Biochemical and Molecular Actions of Nutrients

# Quercetin Attenuates Nuclear Factor-κB Activation and Nitric Oxide Production in Interleukin-1β–Activated Rat Hepatocytes[1]

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ABSTRACT We investigated whether different concentrations of the flavonoid quercetin ameliorate nitric oxide production and nuclear factor (NF)-κB activation in interleukin (IL)-1β–activated rat hepatocytes. Primary cultures of rat hepatocytes were treated with IL-1β alone or with quercetin in concentrations ranging from 5 to 100 μmol/L. The generation of reactive oxygen species, assessed by flow cytometry using dichlorodihydrofluorescein diacetate, was significantly reduced, and the oxidized:reduced glutathione ratio decreased in cultures treated with 50 and 100 μmol/L of quercetin. Quercetin at 100 μmol/L significantly prevented the IL-1β-induced release of nitrite into the culture medium. Western blot and reverse transcription-PCR analyses demonstrated that increased levels of inducible nitric oxide synthase (iNOS) protein and mRNA in hepatocytes stimulated by IL-1β were prevented by 50 μmol/L and 100 μmol/L of quercetin. Electrophoretic mobility shift assay experiments and Western blots indicated that quercetin blocked the activation of NF-κB and decreased the inhibitor κB protein levels induced by IL-1β. In summary, quercetin, a natural flavonol widely distributed in the human diet, inhibits NO production in IL-1β–stimulated hepatocytes through the inhibition of iNOS expression. Although the mode of action remains to be clarified, our findings support the view that the mechanism of action is via inhibition of IL-1β–induced NF-κB activation. J. Nutr. 135: 1359–1365, 2005.

KEY WORDS: • *interleukin-1β* • *nitric oxide* • *nuclear factor-κB* • *quercetin* • *rat cultured hepatocytes*

Interleukin-1β (IL-1β)[3] is a multifunctional cytokine that plays a critical role in inflammation, immunity, antiviral responses, and a variety of diseases (1,2). IL-1β binds to specific high-affinity cell surface receptors and has pleiotropic effects that include costimulation of T lymphocytes, B-cell proliferation, induction of adhesion molecules, and stimulation of the production of other cytokines and inflammatory mediators (3). IL-1β is one of the most important cytokines in the liver, and increased IL-1β levels have been observed in liver diseases (4–6).

Nitric oxide (NO) is a potent biological mediator produced by hepatocytes after exposure to cytokines, including IL-1β (7,8). The production of NO is regulated by intracellular nitric oxide synthases; of the 3 isoforms of NO synthase, the isoform expressed in macrophages and hepatocytes is termed inducible NOS (iNOS). Its activity is regulated at the transcriptional level by cytokines and the exposure of cells to other inflammatory stimuli such as endotoxin or reactive oxygen species (ROS) (9). During inflammation, NO and its metabolites, such as peroxynitrite, are potentially cytotoxic and capable of injuring the invading pathogens and eliminating altered cells (10). However, indiscriminate destruction of cells and tissues by NO and its reactive nitrogen intermediates may be involved in the pathology of many inflammatory conditions; therefore, production of NO induced by iNOS may reflect the degree of inflammation and provide a measure with which to assess the effect of drugs on the inflammatory process (11). Thus, selective inhibition of the iNOS pathway is a rational approach because attenuation of inflammation and suppression of NO production may be effective therapeutic strategies for preventing inflammatory reactions and diseases (12).

Nuclear factor (NF)-κB belongs to the Rel family of transcriptional activator proteins and is induced by a number of pathogens and agents, including IL-1β (13,14). NF-κB activates several different genes important for the inflammatory response, including cytokines, growth factors, and inflammatory enzymes (15). The promoters of murine and human genes encoding iNOS contain a consensus sequence for the binding of NF-κB, which is necessary to confer inducibility by cytokines and lipopolysaccharide (LPS) (16). Because of its ubiquitous role in the pathogenesis of inflammatory gene expression, NF-κB is a current target for treating inflammatory diseases, and inhibition of NF-κB activation may be of therapeutic benefit in various types of inflammation.

Flavonoids are phenolic phytochemicals that represent substantial constituents of the nonenergetic part of the human

[1] Supported by Plan Nacional de I+D, Spain (grant no. BFI2003–03114).
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[3] Abbreviations used: COX, cyclooxygenase; DCF, dichlorofluorescein; DCFH-DA, dichlorodihydrofluorescein diacetate; DMSO, dimethyl sulfoxide; EMSA, electrophoretic mobility shift assay; GSH, reduced glutathione; GSSG, oxidized glutathione; IκBα, inhibitory κBα; IKK, IκB kinase; IL, interleukin; iNOS, inducible nitric oxide synthase; LDH, lactate dehydrogenase; LPS, lipopolysaccharide; NF, nuclear factor; ROS, reactive oxygen species.

0022-3166/05 $8.00 © 2005 American Society for Nutritional Sciences.
Manuscript received 22 December 2004. Initial review completed 6 February 2005. Revision accepted 9 March 2005.



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diet and are thought to promote optimal health through biological functions such as apoptosis-inducing activity, free radical scavenging activity, and antitumorigenic activity (17). They contain a number of phenolic hydroxyl groups, conferring strong antioxidative activity and therapeutic potential for some diseases, including cancer, ischemic heart disease, and atherosclerosis (18,19). Their antiradical property is directed toward the highly reactive species implicated in the initiation of lipid peroxidation. Moreover, flavonoids are soluble chain-breaking inhibitors of the peroxidation process, scavenging intermediate peroxyl and alkoxyl radicals (20).

Several flavonoids were shown to inhibit the expression of NF-κB–dependent cytokines, iNOS, and cyclooxygenase (COX)-2 genes (21). Quercetin (3,5,7,3′,4′-pentahydroxy flavone) is one of the most widely distributed flavonoids; it is present in fruit, vegetables and many other dietary sources. Quercetin was reported to have an inhibitory effect on LPS-induced iNOS gene expression in different LPS-stimulated macrophage lines (9,22,23), rat Kupffer cells (24), and the C6-astrocytic cell line (25). Quercetin inhibits NF-κB activation in cultured human synovial cells (26), primary cultured rat proximal tubule epithelial cells (27), and rat aortic smooth muscle cells (21). However, although different flavonoids such as resveratrol, naringenin, or apigenin inhibit enhanced expression of iNOS in macrophages through downregulation of NF-κB (23), quercetin does not modify NF-κB activity in LPS-stimulated RAW 264.7 macrophages, and it was suggested that it may downregulate iNOS expression by modulating enzyme activity related to signal transduction (28).

In the present study, rat hepatocytes stimulated with IL-1β were used as a model for iNOS induction. We tested whether the induction of iNOS and the NO production could be prevented by quercetin and whether iNOS-induced downregulation involved the inhibition of NF-κB activation.

## MATERIALS AND METHODS

***Animals.*** Male Wistar rats weighing 200–250 g were housed in a room maintained at 22°C with humidity ranging from 45 to 55% and a 12-h dark:light cycle. They had free access to food (standard diet for rats Panlab A04[4]) and water, and were not food deprived before experiments. All study protocols were reviewed and approved by the University of Leon Animal Care Committee and were in accordance with the indications of the Guide to the Care and Use of Experimental Animals.

***Hepatocyte isolation.*** Hepatocytes were isolated by a nonrecirculating in situ collagenase (Sigma) perfusion of liver through the portal vein. Hepatocytes were separated from nonparenchymal cells by differential centrifugation 4 times at 50 × *g*. Hepatocyte purity assessed by microscopy was >98% and cell viability consistently exceeded 95% by the trypan blue exclusion test.

***Cell culture and treatment.*** The isolated hepatocytes were suspended in culture medium at 5.5–6.0 × $10^8$ cell/L, seeded onto plastic dishes (2 mL/dish, 35 mm × 10 mm:9 $cm^2$, Falcon Plastic), and then cultured in monolayers in a 5% $CO_2$ humidified incubator at 37°C. The culture medium used was Williams' medium E (Gibco) supplemented with 10% fetal calf serum, Hepes (5 mmol/L), penicillin (1 × $10^5$ units/L), streptomycin (100 mg/L), and insulin (10 nmol/L). After 4 h, the medium was changed to include IL-1β (0.1 nmol/L) alone or with quercetin (5, 50, or 100 μmol/L) dissolved in dimethyl sulfoxide (DMSO; 0.1%). After the incubation period, culture medium and hepatocytes were collected and frozen at −80°C.

[4] The composition of the standard diet was: 15.4% protein, 2.9% fat, 60.5% carbohydrate, 3.9% fiber, 5.3% minerals, and 12% water.

***Lactate dehydrogenase (LDH) release.*** LDH activity in the culture medium was measured by incubation with β-NADH (0.2 mmol/L) and pyruvic acid (0.4 mmol/L) diluted in PBS. LDH release was calculated using a commercial standard (Merck).

***Nitrite determination.*** Accumulation of nitrite in the medium was used as a measure of NO formation. Nitrite was determined by the Griess method, adapted from Green et al. (29).

***Intracellular generation of ROS.*** Production of peroxides was monitored by flow cytometry using dichlorodihydrofluorescein diacetate (DCFH-DA; Sigma) (30). This dye is a stable nonpolar compound that readily diffuses into cells and yields DCFH. Intracellular $H_2O_2$, low-molecular-weight peroxides, peroxynitrites, and nitric oxide oxidize DCFH to the highly fluorescent compound dichlorofluorescein (DCF). Thus, fluorescence intensity is proportional to the amount of oxidants produced by the cells. At the end of the incubation periods, cells were washed with PBS and immediately detached with trypsin/EDTA and incubated for 30 min in 2 mL of PBS containing 5 μmol/L DCFH-DA at 37°C. The cells were washed twice with PBS to remove the extracellular DCFH-DA, followed by analysis on a FACSCalibur flow cytometer (Becton Dickinson Immunocytometry Systems; excitation 488 nm and emission 525 nm for DCF). Quantification of fluorescence intensity of M2 peaks was expressed as a percentage of control values. ROS production was corroborated by confocal microscopy using a confocal laser scanning microscope Radiance 2000 (Bio-Rad).

***Oxidized:reduced glutathione concentration.*** Oxidized (GSSG) and reduced (GSH) glutathione analysis was performed fluorimetrically in hepatocyte homogenates by the method of Hissin and Hill (31).

***Western blot analysis.*** Protein extraction and Western blotting were performed as described (30). Membranes were probed with anti-iNOS or anti-inhibitory κBα (IκBα) antibodies (Santa Cruz Biotechnology). Bound primary antibody was detected using a peroxidase-conjugated secondary antibody (DAKO) by chemiluminescence using the ECL kit (Amersham Pharmacia Biotech). The densities of the specific iNOS (130 kDa) and IκBα (36 kDa) bands were quantitated with an imaging densitometer. Equal loading of the gels was demonstrated by probing the membranes with an anti-β-actin polyclonal antibody. Blots were developed by enhanced chemiluminescence (Amersham International).

***RT-PCR.*** RNA extraction and reverse transcriptase reaction were performed as described (30). PCR on complementary DNA was performed by using primers purchased from Biosource International. The PCR-primer sequences for rat iNOS were (sense) 5′-CACATCTGGCAGGATGAGAA-3′ and (antisense) 5′-GAAGGCGTAGCTGAACAAGG-3′. The mRNA levels were normalized against β-actin mRNA. The amplified products for iNOS and β-actin contained 201 and 457 bp, respectively. After amplification, PCR products were subjected to electrophoresis in 1% agarose gel and visualized by means of ethidium bromide staining. Fragments were then photographed using a Gelprinter plus photodocumentation system (TDI).

***Electrophoretic mobility shift assay (EMSA).*** Nuclear extracts were prepared from hepatocyte lysates as described previously (32). Activation of transcription factor NF-κB was examined using consensus oligonucleotides of NF-κB (5′-AGT TGA GGG GAC TTT CCC AGG C-3′). Probes were labeled by T4 polynucleotide kinase as described (33). Binding reactions included 10 μg of nuclear extracts in incubation buffer [10 mmol/L Tris-HCl, pH 7.5, 40 mmol/L NaCl, 1 mmol/L EDTA and 4% glycerol and 1 μg poly (dI-dC)]. After 15 min on ice, the labeled oligonucleotide was added and the mixture incubated for 20 min at room temperature. For competition studies, 3.5 pmol of unlabeled NF-κB oligonucleotide was mixed for 15 min before the incubation with the labeled oligonucleotide. The mixture was electrophoresed through a 6% polyacrylamide gel for 90 min at 150 V. The gel was then dried and autoradiographed at −70°C overnight. Signals were analyzed densitometrically.

***Statistical analysis.*** Means and SEMs were calculated. Significant differences between means were evaluated by ANOVA and Newman-Keul's test. A difference was considered significant when $P$ was $<0.05$.

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## RESULTS

***LDH release.*** IL-1$\beta$ increased LDH activity in the culture medium (+68%). This effect was significantly prevented by quercetin at 100 $\mu$mol/L (**Table 1**).

***Generation of ROS.*** We investigated the generation of oxidants by hepatocytes (**Fig. 1**). Analysis of histograms in which the fluorescence, detected with the FL1-H channel, was plotted against the relative number of cells (Fig. 1*A*) and quantification of the corresponding M2 peaks (Fig. 1*B*) indicated that the increase induced by IL-1$\beta$ was significantly blocked by 50 and 100 $\mu$mol/L quercetin concentrations. Representative fluorescent images of DCFH-DA–stained cells obtained by confocal microscopy corroborated the results obtained by flow cytometry (**Fig. 2**).

***Oxidized:reduced glutathione concentration.*** A decrease in the hepatic concentration of GSH (−24%) and an increase in that of GSSG (+42%) and in the GSSG:GSH ratio (+56%) were observed in IL-1$\beta$–treated hepatocytes (**Table 2**). These effects were significantly inhibited by administration of quercetin at 100 $\mu$mol/L.

***Nitrite release and iNOS expression.*** Stimulation of hepatocytes with IL-1$\beta$ caused a significant increase in nitrite ion (oxidation products of NO) release into the culture medium (+65%). This increase was significantly prevented by simultaneous addition of quercetin at 100 $\mu$mol/L (Table 1). Quercetin at 50 and 100 $\mu$mol/L inhibited the induction of iNOS mRNA (**Fig. 3**) and protein (**Fig. 4**) stimulated by IL-1$\beta$ in hepatocytes.

***NF-κB activation and IκBα levels.*** Incubation of hepatocytes with IL-1$\beta$ induced a marked activation of NF-$\kappa$B that was significantly reduced in hepatocytes treated with different concentrations of flavonoid quercetin (**Fig. 5**). Because activation of NF-$\kappa$B correlates with rapid proteolytic degradation of I$\kappa$B$\alpha$, we assessed protein levels of I$\kappa$B$\alpha$. Protein levels were decreased by IL-1$\beta$ and this effect was reduced by 50 $\mu$mol/L quercetin and blocked by 100 $\mu$mol/L quercetin (**Fig. 6**).

## DISCUSSION

In our study, quercetin inhibited the accumulation of nitrite, and this decrease in NO production was consistent with the inhibition of IL-1$\beta$–induced iNOS gene expression as shown by Western blotting and RT-PCR. Several reports demonstrated previously that in LPS-stimulated macrophages or gastric mucosal cells (34,35), inhibition of iNOS expression is mediated by impairment of signaling pathways such as mitogen-activated protein kinases and NF-$\kappa$B. However, results in our study differed from the previous report showing that quercetin inhibits the production of NO by LPS-stimulated Kupffer cells and decreases the amount of iNOS without affecting mRNA levels, an observation suggesting that the inhibition of NO production might occur at a post-transcriptional level (24). In RAW 264.7 macrophages, quercetin and resveratrol were proposed to act more by scavenging NO radicals than by inhibition of iNOS gene expression (36). Our data highlight the divergence between different cell types and suggest that the inhibitory action of quercetin on NO produc-

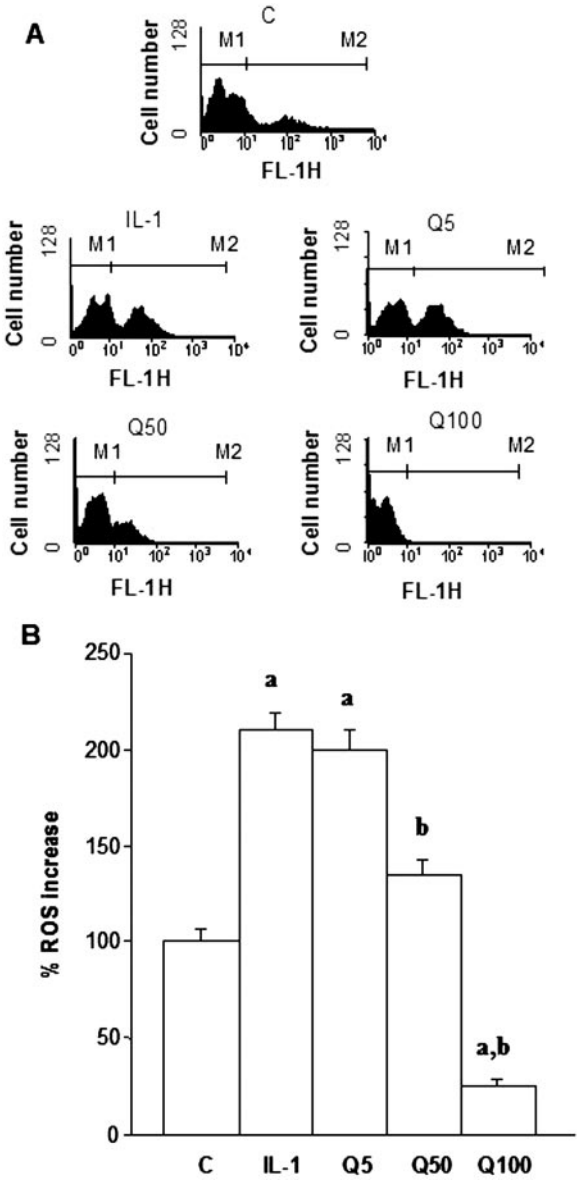


**FIGURE 1** Intracellular oxidant generation in cultures of rat hepatocytes incubated with IL-1$\beta$ alone or with various concentrations of quercetin for 16 h. (*A*) Representative histograms in which the fluorescence (FL1-H) is plotted against the number of cells. (*B*) The quantification of M2 peaks referred by fluorescence intensity as a percentage of the control values. Values are means ± SEM, $n = 5$. Groups: C: control; IL-1: 0.1 nmol/L IL-1$\beta$; Q5: IL-1 + 5 $\mu$mol/L quercetin; Q50: IL-1 + 50 $\mu$mol/L quercetin; Q100: IL-1 +100 $\mu$mol/L quercetin. [a]Different from C, $P < 0.05$; [b]different from IL-1$\beta$, $P < 0.05$.

## TABLE 1

*LDH activity and nitrite concentration in the culture medium of rat isolated hepatocytes incubated with IL-1$\beta$ alone or with various concentrations of quercetin*[1]

| Group | LDH | Nitrite |
|---|---|---|
| | *nmol/(min · 10$^6$ cells)* | *nmol/10$^6$ cells* |
| C | 218.93 ± 10.57 | 3.24 ± 0.85 |
| IL-1 | 541.62 ± 71.23[a] | 9.19 ± 1.30[a] |
| Q5 | 541.21 ± 67.71[a] | 9.09 ± 0.55[a] |
| Q50 | 508.50 ± 27.86[a] | 6.81 ± 1.04[a] |
| Q100 | 335.44 ± 11.64[ab] | 3.57 ± 0.76[b] |

[1] Values are means ± SEM, $n = 5$.
[a] Different from C, $P > 0.05$; [b] different from IL-1$\beta$, $P > 0.05$. Groups: C: control; IL-1: 0.1 nmol/L IL-1$\beta$; Q5: IL-1 + 5 $\mu$mol/L quercetin; Q50: IL-1 + 50 $\mu$mol/L quercetin; Q100: IL-1 + 100 $\mu$mol/L quercetin.

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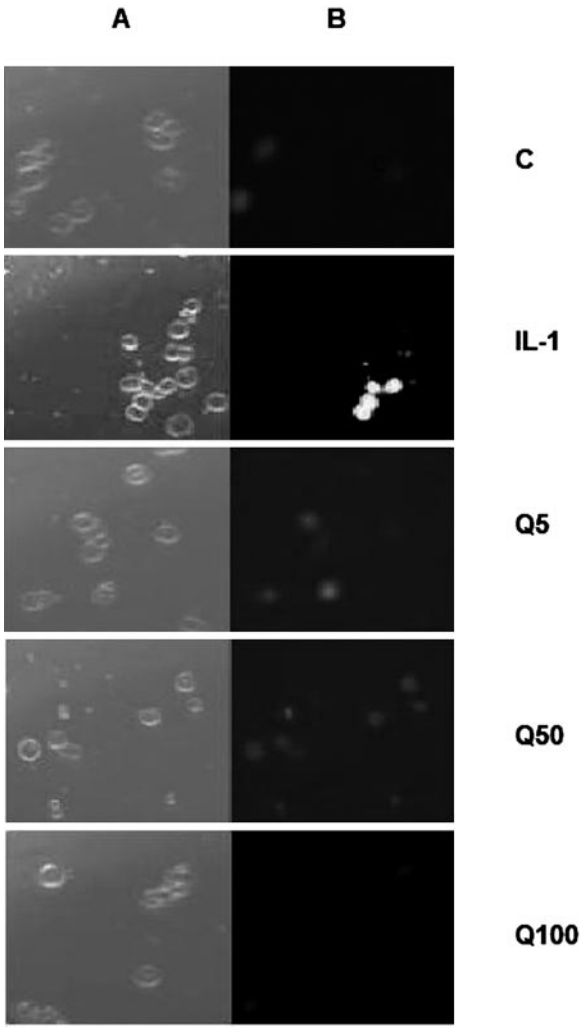


**FIGURE 2** Intracellular peroxide generation in cultures of rat hepatocytes incubated with IL-1$\beta$ alone or with various concentrations of quercetin for 16 h. (*A*) Confocal reflection microscopy images are shown so as to detect unlabeled cells in the population of fluorescently labeled cells. See Figure 1 legend for group definitions. (*B*) Representative fluorescent images.

tion in IL-1$\beta$–stimulated hepatocytes might be due to abrogation of iNOS protein induction by impairment of intracellular signal pathways.

In theory, there are several critical steps at which quercetin may modulate the cascade of molecular events leading to the expression of iNOS in IL-1$\beta$–treated hepatocytes. Certain flavonoids were reported to inhibit protein kinase C, phospholipase C or $A_2$, and phosphodiesterases (37). Another possibility includes modulation of iNOS indirectly by inhibition of the COX and/or lipoxygenase pathways (38). Nevertheless, all pathways of iNOS induction seem to converge in the activation of the essential transcription factor NF-$\kappa$B, and the expression of iNOS is inhibited when NF-$\kappa$B activation is downregulated by proteasome inhibitors or overexpression of I$\kappa$B mutants (39).

However, the relation between quercetin and NF-$\kappa$B is inconsistent, and it was reported that quercetin induces NF-$\kappa$B–dependent apoptosis in L1210 lymphocytic leukemic cells (40) or does not reduce NF-$\kappa$B in the renal cortex of rats with glomerular disease (41). Quercetin and resveratrol (100–200 $\mu$mol/L) inhibit LPS-dependent production of iNOS mRNA

**TABLE 2**

*GSH and GSSG concentrations and the GSSG:GSH ratio in rat isolated hepatocytes incubated with IL-1$\beta$ alone or with various concentrations of quercetin*[1]

| Group | GSSG | GSH | GSSG:GSH × 100 |
|---|---|---|---|
| | *nmol/10$^6$ cells* | | |
| C | 7.18 ± 1.19 | 112.16 ± 8.42 | 6.4 ± 1.4 |
| IL-1 | 12.47 ± 0.53[a] | 84.56 ± 6.44[a] | 14.7 ± 0.9[a] |
| Q5 | 12.12 ± 0.47[a] | 93.95 ± 8.92[a] | 12.9 ± 0.5[a] |
| Q50 | 11.38 ± 0.94[a] | 106.38 ± 7.43[b] | 10.7 ± 1.3[ab] |
| Q100 | 9.19 ± 0.84[b] | 106.91 ± 12.38[b] | 8.6 ± 0.7[b] |

[1] Values are means ± SEM, $n = 5$.
[a] Different from C, $P > 0.05$; [b] different from IL-1$\beta$, $P > 0.05$. See Table 1 for group definitions.

and decrease NO release but do not inhibit activation of NF-$\kappa$B in RAW 264.7 macrophages; this apparently does not support the view that the effects of these flavonoids on genes activated by LPS are mediated by NF-$\kappa$B activation (28). Quercetin is a major component in *Gingko biloba* extract, which was shown to inhibit iNOS mRNA and NO production in LPS/interferon $\gamma$–activated macrophages while having no effect on NF-$\kappa$B activation (42). In LPS-stimulated macrophages, a higher concentration of resvera-

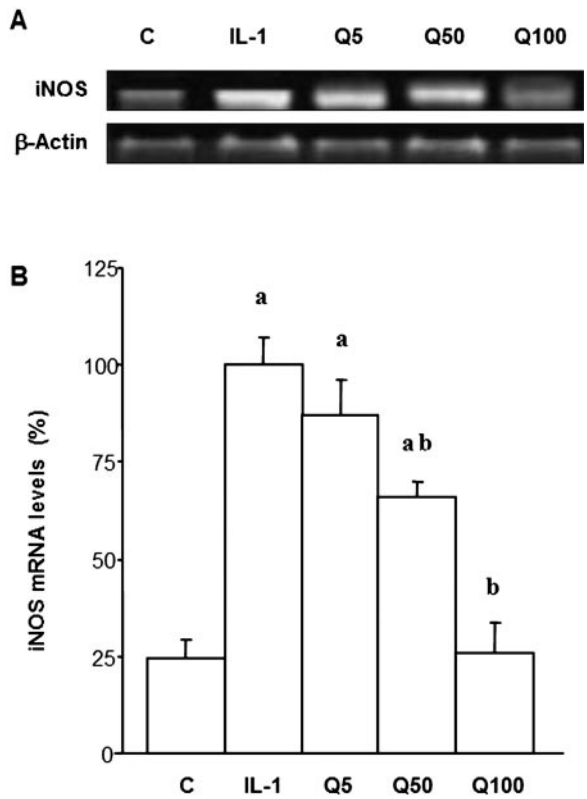


**FIGURE 3** iNOS mRNA levels (RT-PCR) in cultures of rat hepatocytes incubated with IL-1$\beta$ alone or with various concentrations of quercetin for 16 h. (*A*) Representative RT-PCR reactions. (*B*) Values are means ± SEM expressed as a percentage of IL-1$\beta$ values, normalized to $\beta$-actin mRNA, $n = 5$. See Figure 1 legend for group definitions. [a]Different from C, $P < 0.05$; [b]different from IL-1$\beta$, $P < 0.05$.

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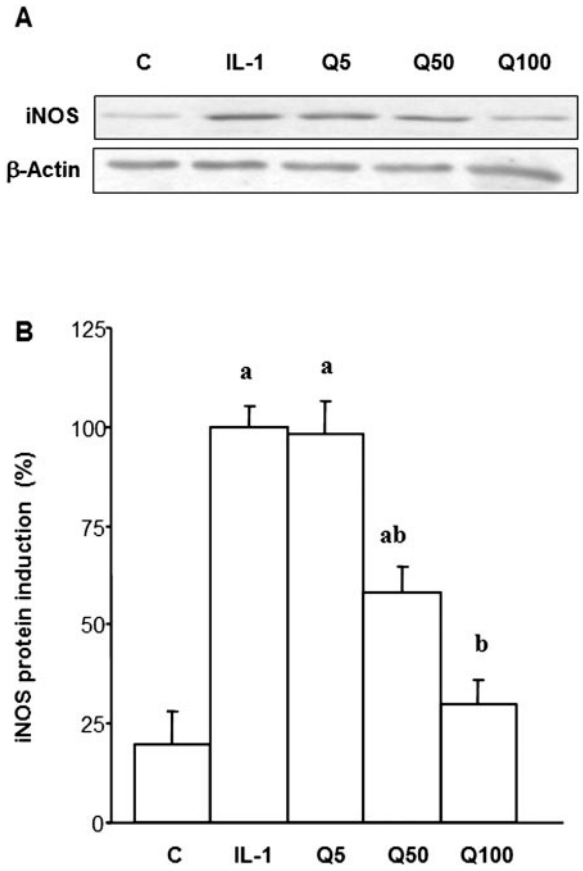


**FIGURE 4** Western blot analysis of iNOS protein in cultures of rat hepatocytes incubated with IL-1$\beta$ alone or with various concentrations of quercetin for 16 h. (*A*) Representative Western blot photographs. (*B*) Values are means ± SEM expressed as a percentage of IL-1$\beta$ values, normalized to $\beta$-actin mRNA, $n = 5$. See Figure 1 legend for group definitions. [a]Different from C, $P < 0.05$; [b]different from IL-1$\beta$, $P < 0.05$.

trol than that needed for the inhibition of NO production is required for the inhibition of NF-$\kappa$B mobilization or iNOS expression (43).

The results of the present study indicate that IL-1$\beta$–stimulated NO production in hepatocytes is associated with activation of the transcription factor NF-$\kappa$B; the EMSA showed that cells exposed to quercetin at concentrations of 50 and 100 $\mu$mol/L caused an inhibition of NF-$\kappa$B activation and a parallel downregulation of iNOS gene expression. A similar effect on NF-$\kappa$B activation was reported previously in aortic smooth muscle cells treated with the same quercetin doses (21), and even lower concentrations of the flavonoid are effective in HepG2 cells treated with $H_2O_2$ (44) or fibroblast L-TK cells treated with IL-1$\alpha$ (45). Our data support the view that antioxidants are cell specific in their ability to inhibit NF-$\kappa$B and clearly suggest that quercetin acts in the hepatocytes on the signal transduction pathways relating IL-1$\beta$ stimulation and NF-$\kappa$B activation.

Although LPS-induced NF-$\kappa$B activation in some cell lines appears to be mediated through its ability to stimulate the production of ROS (46), in RAW 264.7 cells, it was reported that LPS and ROS exhibit differential effects because activation of NF-$\kappa$B is resistant to *N*-acetyl cysteine, resveratrol, or quercetin, whereas some antioxidants inhibit activation induced by $H_2O_2$ (28). There is, however, increasing evidence that ROS are mediators in cytokine signaling pathways and IL-1 stimulates the production of ROS itself (47). On the basis of the present observations, the inhibition of quercetin on NF-$\kappa$B activation induced by IL-1$\beta$ can be explained by ameliorated intracellular oxidative stress in the signaling pathway of IL-1$\beta$ to NF-$\kappa$B activation. Indeed, the suppressive effects of quercetin on NF-$\kappa$B activation at 50 and 100 $\mu$mol/L were parallel to a lower DCF fluorescence, indicating attenuated production of oxidants, and to a reduced GSSG:GSH ratio at these concentrations. Our data coincide with a previous report that genistein at 50 and 100 $\mu$mol/L suppressed NF-$\kappa$B activation and TBARS accumulation, increasing the GSH level and antioxidant enzyme activities (superoxide dismutase and catalase) in RAW 264.7 macrophages (48). iNOS induction in hepatocytes in vivo and in vitro is dependent on intracellular glutathione status and correlates with NF-$\kappa$B binding, and hepatocyte glutathione depletion prevents iNOS induction (49). Moreover, quercetin was shown to elevate the GSH level and the expression of both the regulatory and the catalytical subunits of glutathione cysteine synthetase (50).

NF-$\kappa$B is present in the cytoplasm of unstimulated cells in a complex with the inhibitor I$\kappa$B. When the cells are stimulated with cytokines such as IL-1, I$\kappa$B is phosphorylated and dissociates from NF-$\kappa$B, allowing its migration to the nucleus, where it activates its target genes (51). The signal-induced phosphorylation of I$\kappa$B involves 2 I$\kappa$B kinases, IKK$\alpha$ and IKK $\beta$. I$\kappa$B$\alpha$ degradation results in rapid changes in NF-$\kappa$B induc-

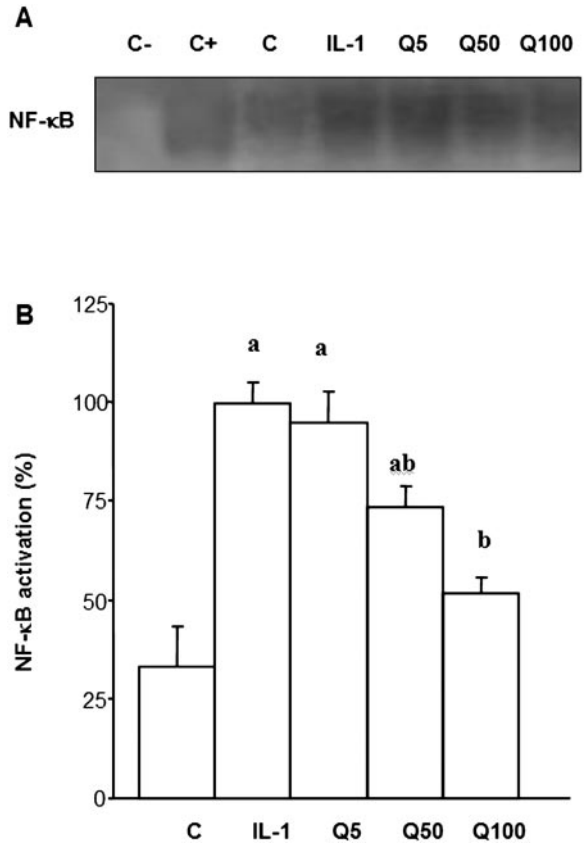


**FIGURE 5** NF-$\kappa$B activation in cultures of rat hepatocytes incubated with IL-1$\beta$ alone or with various concentrations of quercetin for 30 min. Specific binding was verified by addition of unlabeled (cold) oligonucleotide (competitor, C−) or labeled oligonucleotide mutate (noncompetitor, C+). (*A*) A representative EMSA. (*B*) Values are means ± SEM expressed as a percentage of IL-1$\beta$ values, $n = 5$. See Figure 1 legend for group definitions. [a]Different from C, $P < 0.05$; [b]different from IL-1$\beta$, $P < 0.05$.

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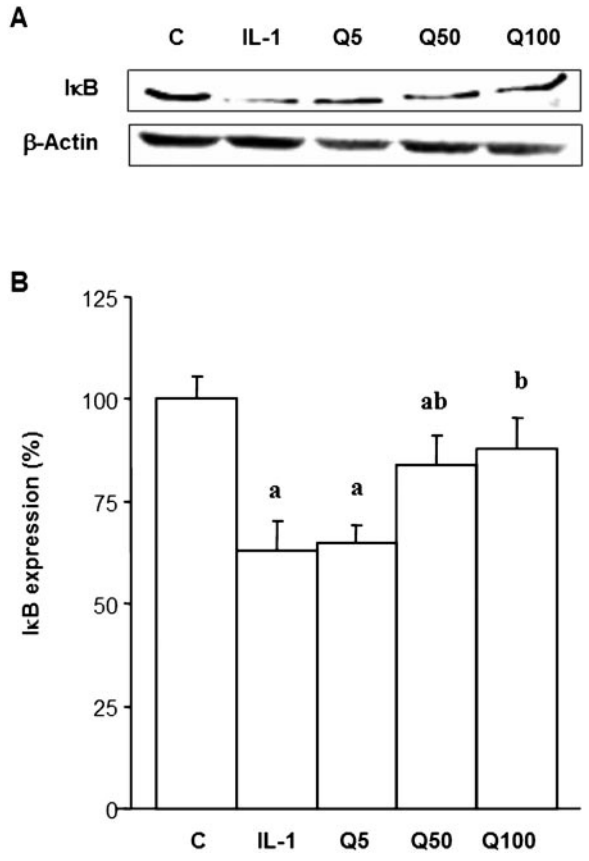


**FIGURE 6** Western blot analysis of IκB-$\alpha$ protein in cultures of rat hepatocytes incubated with IL-1$\beta$ alone or with various concentrations of quercetin for 30 min. (*A*) Representative Western blot photographs. (*B*) Values are means ± SEM expressed as a percentage of IL-1$\beta$ values, normalized to $\beta$-actin mRNA, $n = 5$. See Figure 1 legend for group definitions. [a]Different from C, $P < 0.05$; [b]different from IL-1$\beta$, $P < 0.05$.

tion, whereas IκBβ degradation is associated with prolonged NF-κB activation (52). Some flavonoids were reported to inhibit NF-κB through the activation of IκB kinases (53,54) and downregulation of iNOS expression is associated with the suppression of the release of NF-κB into LPS-stimulated macrophages (34). We found that IL-1$\beta$ stimulation induced a significant decrease in IκBα; in contrast, quercetin partially prevented IL-1$\beta$–induced IκBα degradation. Thus, this mechanism seems to be involved in the action of quercetin on iNOS induction in IL-1$\beta$–treated hepatocytes.

In summary, the data presented in this study indicate that quercetin, a natural flavonol widely distributed in the human diet, inhibits NO production in IL-1$\beta$–stimulated hepatocytes through the inhibition of iNOS expression. However, these effects did not occur at 5 $\mu$mol/L, but were evident only at high doses (50 or 100 $\mu$mol/L). The high levels of quercetin are not likely achievable physiologically even with extremely high intakes (55), but it is possible that quercetin and other phytochemicals could be used as lead molecules to develop a new generation of drugs for controlling various acute and chronic inflammatory diseases associated with induction of iNOS. Although the mode of action remains to be clarified, our findings support the view that the mechanism of action is via inhibition of the IL-1$\beta$–induced NF-κB/IκB transduction pathway.

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