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Effect of Dietary Genistein on Phase II and Antioxidant Enzymes in Rat Liver

HEIKE WIEGAND[1], ANIKA E. WAGNER[1], CHRISTINE BOESCH-SAADATMANDI[1], HANS-PETER KRUSE[2], SABINE KULLING[2] and GERALD RIMBACH[1]

[1]*Institute of Human Nutrition and Food Science, Christian-Albrechts-University, 24118 Kiel;*
[2]*Institute of Nutritional Science, Food Chemistry, University of Potsdam, 14558 Nuthetal, Germany*

Abstract. *Isoflavones are thought to be biologically active components in soy that play a role in the prevention of chronic diseases including cancer. How isoflavones may mediate their beneficial effects has not yet been fully established. Potential mechanisms of cellular action of isoflavones may include their ability to modulate gene expression and the activity levels of enzymes involved in antioxidant defence and the metabolism of xenobiotics including NAD(P)H (Nicotinamide-adenine-dinucleotide-phosphate) quinone oxidoreductase 1 (NQO1) and glutathione S-transferase (GST). Although there is increasing evidence from cell culture studies that genistein, the major isoflavone present in soy, may regulate the expression of genes encoding for phase II and antioxidant enzymes, little is known about its effect in vivo. Feeding rats over 3 weeks with semisynthetic diets enriched with genistein (2 g/kg) significantly increased both the hepatic mRNA and activity levels of NQO1. The total GST activity did not change in response to dietary genistein supplementation, whereas the mRNA levels of individual GST isoenzymes were differentially modulated. The hepatic mRNA level of Gsta2 (class alpha 2) was significantly increased whereas the mRNA levels of Gstm2 (class mu 2) and Gstp1 (class pi 1) were significantly lowered due to genistein supplementation. The protein level of Nrf2 (Nuclear factor E2-related factor 2), a transcription factor involved in the regulation of phase II enzymes, was not altered by dietary genistein. Furthermore, genistein did not affect the hepatic enzyme activity of the antioxidant enzymes catalase (CAT), glutathione peroxidase (GPx) and superoxide dismutase (SOD) or liver lipid peroxidation and glutathione levels. The induction of NQO1 may be one mechanism by which dietary genistein improves the capacity of the liver to detoxify carcinogens.*

Soy products contain a group of compounds called isoflavones, with genistein being the most abundant. Dietary isoflavones and other polyphenols may have beneficial effects in a multitude of diseases including cancer (1, 2) and cardiovascular diseases (3, 4). Some of the biological actions of isoflavones have been partly attributed to their antioxidant properties, either through their reducing capacities *per se* or through their influence on the intracellular redox status (*e.g.* GSH:GSSG ratio, reduced glutathione: oxidised glutathione) (5). However, the classical hydrogen donating antioxidant activity (6) of genistein is unlikely to be the sole explanation for its cellular effects as genistein is extensively metabolised *in vivo*, resulting in significant alterations of its redox potential (7, 8). Furthermore, our previous electron spin resonance and spin trapping spectroscopy studies have indicated that genistein is a rather weak scavenger of reactive oxygen and nitrogen species (9). Instead, genistein may exert its effects *via* mechanisms such as modulation of cell signalling pathways and effects on gene expression (5, 10-13).

The cellular activities of genistein may also depend on its effect on phase II enzymes including glutathione-S transferase (GST) and NAD(P)H (Nicotinamide-adenine-dinucleotide-phosphate) quinone oxidoreductase 1 (NQO1). GST catalyzes the conjugation of endogenous and exogenous electrophiles with glutathione (14). The major cytosolic GST subclasses present in humans and rats are GSTA, GSTM and GSTP (14, 15). NQO1 prevents the generation of toxic semiquinone radicals and reactive oxygen species (16). NQO1 is primarily localized in the cytosol and is found in the liver, the kidney and the gastrointestinal tract (17).

Abbreviations: GST, glutathione S-transferase; NQO1, NAD(P)H quinone oxidoreductase 1; CAT, catalase; GPx, glutathione peroxidase; SOD, superoxide dismutase; Nrf2, nuclear factor E2-related factor 2; ARE, antioxidant responsive element.

Correspondence to: Professor Gerald Rimbach, Institute of Human Nutrition and Food Science, Christian-Albrechts-University; Olshausenstrasse 40, 24118 Kiel, Germany. Tel: +49 4318802583, Fax: +49 4318802628, e-mail: rimbach@foodsci.uni-kiel.de

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There is evidence from numerous cell culture (18-21) and a few *in vivo* (22-24) studies that genistein may affect phase II and antioxidant enzymes. However, the effects on the transcriptional level of individual GST isoenzymes and NQO1 by genistein *in vivo* have not yet been described.

Genes that encode for cytoprotective enzymes, such as GST and NQO1, contain an antioxidant responsive element (ARE, also referred to as electrophile responsive element, EpRE) in their promoter region (25). Itoh *et al*. (26) were the first to describe that the gene expression of phase II enzymes is regulated through activation of the transcription factor Nrf2 (nuclear factor E2-related factor 2) that binds to the ARE region in the promoter and initiates the transcription of phase II enzymes. Furthermore, we have previously shown that genistein significantly increased the nuclear translocation of Nrf2 in cultured cells (5). Secondary plant compounds, such as quercetin (27), have been shown to induce phase II enzyme gene expression and activity through the transactivation of Nrf2 (28).

The present study aimed to investigate the *in vivo* effects of dietary genistein supplementation on mRNA and activity levels of individual GST isoenzymes and NQO1 in growing rats. Since Nrf2 partly regulates the transcription of phase II and antioxidant enzymes, hepatic Nrf2 protein levels were determined and since Nrf2 exhibits an ARE-like sequence in its promoter region and partly regulates its own gene expression (29), the mRNA levels of Nrf2 were also measured. Furthermore, genistein was studied regarding its effect on Nrf2 transactivation by the use of a reporter gene assay which was conducted in Huh7 cells. In addition, the activity of the hepatic antioxidant enzymes catalase (CAT), glutathione peroxidase (GPx) and superoxide dismutase (SOD) as well as the level of glutathione, the mRNA levels of both isoenzymes of γ-glutamylcysteine synthetase, which is the rate limiting enzyme in glutathione synthesis (30), and lipid peroxides were measured in response to dietary genistein supplementation in the rat liver.

Materials and Methods

Chemicals and reagents. Genistein was purchased from LC Laboratories (Woburn, MA, USA). CDNB (1-chloro-2,4-dinitrobenzene), DTNB (5,5'-dithiobis(2-nitrobenzoic acid)), TEP (1,1,3,3-tetraethoxypropane), menadione (2-methyl-1,4-naphthoquinone) and TCA (trichloroacetic acid) were obtained from Sigma-Aldrich (Schnelldorf, Germany) and NADPH (β-nicotinamide-adenine-dinucleotide-phosphate, tetrasodium salt) and MTT (3-(4,5-dimethylthiazo-2-yl)-2,5-diphenyltetrazolium bromide) were purchased from Roth (Karlsruhe, Germany).

Animals and diets. Twelve male Wistar Unilever rats (HsdCpb:WU, Harlan and Winkelmann, Germany) were housed in pairs in Macrolon cages with spruce and fir wood bedding in a controlled environment (21±2˚C and 55±5% relative humidity, 12 h light-dark cycle). The animals were fed either a flavonoid-free, semi-synthetic diet (ssniff special diets GmbH, Germany) (Table I) or the same diet enriched with genistein at a concentration of 2 g/kg. The experimental diet was prepared weekly and stored at 4˚C. Diets and water were fed *ad libitum*.

Table I. *Composition of the basal diet*[1].

Ingredients	g/kg
Cornstarch	472
Glucose	110
Cellulose	50
Casein	240
Coconut oil concentrate	38
Corn oil	20
Mineral premix	60
Vitamin premix	10

[1]Genistein was added to the basal diet at a concentration of 2 g/kg.

The rats were randomly divided into 2 groups of six animals each with an initial body weight of 89±1.1 g and fed the experimental diets for 22 days. Body weight and food intake were recorded weekly. At the end of the experiment, the rats were food deprived for 12 h, anaesthetized with carbon dioxide and decapitated. Liver tissue was quickly excised, rinsed with 0.9% NaCl solution, frozen in liquid nitrogen and stored at –80˚C until further analysis.

RNA isolation and real time qRT-PCR. The total RNA was isolated from the rat liver tissue using a RNeasy Mini kit (Qiagen, Hilden, Germany). The RNA was quantified photometrically and RNA quality checked by gel electrophoresis. Real-time PCR was performed as a one step procedure with a QuantiTect SYBR Green RT-PCR kit (Qiagen). The transcription levels of the target genes were related to the transcription levels of the housekeeping gene β-actin. The primers were designed with a standard program (primer3, NCBI Spidey and Blast) and purchased from MWG (Ebersberg, Germany). The primer specifications are given in Table II.

Liver tissue preparation. Liver homogenates were prepared 1:10 with ice cold phosphate buffered saline (PBS, pH 7.4) and centrifuged at 3800 × g at 4˚C for 10 min. The supernatant was stored at –80˚C until further use. For the preparation of the cytosolic fraction, 1 g of liver tissue was homogenized with 4× v/w of ice cold PBS and centrifuged at 10,000 × g at 4˚C for 20 min (Eppendorf 5804 R, Rotor F34-6-38, Wesseling-Berzdorf, Germany). The supernatant was transferred into a new tube and centrifuged at 100,000 × g at 4˚C for 1 h (Kontron Instruments, Centrikon T-1065 Ultra centrifuge, Rotor TST 28.38, Neufahrn, Germany). The resulting supernatant represented the cytosolic fraction.

Enzyme activity measurements. Total cytosolic GST activity was measured according to the method of Habig *et al*. (31) using CDNB as substrate at 340 nm. A standard curve was generated with equine liver GST (Sigma-Aldrich) for calculating the enzyme activity. NQO1 activity was measured in the cytosolic fraction based on the method of Prochaska and Santamaria (32), in which NQO1 catalyzes the reduction of menadione to menadiol by NADPH utilization. Simultaneously MTT is reduced by menadiol producing a blue dye that can be quantified at 610 nm by spectrophotometry.

Table II. *Nucleotide sequences of primers used for the real-time qRT-PCR experiments.*

Gene	Sequence (5'-3')		Gene	Sequence (5'-3')	
Gsta2	F	GGAGAGAGCCCTGATTGACA	Gsta3	F	GGGAAGCCAGTCCTTCACTA
	R	TTCAAAGGCAGGCAAGTACC		R	GGTCATCCCGAGTTTTCAGA
Gstp1	F	CTTTTGAGACCCTGCTGTCC	Gstm2	F	TTCGCCTGTTCCTGGAGTAT
	R	GAGCCACATAGGCAGAGAGC		R	TTGCTCTGGGTGATCTTGTG
Nqo1	F	CGCAGAGAGGACATCATTCA	Nrf2	F	GCAACTCCAGAAGGAACAGG
	R	CGCCAGAGATGACTCAACAG		R	TCTCTGCCAAAAGCTGCATA
Gclc	F	CTGGGGAGTGATTTCTGCAT	Gclm	F	TGTGTGATGCCACCAGATTT
	R	AGATCTCCGTGTCGATGGTC		R	GCTTTTCACGATGACCGAGT
Actb	F	GGGGTGTTGAAGGTCTCAAA			
	R	TGTCACCAACTGGGACGATA			

F forward primer; R reverse primer; Gst glutathione S-transferase; a, class alpha; p, class pi; m, class mu; Nqo1, NAD(P)H quinone oxidoreductase 1; Nrf2, nuclear factor E2-related factor 2; Gclc, glutamate-cysteine ligase, catalytic subunit; Gclm, glutamate cysteine ligase, modifier subunit; Actb, beta-actin.

NQO1 activity was calculated by the use of a standard curve with human recombinant diaphorase (Sigma-Aldrich). The antioxidant enzyme activities were measured in the liver homogenates according to the method of Marklund and Marklund (33) for SOD, Lawrence and Burk (34) for GPx and Johansson and Borg (35) for CAT. The enzyme activities were expressed as units per milligram protein. The protein concentration was determined with a commercial kit (Pierce BCA Protein Assay, Rockford, IL, USA) using bovine serum albumin as a standard.

Plasma genistein analysis. Rat plasma sample clean-up and extraction were performed as previously described (36). [3,4,1'-$^{13}C_3$]Genistein, purchased from Dr. N. Botting (University of St. Andrews, Scotland), was used as an internal standard. The extract residue was reconstituted in mobile phase and a 10 µl volume was analyzed using a Shimadzu LC system (Shimadzu Deutschland GmbH, Duisburg, Germany) (equipped with two 10AD-VP pumps, a SIL-20AC autosampler, a CTO (Column temperature oven)-20AC column oven, a gradient former FCV (flow-changeover valve)-10ALVP pump, a DGU-20AS degassing module, a SPD-M20A photodiode array detector and a 2010-EV single quadrupole mass analyser.

Chromatography was performed using a PerfectSil Target ODS-3 column (Octadecyl-bonded silica column, 250×3 mm, 3 µm particle size, MZ-Analysentechnik GmbH, Mainz, Germany) with a gradient elution (95% 0.1% formic acid/5% acetonitrile for 5 min, 5% up to 75% acetonitrile in the following 36 min, held at 75% for 4 min, and reequilibrated at starting conditions for 10 min). The flow rate was 0.3 ml/min. The temperature of the column oven was set at 40˚C. Quality control samples containing a mixture of labeled and unlabeled genistein were analyzed throughout each sample set to provide a check of instrument responses and the recovery of internal standard.

The chromatograms were recorded in the selected ion-monitoring (SIM) mode at *m/z* 271 and 274, which corresponds to the molecular ions $[M + H]^+$ of genistein and its labeled analogue. The analytical conditions for the LC-MS QP-2010 (Liquid chromatographic-mass spectrometry quadrupole-2010) were the following: probe (interface) voltage, 4.5 kV (ESI (Electrospray) positive mode); nebulizing gas flow, 1.5 L/min; drying gas pressure, 0.2 MPa; CDL (Curved desolvation line) temperature, 200˚C; CDL voltage 0 V; Q-array DC (Direct Current) voltage, 20 V; Q-array RF (Radio Frequency), 85-95 V; block heater temperature, 230˚C.

Western blot analysis of Nrf2. Liver tissue was homogenized with ice cold RIPA buffer, pH 7.2 (50 mM Tris-HCL, 150 mM NaCl, 0.5% Na-deoxycholat, 1% SDS, 1% NP-40, 2 mM EDTA, protease inhibitor cocktail), incubated on ice for 30 min and centrifuged at 12,000 xg at 4˚C for 25 min. The supernatant was removed and the protein concentration determined, then 30 µg protein was mixed with loading buffer (0.5 M Tris buffer, pH 6.8, 87% glycerol, 10% SDS, 0.5% bromphenol blue, H_2O, 0.05% β-mercaptoethanol) and denaturated at 95˚C for 5 min. Subsequently, the samples were separated on a 10% SDS/polyacrylamide gel and blotted onto an immuno-blot PVDF (polyvinylidene difluoride) membrane. The membrane was incubated with the polyclonal rabbit anti-Nrf2 antibody (Santa Cruz, Heidelberg, Germany) and with the polyclonal rabbit anti-actin antibody (Santa Cruz) at 4˚C overnight. The membranes were then incubated with a goat anti-rabbit IgG secondary antibody conjugated with horseradish peroxidase. The bands were visualized by Immun-Star™ detection reagent on a ChemiDoc system (Bio-Rad, Munich, Germany).

Reporter gene assay in Huh7 cells. Huh7 cells (human hepato cellular carcinoma cell line) were transfected with pARE_Luc, a luciferase reporter gene under the control of the ARE region in the promoter of the human gastrointestinal glutathione-peroxide (kindly provided by Dr. A. Banning, DIFE, Nuthetal, Germany). pRenilla reporter gene (Promega, Mannheim, Germany) was used for the normalization purpose using FugeneHD transfection reagent according to the manufacturer's protocol (Roche, Penzberg, Germany).

The transfection medium was replaced with standard medium after 12 hours. The Huh7 cells were treated with solvent (0.1% dimethyl sulfoxide (DMSO); vehicle control), 5 µM sulforaphane (SFN; positive control) or 0.1; 1.0 and 10 µM genistein, respectively. The isothiocyanate SFN is a well known inducer of several phase II enzymes mediating its effects *via* the transcription factor Nrf2 (37, 38). Then 24 h later luciferase activity (Firefly and Renilla) was determined as described by the manufacturer (Promega). The luciferase values were normalized by the Renilla values. The experimental data represent the mean of three independent experiments.

Table III. *Body weight and estimated food intake of rats fed either the control diet or the diet supplemented with genistein (2 g/kg).*

	Body weight (g)		Food intake (g/d)	
week	Control	Genistein	Control	Genistein
0	89±1.6	89±1.7	-	-
1	139±2.7	143±3.0	14±0.5	15±1.2
2	188±3.9	197±5.2	18±0.5	20±0.3
3	237±4.9	248±6.2	21±0.3	22±0.6

Glutathione concentration. The total concentration of glutathione was measured according to the method of Dringen and Hamprecht (39). Glutathione reacts spontaneously with DTNB whereby free 2-nitro-5-thiobenzoate (TNB) molecules are generated. TNB is a chromogenic thiol compound that can be spectrophotometrically quantified at 405 nm.

Lipid peroxidation. Lipid peroxidation was fluorometrically assayed as thiobarbituric acid reactive substances (TBA-RS) in the liver homogenates following protein precipitation with TCA and extraction in 1-butanol. The samples were measured with and without ferrous provocation. Excitation and emission wavelengths were 520 nm and 560 nm, respectively. The calibration curve was prepared with TEP as an external standard (40).

Statistical analysis. The statistical analysis was performed using the statistical software SPSS (Statistical Package for the Social Sciences, Version 15.0, SPSS GmbH Software, Munich, Germany). Normally distributed data were compared *via t*-test, otherwise, the non-parametric Mann-Whitney *U*-test and the Games-Howell post hoc test were used. Differences were considered significant if $p<0.05$. The results are expressed as the mean with standard error of mean (SEM).

Results

Body weight gain and food intake. Feeding growing rats with the genistein-supplemented diet did not significantly affect their body weight and food intake over the 3 weeks experimental period (Table III).

Plasma genistein levels. In the control rats no genistein in plasma could be detected, whereas in the genistein supplemented rats average plasma genistein concentration was 7.6±1.0 μM.

Hepatic phase II enzyme activity and mRNA levels. Feeding the genistein-supplemented diet significantly increased the cytosolic NQO1 activity in the rat liver (Table IV). Nqo1 mRNA levels were more than 2-fold higher in the genistein-fed rats as compared to the control animals.

The GST activity was not affected by feeding the genistein-supplemented diet (Table V). However, the mRNA levels of the individual GST isoenzymes were differentially modulated: Gsta2 mRNA levels were significantly increased whereas the mRNA levels of Gstm2 and Gstp1 were significantly lowered by dietary genistein. Gsta3 mRNA level did not change in response to dietary genistein supplementation.*Hepatic Nrf2 mRNA and protein levels*.

Table IV. *Hepatic cytosolic NQO1 activity and mRNA level in rats fed either the control diet or the diet supplemented with genistein (2 g/kg).*

	Control	Genistein
NQO1 (mU/mg protein)	57.0±4.80	116±35.1#
Nqo1 mRNA level	1.00±0.15	2.30±0.52*

#(Mann-Whitney-test) and *(*t*-test) indicate significant differences ($p<0.05$) as compared to control animals.

Table V. *Hepatic cytosolic GST activity and mRNA level in rats fed either the control diet or the diet supplemented with genistein (2 g/kg).*

	Control	Genistein
GST (mU/mg protein)	188±12.5	181±9.50
Relative mRNA level of		
Gsta2	1.00±0.15	1.62±0.22*
Gsta3	1.00±0.10	0.99±0.10
Gstm2	1.00±0.06	0.67±0.08*
Gstp1	1.00±0.11	0.65±0.06*

*Indicates significant differences ($p<0.05$) as compared to control animals (*t*-test).

Table VI. *Hepatic antioxidant enzyme activities, TBA-RS, glutathione concentrations and mRNA level of the γ-GCS subunits (Gclc and Gcml) in rats fed either the control diet or the diet supplemented with genistein (2 g/kg).*

	Control	Genistein
GPx (mU/mg protein)	288±23	315±13
SOD (U/mg protein)	33.9±1.2	34.0±2.1
CAT (U/mg protein)	559±60	480±51
TBA-RS (nM/g liver)	35.0±2.4	38·8±1·9
$TBA\text{-}RS_{ferrous}$ (nM/g liver)	73.9±3.6	78.7±2.4
GSH (μM/g protein)	24.9±2.1	32.2±4.3
Relative mRNA level of		
Gclc	1.00±0.1	0.84±0.1
Gclm	1.00±0.2	0.90±0.1

No significant differences between groups. GPx, glutathione peroxidase; SOD, superoxide dismutase; CAT, catalase; TBA-RS, thiobarbituric acid reactive substances with and without ferrous provocation; GSH, glutathione (red.); Gclc, glutamate-cysteine ligase, catalytic subunit; Gclm, glutamate cysteine ligase, modifier subunit.

Hepatic Nrf2 mRNA levels were not significantly altered by feeding the genistein-supplemented diet (0.99±0.07) as compared to the control animals (1.00±0.09).

The Nrf2 protein levels of the control and genistein-supplemented rats are shown in Figure 1. Actin was used as the endogenous control. Two bands at 68 kDa and 100 kDa were detected in the Western blots as described previously (41). The protein band at 100 kDa indicates a complex of Nrf2 with actin (42, 43) and the 68 kDa indicates the Nrf2 protein *per se*. However, no differences in the Nrf2 protein levels were detected between the control and treatment groups.

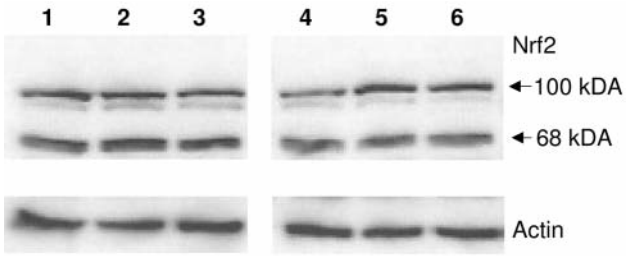

Figure 1. *Hepatic Nrf2 protein levels in rats fed either the control diet or the diet supplemented with genistein (2 g/kg). Lane 1-3, genistein-fed animals; lane 4-6, control animals. Actin was used as loading control.*

ARE reporter gene assay in Huh7 cells. Luciferase reporter gene activity driven by the human gastrointestinal GPx ARE promoter element transfected into Huh7 cells was significantly induced following treatment with 10 μM genistein compared to the control cells (Figure 2). Concentrations of 0.1 and 1.0 μM genistein did not affect the ARE transactivation.

Hepatic antioxidant enzyme activity, glutathione and lipid peroxidation levels. There was no statistically significant effect of dietary genistein on the liver activity of the antioxidant enzymes CAT, GPx and SOD (Table VI). Furthermore hepatic glutathione as well as liver lipid peroxidation levels, as measured by thiobarbituric acid reactive substances (TBA-RS), in the absence and presence of Fe^{2+}, remained unchanged in response to the genistein treatment. The mRNA levels of both isoenzymes of γ-glutamylcysteine synthetase were similar between the control rats and the rats fed the diet supplemented with genistein (Table VI).

Discussion

An important finding of this study was that dietary genistein significantly increased NQO1 mRNA and activity levels in rat liver, and this may be one mechanism by which dietary genistein improves the capacity of the liver to detoxify xenobiotics.

The dietary genistein concentration (2 g/kg) used in this rat study refers to a genistein concentration of approximately 200 mg/kg body weight and is many times higher than the genistein concentrations which can be normally achieved in the plasma of rats (44, 45) or in humans (46, 47) consuming isoflavone-rich diets *in vivo*. However it should be taken into account that genistein is also available in a purified form for human consumption and commercially available supplements contain isoflavone concentrations up to 80 mg/capsule (48).

In the present study, the treatment of Huh7 cells with 10 μM genistein for 24 h resulted in a significant increase of ARE transactivation whereas lower genistein concentrations did not have any effect on ARE transactivation. The hepatic tissue genistein concentration in the genistein-fed rats was possibly too low to induce NQO1 *via* Nrf2, since it has been reported that genistein concentrations in the liver are in the nanomolar range (49, 50) although plasma levels were in the micromolar range, as observed in the present study. The hepatic Nrf2 protein levels did not show significant differences between the control and genistein-fed rats suggesting that the induction of NQO1 following genistein administration was, most likely, not mediated *via* a Nrf2 dependent signal transduction pathway.

Because of the structural similarity of genistein to estradiol, genistein is able to bind to the estrogen receptor (ER) (51-53). It has been reported that the transcriptional up-regulation of NQO1 can be also mediated by a phytoestrogen-ER complex binding to the ARE site (54-56). The ER-dependent transactivation of ARE by phytoestrogens depends on the presence of ER alpha and beta, which are both present in the liver although ER alpha is predominantly expressed in the hepatic tissue. It may be possible that the induction of NQO1 due to dietary genistein, as observed in this study, may be due to ER dependent mechanisms. On the other hand, it has been suggested that estrogens, such as estradiol, are metabolised to the catechol estrogen 4-hydroxyestradiol through cytochrome P450 dependent enzymes, which in turn transactivates the ARE by a phosphatidyl-inositol 3-kinase-Nrf2 dependent pathway, not involving ER (57).

NQO1 may also be induced due to elevated oxidative stress (58). In fact, it has been shown that dietary flavonoids may act also as prooxidants (59). However, it seems unlikely that the induction of hepatic NQO1, as evident in the rats in the present study, was due to oxidative stress since liver lipid peroxidation and glutathione levels as well as the activity levels of the antioxidant enzymes CAT, SOD, GPx remained largely unchanged in response to the genistein treatment.

Genistein feeding did not result in any significant effects on total GST activity in the rat liver compared to the control group. The mRNA level of GST isoenzyme a2 was

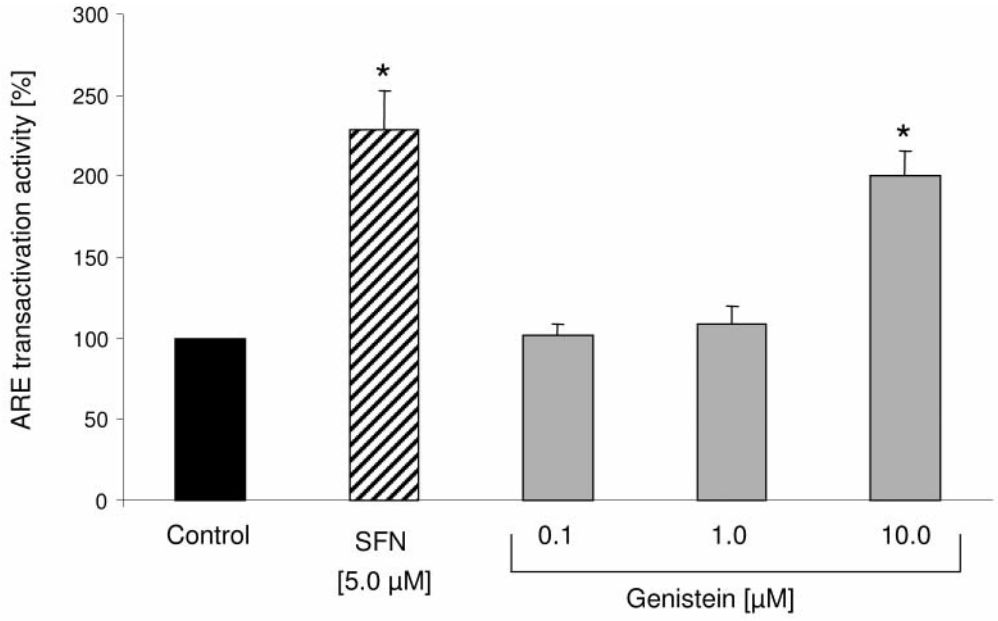

Figure 2. *ARE transactivation in Huh7 cells, transfected with pARE_Luc, after 24 h incubation with genistein and sulforaphane (SFN, 5.0 µM) as positive control. Dimethylsulfoxide (0.1%) was used as vehicle control. Bar graph represents mean±SEM (%) of luciferase activity from 3 independent experiments (vehicle control as 100%). *indicates significant differences (p<0.05) as compared to control (Games Howell-test).*

significantly increased whereas Gstm2 and Gstp1 were significantly decreased by genistein. To the best of our knowledge differential effects on the transcriptional level of GST isoenzymes by genistein *in vivo* have not yet been reported in the literature. It should be taken into account that CDNB, the substrate used for the measurement of total GST activity in this study, is accepted as a general substrate for all GST isoenzymes. However, individual GST isoenzymes exhibit different substrate affinity to CDNB. For example, isoenzymes of class alpha have the highest affinity towards CDNB (31, 60). Thus differences in mRNA levels of individual Gst may not be fully reflected by the measurement of total GST activity as conducted in the present study. Therefore further studies are warranted to investigate the effect of dietary genistein on the activity levels of individual GST.

In summary, dietary genistein did significantly increase the mRNA and activity levels of NQO1 in the rat liver. GST activity, however, which is the sum of different isoenzyme activities, was not changed in response to the dietary genistein treatment, although the hepatic mRNA level of Gsta2 was markedly increased, and the mRNA levels of Gstm2 and Gstp1 were significantly decreased. The induction of NQO1 gene expression is possibly not mainly mediated *via* a Nrf2 dependent signal transduction pathway. The induction of NQO1 in the liver due to dietary genistein may be one mechanism by which the generation of toxic semiquinone radicals and reactive oxygen species, may be partly prevented.

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