FLAVONOIDS INCREASE THE INTRACELLULAR GLUTATHIONE LEVEL BY TRANSACTIVATION OF THE \( \gamma \)-GLUTAMYL-CYSTEINE SYNTHETASE CATALYTICAL SUBUNIT PROMOTER

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Abstract—Fruits and vegetables protect against cancer by so far not well-characterized mechanisms. One likely explanation for this effect is that dietary plants contain substances able to control basic cellular processes such as the endogenous defense against oxidative stress. Oxidative stress is pivotal in many pathological processes and reduced oxidative stress is implicated in prevention of disease. Our results demonstrate that extract from onion and various flavonoids induce the cellular antioxidant system. Onion extract and quercetin were able to increase the intracellular concentration of glutathione by approximately 50%. Using a reporter construct where reporter expression is driven by the \( \gamma \)-glutamyl-cysteine synthetase (GCS) heavy subunit (GCS\(_h\)) promoter we show that onion extract, quercetin, kaempferol, and apigenin increased reporter gene activity, while a fourth flavonoid, myricetin and sugar conjugates of quercetin were unable to increase reporter expression. Quercetin was also able to induce a distal part of the GCS\(_h\) promoter containing only two antioxidant-response/electrophile-response elements (ARE/EpRE). Our data strongly suggest that flavonoids are important in the regulation of the intracellular glutathione levels. This effect may be exerted in part through GCS gene regulation, and may also contribute to the disease-preventing effect of fruits and vegetables. © 2002 Elsevier Science Inc.

Keywords—\( \gamma \)-Glutamyl-cysteine synthetase, Glutathione, Antioxidant-response element, Electrophile-response element, Flavonoids, Free radicals

INTRODUCTION

Epidemiological studies show convincingly that fruits and vegetables reduce the risk of developing cancer [1]. These observations are supported by studies both in animal models and cell cultures demonstrating that substances found naturally in plant food possess the ability to inhibit chemically induced cancer [2,3]. The mechanisms explaining the protective effects are not fully resolved but it is possible that plant constituents can improve the cell’s overall defense [4]. Many compounds in plants have the ability to scavenge reactive oxygen species (ROS), thereby reducing oxidative stress directly, or they may offer an indirect protection by activating endogenous defense systems evolved to combat insults. These defense mechanisms include phase 2 detoxification and antioxidant enzymes. The tripeptide \( \gamma \)-glutamylcysteinylglycine or glutathione (GSH) is a ubiquitous nonprotein present in all cell types in millimolar concentration. The major roles of GSH are to maintain the intracellular redox balance and to eliminate xenobiotics and ROS. Thus, glutathione provides the cell with multiple defenses not only against ROS but also against other toxic insults [5]. The two-step synthesis of GSH is catalyzed by \( \gamma \)-glutamylcysteine synthetase (GCS) and glutathione synthetase [6], with the former being the rate-limiting enzyme. Ample data have shown that a change in GCS activity is accompanied by a change in the GSH level suggesting that regulation of GCS is important for GSH levels [7]. GSH can regulate its own synthesis by a feedback loop on GCS [8]. GCS consists of two subunits, the heavy subunit (GCS\(_h\)) with catalytic activity and the light subunit (GCS\(_l\)) with regulatory activity [9–11]. Regulation of enzyme activity occurs through multiple mechanisms affecting one or both subunits. The heavy subunit in particular is regulated by both transcriptional and post-transcriptional mechanisms [12]. Transcriptional control of GCS\(_h\) is mediated by a region

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spanning approximately 5 kb of the 5’ flanking sequence of the GCS_h gene [13]. Analysis of this region has revealed the presence of several response elements including AP-1 sites, one NF-kB site, one xenobiotic-response element (XRE), and four antioxidant-response elements also called electrophile-response elements (ARE/EpRE). Nrf1 and Nrf2 are transcription factors in the CNC bZIP family that binds to ARE/EpRE elements and are implicated in the regulation of GCS [14,15]. We have recently shown that overexpression of Nrf1 led to an increase in the cellular glutathione level by transcriptional upregulation of GCS_h [16]. ARE/EpRE motifs are also found in promoters of other genes that participate in the defense against free radicals and toxic insults. Among such genes are NADPH-quinone oxidoreductase [17], glutathione S-transferase [18], UDP-glucuronosyl transferase [19], thioredoxin [20], heme oxygenase-1 [21], and ferritin [22]. Flavonoids are phenolic compounds and comprise a large group of naturally occurring substances found in all parts of the plant. Intake of flavonoids through human diet varies, and is estimated to be in the range of 20–80 mg/day based on several independent studies [23]. Recent data shows that the naturally occurring flavonoid quercetin is able to induce ARE/EpRE-dependent expression in MCF-7 cells, a human breast carcinoma cell line [24].

In this study we have investigated the possible regulatory role of dietary flavonoids on the glutathione level in COS-1 cells. The cells were incubated with either crude extract from onion or pure flavonoids and assayed for GSH content or transactivation of the GCS_h promoter. A significant increase in GSH concentration was observed with both extract and the flavonoid quercetin, prompting us to investigate whether the increase was mediated through increased expression of the genes coding for GCS. mRNA quantitation showed an increase in both GCS_h and GCS_l transcripts. By transfecting COS-1 cells with a reporter construct containing the GCS_h promoter, and subsequent treatment with quercetin, kaempferol, apigenin, or onion extract, we could show that all treatments increased reporter gene activity, whereas a fourth flavonoid, myricetin, was unable to increase the GCS_h promoter activity. Quercetin was also able to induce the expression of a reporter construct containing the two distal ARE/EpRE elements of the GCS_h promoter.

MATERIALS AND METHODS

Cell culture

COS-1 cells, purchased from American Type Culture Collection, were grown in Dulbecco’s modified Eagle’s medium supplemented with 2 mM l-glutamine, 5 units/ml penicillin, 50 μg/ml streptomycin (Sigma, St. Louis, MO, USA), and 10% fetal calf serum (Integro b.v.) (COS-1 medium). The cell cultures were contained in a humidified atmosphere with 5% CO2 at 37°C. Lactate dehydrogenase (LDH) release was measured as control of toxicity according to the manufactures protocol (Roche Diagnostics, Ottweiler, Germany). Cells were examined microscopically for morphological changes after the different treatments.

Construction of plasmids

A reporter construct consisting of 3.8 kb of the GCS_h promoter in front of luciferase was kindly provided by R. T. Mulcahy [13]. Briefly, a 4.2 kb fragment was obtained from the 5’ flanking region of the human GCS_h gene and inserted into Hind III/Neo I sites of the pGL3 basic vector (Promega, Madison, WI, USA) resulting in the −3802/GCS_h-luc. The distal GCS_h-luc construct was obtained by amplification of a 312 bp fragment containing ARE/EpRE3 and ARE/EpRE4 from human genomic DNA by PCR (primers: 5′-ACT GCG GCA ATC CTA GCA GC-3′ and 5′-AAG CTT CTG GAC CGT GGA GAT CC-3′). The resulting fragment was cloned into the pCR 2.1-TOPO vector from Invitrogen (Carlsbad, CA, USA). This plasmid was digested with Hind III and the resulting fragment was ligated into the Hind III site of a TATA luc vector [25], resulting in the −3258: −2946/ GCS_h-luc recombinant plasmid. The proximal GCS_h-luc construct was obtained by digestion of the recombinant −3802/GCS_h-luc plasmid with Sac I. The resulting 3217 bp fragment was isolated and religated to obtain the recombinant -2752/GCS_h-luc.

Transient transfection of COS-1 cells

The cells were plated in 35 mm tissue culture wells the day before transfection at a density of approximately 60% confluence and transfected using a standard dextran-chloroquin method as described previously [26] with 0.7 μg DNA in each well. The cells were subjected to a DMSO shock (Sigma, St. Louis, MO, USA) (10% in PBS), after which they were incubated overnight in COS-1 medium. At the conclusion of this incubation period the medium was replaced with fresh COS-1 medium containing either DMSO or onion extract, quercetin, quercetin-3-glucoside, quercetin-3-rhamnoglucoside, kaempferol, myricetin, apigenin dissolved in DMSO. The flavonoids were purchased from Sigma.

Luciferase measurements

Luciferase activity was measured in cell lysates according to the manufacturers protocol (Promega).
Briefly, the cells were washed in PBS without Ca\(^{2+}\) or Mg\(^{2+}\) prior to the addition of 300 \(\mu\)l lysis buffer. The cells were incubated at room temperature for 20 min before collection by scraping and subsequent lysis by vigorous vortexing. The lysate was then briefly centrifuged to remove cell debris. Luciferase activity was measured by adding 100 \(\mu\)l luciferase assay solution to 20 \(\mu\)l of the lysate and luminescence was detected in a TD 20/20 luminometer (Turner Design, Sunnyvale, CA, USA). Protein concentration was measured in the cell lysate using Coomassie brilliant blue reagent from Bio-Rad Laboratories (Hercules, CA, USA).

**Onion extraction**

The three outer layers of onions were homogenized and lyophilized. One gram of dry material was extracted overnight at 4°C in 20 ml 70% methanol. The extract was filtered through a 0.5 \(\mu\)m Millipore filter type FH (Millipore, Waltham, MA, USA). The filtrate was concentrated by evaporation to a volume of 500 \(\mu\)l and diluted with 500 \(\mu\)l 100% DMSO (1 g freeze-dried onion/ml). From this stock solution various volumes were added to cell cultures in the experiments as indicated.

**Glutathione measurements**

Cells were harvested in a sodium phosphate buffer (10 mM, pH 6) containing 10 \(\mu\)M EDTA (Sigma) and thereafter lysed by several freeze-thaw cycles. Proteins were precipitated with approximately 2% TFA, and the resulting supernatant was neutralized to pH 2 with 1 M KOH. GSH was precipitated with approximately 2% TFA, and the resulting precipitate was analyzed by evaporation to a volume of 500 \(\mu\)l and diluted with 500 \(\mu\)l 100% DMSO (1 g freeze-dried onion/ml). Protein concentration was measured in the cell lysate using Coomassie brilliant blue reagent from Bio-Rad Laboratories (Hercules, CA, USA).

**Quantitative mRNA analysis**

The cells were plated in 35 mm tissue culture wells 2 d before mRNA isolation at a density of approximately 60% confluence. mRNA was isolated by oligo (dT)\(_{16}\) beads according to the manufacturers protocol (Genovision, Oslo, Norway). mRNA was eluted from the beads in 20 \(\mu\)l DEPC-treated water and cDNA synthesis was performed with Omniscript kit (Qiagen, Hilden, Germany) according to the manufacturer’s instructions. cDNA from each sample was analyzed by PCR amplification using LightCycler (Roche Diagnostics, Ottweiler, Germany) with Fast Start DNA Master Sybrgreen I kit (Roche Diagnostics). The PCR conditions were 4 mM MgCl\(_2\) for GCS\(_h\) and GCS\(_l\) or 3 mM MgCl\(_2\) for \(\beta\)-actin, 10 pmol of each PCR primer (GCS\(_h\) primers: 5’-AGA GAA GGG GGA AAG GAC AA-3’ and 5’-GTG AAC CCA GGA CAG CCT AA-3\(’\), GCS\(_l\) primers: 5’-TCA GTC CTT GGA GTT GCA CA-3’ and 5’-AAA TCT GGT GGC ATC ACA CA-3’; \(\beta\)-actin primers: 5’-TCG TGG AGT TTT TCC AGG AG-3’ and 5’-AGC ACT GTG TTG GCG TAC AG-3’), 2 \(\mu\)l of LightCycler DNA Master Mix, and cDNA to a final volume of 20 \(\mu\)l. After 10 min preincubation at 95°C, 45 PCR cycles were performed with 15 s denaturation at 95°C, 5 s annealing at 55°C (GCS\(_h\)), 55°C (GCS\(_l\)), and 12 s extension at 72°C. cDNA obtained by RT-PCR of GCS\(_h\), GCS\(_l\), and \(\beta\)-actin was cloned into a pCR 2.1-TOPO vector (Invitrogen, Carlsbad, CA, USA) to obtain a standard plasmid used to calculate the number of cDNA copies in a sample. After amplification, data analyses were performed using the second derivative maximum method. The second derivative maximum values were used to plot cycle number versus log concentration of the standard plasmid. The actual number of GCS\(_h\) and GCS\(_l\) cDNA copies was related to that of \(\beta\)-actin in each sample. Contamination of mRNA samples with genomic DNA was ruled out by omitting the reverse transcriptase in control reactions. The identity of the PCR products was confirmed by melting curve analyses and size determination.

**Statistical analysis**

Statistical analysis was carried out using Student’s \(t\)-test for unpaired two-tailed comparisons; \(p\) values less than .05 were considered significant.
RESULTS

Glutathione levels are increased in COS-1 cells incubated with onion extract or quercetin

To test the hypothesis that dietary flavonoids increase cellular endogenous antioxidants, COS-1 cells were treated with an extract of yellow onion known to contain high concentrations of flavonoids [27]. Exposing the cells to an extract containing 2 mg freeze-dried onion per ml medium (A) or increasing concentrations of quercetin (B) for 24 h resulted in a 47% increase in the GSH level (Fig. 1A). The cells were thereafter lysed by several freeze-thaw cycles. Proteins were precipitated and the resulting supernatant was analyzed for GSH and GSSG concentrations on a HPLC system. The amount of GSH and GSSG was related to the protein concentration in each sample. Data is given as % GSH related to that of control (0.1% DMSO) or as % GSSG of GSH (C). The mean values ± SD are presented. * p < .05 (n = 6).

mRNA of GCS₉ and GCS₁ are increased in COS-1 cells incubated with quercetin

To determine at what stage substances in plant extract exert their GSH increasing activity, mRNA levels of GCS₉ and GCS₁ were analyzed by quantitative RT-PCR after incubating cells with 5 and 25 μM quercetin for 17 h. The mRNA levels of both GCS₉ and GCS₁ were increased dose-dependently reaching a 2-fold increase with 25 μM quercetin (Fig. 2), suggesting that the increase in the glutathione level. Five and 25 μM quercetin significantly increased the GSH level by 34 and 51%, respectively (Fig. 1B). The level of oxidized glutathione (GSSG) constituted less than 0.2% of GSH both in untreated cells and in cells treated with concentrations of quercetin up to 50 μM (Fig. 1C). To rule out cytotoxic effects of quercetin we measured LDH release from the COS-1 cells after quercetin treatment. Five or 25 μM quercetin did not significantly influence LDH release as compared with DMSO-treated control cells (Table 1).

Table 1. LDH Release as a Measure of Loss of Membrane Integrity

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% LDH-release</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triton X-100 (2%)</td>
<td>100</td>
</tr>
<tr>
<td>DMSO (0.1%)</td>
<td>5.24 ± 3.03</td>
</tr>
<tr>
<td>Quercetin 5 μM</td>
<td>5.13 ± 0.45</td>
</tr>
<tr>
<td>Quercetin 25 μM</td>
<td>2.41 ± 0.16</td>
</tr>
</tbody>
</table>

LDH was measured 17 h after incubation with the indicated reagent. Quercetin was dissolved in DMSO and added to the cells in a final DMSO concentration of 0.1%. LDH release is expressed as % of the value measured in Triton X-100 (2%) treated cells. Data are presented as mean of values ± SD (n = 6).

Fig. 1. Effect of onion extract and quercetin on GSH levels in COS-1 cells. COS-1 cells were treated with an extract containing 2 mg freeze-dried onion per ml medium (A) or increasing concentrations of quercetin (B) for 24 h. The cells were thereafter lysed by several freeze-thaw cycles. Proteins were precipitated and the resulting supernatant was analyzed for GSH and GSSG concentrations on a HPLC system. The amount of GSH and GSSG was related to the protein concentration in each sample. Data is given as % GSH related to that of control (0.1% DMSO) or as % GSSG of GSH (C). The mean values ± SD are presented. * p < .05 (n = 6).

Fig. 2. Effect of quercetin on GCS₉ and GCS₁ mRNA levels. COS-1 cells were incubated with 5 μM quercetin (white bars) or 25 μM quercetin (gray bars) for 17 h using 0.1% DMSO (black bars) as control. The cells were harvested and quantitative RT-PCR was performed as described in Materials and Methods. The mRNA level of GCS₉ or GCS₁ is related to the mRNA level of β-actin in each sample. The results are given as fold increase compared to control levels and represent the mean values ± SD. * p < .05 (n = 8).
tathione level by quercetin is mediated by transcriptional induction of both GCS<sub>n</sub> and GCS<sub>h</sub> mRNA. To confirm this, we incubated COS-1 cells with 5 or 25 μM quercetin in the absence or presence of 1 μg/ml actinomycin D. We observed no increase in the cellular GSH levels in the presence of actinomycin D (data not shown).

The complete promoter of GCS<sub>h</sub> is induced in COS-1 cells incubated with various flavonoids or onion extract

To demonstrate that onion extract transactivated the GCS<sub>h</sub> promoter, extracts at various concentrations were added to COS-1 cells transfected with a luciferase reporter construct controlled by the 3.8 kb 5′ region of the GCS<sub>h</sub> gene (−3802/GCS<sub>h</sub>-luc, Fig. 5). Figure 3A shows that various extracts ranging from 0.5 to 2 mg freeze dried onion per ml medium induced the GCS<sub>h</sub> promoter activity in a concentration-dependent manner after 17 h incubation. The highest induction (2.7-fold increase) was found with 2 mg/ml onion extract while 0.5 and 1 mg/ml extract gave 1.7- and 2-fold increase in luciferase activity compared to control, respectively.

We then added quercetin to COS-1 cells transfected with the GCS<sub>h</sub> promoter luciferase construct to explore whether the effect of quercetin on the mRNA level of GCS<sub>h</sub> was due to transactivation of the GCS<sub>h</sub> promoter. After a 20 h post-transfection recovery period the cells were incubated with 5–75 μM quercetin for 17 h. The highest inductions were measured after exposing the cells to 25 and 50 μM quercetin (4.5- to 5-fold) while at the highest concentration (75 μM) a slightly lower induction was observed (4-fold). The lowest induction was observed with 5 μM (1.7-fold increase) (Fig. 3B). This is in line with the observed induction of GCS<sub>h</sub> mRNA at 5 μM quercetin, as measured by quantitative RT-PCR (Fig. 2). The human cell lines HepG2 and Jurkat were also subjected to treatment with quercetin under similar conditions as COS-1 cells. In both of these cell lines quercetin induced reporter gene activity to nearly the same extent as in COS-1 cells (data not shown). To rule out transactivation by quercetin-mediated hydrogen peroxide production in the cell medium, we incubated transfected cells with up to 250 μM hydrogen peroxide before measuring luciferase activity in cell homogenates. No increase in luciferase activity was observed after incubation of cells with hydrogen peroxide under the conditions used (data not shown).

In fruits and vegetables flavonoids are conjugated to sugar residues and are thus ingested in this form. We wanted to investigate whether quercetin containing sugar moieties also induced the GCS<sub>h</sub> promoter in COS-1 cells. The two derivates of quercetin, quercetin-3-glucoside or quercetin-3-rhamnoglucoside (5 or 25 μM), were added to COS-1 cells transfected with the reporter construct. The two quercetin conjugates had no effect on the GCS<sub>h</sub> promoter activity, whereas the aglycone form of quercetin potently induced luciferase activity through the GCS<sub>h</sub> promoter, as shown in Fig. 3C.

To investigate whether flavonoids in general can activate the GCS<sub>h</sub> promoter we incubated COS-1 cells with four of the most common flavonoids in dietary plants, namely quercetin, kaempferol, myricetin, and apigenin. Quercetin...
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Fig. 4. Induction of GCS<sub>n</sub> by various flavonoids in COS-1 cells. COS-1 cells transiently transfected with the complete GCS<sub>n</sub> luc were incubated with either 25 μM of quercetin (n = 12), kaempferol (n = 9), or myricetin (n = 9) or 10 μM apigenin (n = 9) for 17 h. The cells were thereafter washed in PBS, lysed, and scraped from the wells. Cell lysates were collected and luciferase activity was measured. Luciferase activity was related to protein concentrations in each well. Data is given as fold increase related to that of 0.1% DMSO (control) and each bar graph represent the mean value ± SD. * p < .05.

(25 μM), kaempferol (25 μM), and apigenin (10 μM) induced luciferase activity 3-fold, 2.2-fold, and 1.8-fold, respectively after 17 h of incubation (Fig. 4). Apigenin was toxic to the cells when added at concentrations higher than 10 μM as measured by release of LDH (data not shown). Myricetin treatment of transfected cells had no effect on transcription of the reporter construct.

Quercetin induce both distal and proximal parts of the GCS<sub>n</sub> promoter

As outlined in Fig. 5A, the 3.8 kb GCS<sub>n</sub> promoter contains several response elements that could mediate transactivation by the different flavonoids. These include 4 ARE/EpRE motifs, two located at the distal end of the promoter (ARE/EpRE3 and 4) and two located at the more proximal end (ARE/EpRE1 and 2). ARE/EpRE3 and 4 perfectly match the consensus ARE/EpRE sequence as defined by Nguyen and Pickett [29] while ARE/EpRE1 and 2 show differences in the first and the last position, respectively. To determine the part of the promoter through which the quercetin exerts its effect, we constructed reporter plasmids where luciferase are either driven by the 5' flanking region of GCS<sub>n</sub> spanning from −3258 to −2946 (ARE/EpRE3 and 4, Fig. 5) or −2752 to −1 (ARE/EpRE1 and 2, Fig. 5). Quercetin was thereafter tested for its ability to induce the expression of luciferase from the two constructs. As shown in Fig. 5, quercetin induced both the distal and the proximal part of the promoter. The induction of luciferase activity by quercetin was, however, higher when cells were transfected with a construct containing the 3.8 kb promoter, suggesting that the different promoter elements operate together.

DISCUSSION

In a recent study quercetin and other flavonoids were found to efficiently protect neuronal cells from oxidative glutamate toxicity and other forms of oxidative injury. Interestingly, increasing the production of GSH was one of three mechanisms suggested by the authors for the protective effects of quercetin [30]. In our study using COS-1 cells we show that indeed quercetin elevates the GSH level and the expression of both the regulatory and the catalytical subunit of GCS. In addition we find that quercetin potently transactivates the GCS<sub>n</sub> promoter. Thus, our data strongly suggest that the elevation of GSH by flavonoids is due to an increased synthesis of GCS. The activity of GCS is regu-
lated both at the transcriptional and post-transcriptional level, including post-translational regulation. Thus, the overall regulation related to transcriptional regulation of GCS₉ as described in the present work is not clear. The nonlinear relationship between GCS₉ promoter transactivation, GCS₉ mRNA levels, and GSH suggest that indeed other regulatory mechanisms also contribute to the observed GSH regulation. The ability to regulate GSH production may be one of the mechanisms cells have evolved to mount a defense against various stress conditions, since GSH can act as a versatile compound in the combat against a battery of different insults [31].

Flavonoids are usually present in plant food as sugar conjugates. Neither quercetin-3-glucoside nor quercetin-3-rhamnogluconoside (rutin) were able to activate gene transcription through GCS₉ promoter (Fig. 3D) in our experiments. This indicates that the transcriptional active compound is the aglycone form of the flavonoids, or that the cell did not efficiently take up the sugar-conjugates of quercetin. It has been suggested that quercetin-gluconides are taken up through a Na-dependent glucose transport [32]. As our experiments are carried out in medium with high glucose it is possible that uptake of quercetin-gluconides is competed out with glucose. Alternatively, quercetin is rendered more hydrophilic by the sugar moieties and thus has less potential to passively diffuse across the cell membrane. Quercetin was recently shown to activate the promoter of NADPH-quinone oxidoreductase through ARE/EpRE-dependent mechanisms [24]. This observation together with our results strongly indicates that dietary flavonoids can stimulate transcription of antioxidant and detoxification defense systems through ARE elements. The observation that different flavonoids induced the reporter with different efficiency suggests that induction through ARE/EpRE distinguishes between subtle differences in flavonoids structure. Also, Ishige and co-workers observed differences in the ability of flavonoids to regulate cellular GSH content. Quercetin and fisetin increased the total GSH level, whereas kaempferol and luteolin did not [30]. The structure-activity relationships may be related to their antioxidant capacity or to their potential function as ligands for hitherto unknown receptors. Alternatively, the flavonoids may be able to influence an intracellular sensor protein, Keap1. Keap1 is critical in transcriptional activation of proteins participating in cellular defense [31,33]. It has been shown that Keap1 sequesters Nrf2 in the cytosol, preventing it from ARE/EpRE-dependent transactivation [33]. We speculate that flavonoids are able to modify the interaction between Keap1 and Nrf2, thereby releasing Nrf2, which can translocate into the nucleus and transactivate the GCS₉ promoter. The nature of the Keap1 Nrf2 interaction is still not clear.

Dinkova-Kostova et al. have made further advances by providing evidence that the potentials for phase 2 enzyme induction, increase in cellular GSH levels, and thiol reactivity are closely related [34]. Flavonoids have been shown to react with sulphydryl groups [35], supporting the hypothesis that flavonoids can modulate one or several sensor protein(s), of which Keap1 could be a member. Further experiments are needed to explore these possibilities.

Induction of expression of protective enzymes may be an important aspect of the chemopreventive effects of fruits and vegetables. Chemopreventive agents that enhance the cellular capacity to combat oxidative stress have been shown to inhibit chemically induced carcinogenesis in animal models [36–38]. Humans ingest approximately 1 g/d of polyphenols, of which 20–80 mg are flavonoids [39]. Ingested flavonoids are then extensively metabolized both in the small intestine and after eventual absorption. We obtained GSH induction with only the aglycone of quercetin, suggesting that the cells most efficiently take up this form of quercetin. Whether quercetin exerts its effect on the GCS₉ promoter before or after conversion to another flavonoid or conjugation cannot be determined from our experiments. However, the results suggest that even if quercetin-gluconide may be absorbed in the intestine it does not transactivate GCS₉. The use of purified compounds in experimental systems may provide insight into chemoprevention, however, the human body has adapted to a high intake of naturally occurring phenolic compounds. Chemoprevention is therefore most likely to succeed with recommendation of a high intake of fruits and vegetables.

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REFERENCES


ABBREVIATIONS
ARE/EpRE—antioxidant-response element/electrophile-response element
DMSO—dimethyl sulfoxide
GCS—γ-glutamylcysteine synthetase
GSH—glutathione
GSSG—oxidized glutathione
LDH—lactate dehydrogenase
ROS—reactive oxygen species
RT-PCR—reverse transcriptase PCR
TFA—trifluoroacetic acid
XRE—xenobio-tica-response element