,23} The increased antioxidant capacity, decreased lipid peroxidation and inhibition of low-density lipoprotein (LDL) oxidation after quercetin treatment have been reported in various studies.^{2,24–26} In this study, we also provided similar data, confirming that quercetin supplementation was effective in increasing serum antioxidant capacity in rats as measured by the FRAP assay. Meanwhile, the levels of serum ascorbic acid and VE were also elevated significantly in the quercetin-treated rats compared with the control rats, implying that quercetin may exert a sparing action on the antioxidant vitamins *in vivo*. However, a few human studies have yielded conflicting results in regard to the antioxidant effects of quercetin. For example, Egert *et al.*²⁷ found that six weeks of quercetin supplementation decreased oxidized LDL levels in obese subjects, while Shanely *et al.*²⁸ reported that quercetin supplementation over 12 weeks had no influence on antioxidant status in humans. Currently, there is no satisfactory

explanation for this discrepancy. The relatively high inter-individual variation in study subjects and the impacts of dietary and non-dietary factors during the supplementation period may possibly have compromised the beneficial effects of increased quercetin intake.

In the present study, we demonstrated that two-week quercetin supplementation altered the hepatic gene expression profile significantly in rats. Many antioxidant/detoxifying genes were up-regulated after quercetin treatment, including *Aldh9a1*, *Aldh7a1*, *Car3*, *Cyp2c7*, *Cyp2d26*, *Cyp2c40*, *Cyb5r3*, *Dia1*, *Ephx1*, *Gsta2*, *Sult2a2*, *Ugt1a1* and *Ugt1a2*. This is not surprising, because quercetin is extensively biotransformed by phase 2 enzymes in the liver.¹³ The results obtained in this study are basically in line with the studies conducted by Angeloni *et al.* and Natsume *et al.* They also showed that some genes involved in phase 2 metabolism were up-regulated in response to quercetin treatment in mouse intestinal epithelium and primary rat cardiomyocytes.^{11,12} We consider that increased expression of phase 2 enzyme genes is beneficial, because the hepatic capacity for the detoxification of carcinogens is also potentially increased, which may provide a partial explanation for the anticarcinogenic action of quercetin.⁵

The ABC superfamily comprises more than 400 proteins and is widely distributed in various organisms.²⁹ The *Abcb11* gene is known to code for the human bile salt export pump, which is located in the canalicular membrane of hepatocytes, and can mediate the secretion of numerous conjugated bile salts into the bile canaliculus.³⁰ The *Abca8* gene is responsible for encoding a protein which has been identified as a new member of the xenobiotic transporter in the ABC subfamily.³¹ In this study, more than a two-fold increase in expression has been demonstrated for these two genes, suggesting that biliary excretion of quercetin metabolites is accelerated significantly after quercetin supplementation. Recently, Matsukawa *et al.*³² have demonstrated a higher biliary excretion of quercetin metabolites than urinary excretion in rats fed a diet containing 1% quercetin glycoside.

Apolipoproteins are proteins that bind to lipids to form lipoproteins, which transport the lipids through the lymphatic and circulatory systems. Apolipoproteins also serve as enzyme co-factors, receptor ligands and lipid transfer carriers that regulate the metabolism of lipoproteins and their uptake in different tissues.³³ In the present study, we found that two genes, *Apob* and *Apof*, related to lipid metabolism were up-regulated significantly. Apolipoprotein B, encoded by *Apob* gene, is the primary apolipoprotein of LDL, responsible for carrying cholesterol from the liver to other organs or tissues.³⁴ Apolipoprotein F, encoded by the *Apof* gene, is involved in the transport and/or

Table 1 Serum FRAP value and contents of ascorbic acid, VE, GSH and MDA

Group	FRAP value (nmol/L)	Ascorbic acid (mg/L)	VE (mg/L)	GSH (g/L)	MDA (μmol/L)
Control	0.48 ± 0.07	17.2 ± 3.1	10.4 ± 1.3	0.6 ± 0.1	3.97 ± 1.86
Quercetin	0.58 ± 0.04**	21.5 ± 3.7*	13.0 ± 2.7*	0.8 ± 0.3	3.51 ± 1.47

FRAP, ferric reducing antioxidant power; GSH, glutathione; VE, vitamin E; MDA, malondialdehyde

Values are mean ± standard deviation (*n* = 10)

Student's *t*-test, ***P* < 0.01, **P* < 0.05 versus control

Table 2 Up-regulated (≥ 2.0 -fold) and down-regulated (≤ -2.0 -fold) genes in rat livers after quercetin administration

Genbank ID	Gene symbol	Gene description	Fold change
Metabolic process			
XM_341497	Auh	AU RNA-binding protein/enoyl-coenzyme A hydratase	2.01
NM_012683	Ugt1a1	UDP glucuronosyltransferase 1 family, polypeptide A1	2.04
NM_031760	Abcb11	ATP-binding cassette, subfamily B (MDR/TAP), member 11	2.05
NM_017013	Gsta2	Glutathione S-transferase A2	2.08
NM_022273	Aldh9a1	Aldehyde dehydrogenase 9 family, member A1	2.13
XM_214535	Aldh7a1	Aldehyde dehydrogenase 7 family, member A1	2.21
NM_201423	Ugt1a2	UDP glucuronosyltransferase 1 family, polypeptide A2	2.25
Electron transport			
NM_017158	Cyp2c7	Cytochrome P450, family 2, subfamily c, polypeptide 7	2.01
NM_138877	Cyb5r3	Cytochrome b5 reductase 3	2.01
NM_012730	Cyp2d2	Cytochrome P450, family 2, subfamily d, polypeptide 2	2.20
NM_031572	Cyp2c12	Cytochrome P450, family 2, subfamily c, polypeptide 12	2.35
Lipid metabolic process			
NM_198780	Pck1	Phosphoenolpyruvate carboxykinase 1, cytosolic	2.01
NM_001024351	ApoF	Apolipoprotein F	2.14
NM_019287	ApoB	Apolipoprotein B (including Ag(x) antigen)	2.16
XM_235538	Pnpla5	Patatin-like phospholipase domain containing 5	-2.23
XM_341791	Sult2a2	Sulfotransferase family 2A, dehydroepiandrosterone (DHEA)-preferring, member 2 (Sult2a2), mRNA.	2.85
NM_012695	Sult2a11	Sulfotransferase family 2A, dehydroepiandrosterone (DHEA)-preferring-like 1	2.93
Regulation of progression through cell cycle			
NM_019257	Sfrs5	Splicing factor, arginine/serine-rich 5	2.22
XM_213484	Vat1	Vesicle amine transport protein 1 homolog (T californica) (Vat1), mRNA	2.26
NM_022671	Onecut1	One cut homeobox 1	2.33
NM_024385	Hhex	Hematopoietically expressed homeobox	3.15
Amino acid biosynthetic process			
NM_030850	Bhmt	Betaine-homocysteine methyltransferase	-2.17
XM_215528	Tloc1	Translocation protein 1	3.07
NM_013218	Ak3	Adenylate kinase 3	2.03
NM_017074	Cth	Cystathionase (cystathionine gamma-lyase)	2.05
Response to oxidative stress			
NM_053769	Dusp1	Dual specificity phosphatase 1	2.00
NM_019292	Car3	Carbonic anhydrase 3	2.01
Transport			
XM_221100	Abca8a	ATP-binding cassette, subfamily A (ABC1), member 8a	2.09
NM_198753	Rpl3	Ribosomal protein L3	2.01
Proteolysis			
NM_138900	C1s	Complement component 1, s subcomponent	2.12
NM_012844	Ephx1	Epoxide hydrolase 1, microsomal	2.81
Biological process			
XM_221100	Abca8a	ATP-binding cassette, subfamily A (ABC1), member 8a	2.09
tRNA processing			
NM_001007706	MGC94251	GCD14/PCMT domain containing protein	-2.10
Cell adhesion: oligopeptide transport			
NM_053977	Cdh17	Cadherin 17	-3.23
RNA processing: mRNA metabolism			
XM_343338	Pcbp2	poly(rC) binding protein 2 (Pcbp2), mRNA	2.28
Acute-phase response: inflammatory response			
NM_023103	Mug1	Murinoglobulin 1	2.09
Complement activation			
XM_342977	Masp2	Mannan-binding lectin serine peptidase 2	2.01
Response to virus			
XM_227821	Ifi44	Interferon-induced protein 44	-2.22
Nuclear mRNA splicing, via spliceosome			
XM_342684	Hnnpa2b1	Heterogeneous nuclear ribonucleoprotein A2/B1	2.02
Apoptosis			
NM_022526	Dap	Death-associated protein	2.02
Others			
XM_346120	XM_346120	Similar to liver carboxylesterase 1 precursor (carboxylesterase ES-1) (E1) (ES-THET) (LOC367389), mRNA	2.20
NM_001002826	Mug2	Murinoglobulin 2	3.22
XM_227045	LOC310330	Similar to Zbed4 protein (LOC310330), mRNA	2.33
XM_343667	LOC363328	Similar to AW046014 protein (LOC363328), mRNA	-2.65
XM_341168	RGD1310587	Similar to hypothetical protein FLJ14146	2.08
XM_344411	XM_344411	Similar to ribosomal protein L23a; 60S ribosomal protein L23a; melanoma differentiation-associated gene 20	-3.64

(Continued)

Table 2 Continued

Genbank ID	Gene symbol	Gene description	Fold change
NM_017004	Es2	Esterase 1	2.29
AL410264	AL410264	Unknown	-2.66
BF555121	BF555121	Unknown	-2.06
BF285957	BF285957	Unknown	4.01
AA848601	AA848601	Unknown	-2.03
BF548548	BF548548	Unknown	-2.21
	TC557487	Unknown	2.70
	ENSRNOT00 000023913	Unknown	2.15
	A_44_P667548	Unknown	2.09
	TC558884	Unknown	2.04
	TC561882	Unknown	2.57
	A_44_P775779	Unknown	2.03
	TC521231	Unknown	2.07

esterification of cholesterol by forming complexes with lipoproteins.³⁵ It is, therefore, indicated that quercetin may increase the transportation of cholesterol by up-regulating the expression of *ApoB* and *ApoE* genes. Recently, Egert *et al.*²⁷ found that quercetin administration resulted in an increased LDL:HDL cholesterol ratio in overweight patients with apoE4 genotype. They hypothesized that altered expression or activities of enzymes associated with apoE genotype might be involved. However, the precise mechanisms behind this action need to be further investigated.

The expression of two genes involved in the metabolism of homocysteine was also found to be regulated differentially after quercetin supplementation. The *Bhmt* gene is known to code for betaine-homocysteine methyltransferase, which functions in catalyzing the conversion of homocysteine to methionine, whereas the *Cth* gene encodes cystathionine gamma-lyase, a critical enzyme involved in the conversion of cystathionine to cysteine and α -ketobutyrate.³⁶ In the present study, the *Bhmt* gene was down-regulated, whereas the expression of *Cth* gene was up-regulated in response to quercetin supplementation, indicating that the conversion of cystathionine to cysteine and α -ketobutyrate was promoted. The cysteine generated in the reaction could serve as a substrate for GSH synthesis. Boots *et al.*^{37,38} found that quercetin could be oxidized to form a thiol-reactive quinone and led to increased GSH consumption preferentially. This is also in line with the finding in this study that the expression of *Gsta2*, which encodes GSH-S-transferase, was also increased remarkably after quercetin supplementation. It is plausible to speculate that the GSH synthesis is increased in order to compensate for its increased consumption in the rats exposed to quercetin treatment. However, an interesting possibility raised here is that quercetin treatment may provide an alternative in combating homocysteinemia, which is an important risk factor in the development of cardiovascular diseases.^{36,39} Further studies are warranted to confirm the possible action of quercetin on homocysteinemia.

Three genes involved in energy metabolism, *Ak3*, *Auh* and *Pck1*, were up-regulated in expression in response to quercetin supplementation, suggesting that the process of energy production is altered. The expression of several genes related to the activity of cell cycle or differentiation, such as *Hhex*, *Onecut1* and *Sfrs5*, was also modulated by

quercetin. Currently, we are not able to explain these changes in detail, because not enough data are available or the functions of these genes are not well elucidated.

In summary, we demonstrated that dietary quercetin supplementation increased serum antioxidant capacity and altered hepatic gene expression profile significantly in rats. Many genes involved in the phase 2 reaction were up-regulated remarkably in response to quercetin supplementation. Several genes coding for enzymes involved in the metabolism of cholesterol and homocysteine were also altered in expression by quercetin treatment. Future studies should be carried out to confirm these changes by reverse transcriptase polymerase chain reaction analysis and to elucidate the potential connection between gene expression and related actions of quercetin *in vivo*.

Author contributions: All authors participated in the design, interpretation of the study, analysis of the data and review of the manuscript. LZ, JWu, JY, JWei and WG conducted the experiments. LZ and CG wrote the manuscript. All authors have read and approved the final version of manuscript.

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