A Resveratrol and Polyphenol Preparation Suppresses Oxidative and Inflammatory Stress Response to a High-Fat, High-Carbohydrate Meal

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Background: High-fat, high-carbohydrate (HFHC) meals are known to induce oxidative and inflammatory stress, an increase in plasma endotoxin concentrations, and an increase in the expression of suppressor of cytokine signaling-3 (SOCS-3).

Hypothesis: The intake of a nutritional supplement containing resveratrol and muscadine grape polyphenols reduces HFHC meal-induced oxidative and inflammatory stress and stimulates the activity of the antioxidant transcription factor, NF-E2-related factor-2 (Nrf-2), and its downstream targets.

Methods: Ten normal, healthy subjects were given a 930-kcal HFHC meal either with placebo or with the supplement. Indices of oxidative stress, inflammation, Nrf-2 binding activity, the concentrations of endotoxin (lipopolysaccharide) and lipoprotein binding protein (LBP), and the expression of toll-like receptor 4 (TLR-4), CD14, IL-1β, TNFα, SOCS-3, Keap-1, NAD(P)H:quinone oxidoreductase-1 (NQO-1), and GST-P1 were measured.

Results: The intake of the supplement suppressed the meal-induced elevations of plasma endotoxin and LBP concentrations, the expression of $p47^{phox}$, TLR-4, CD14, SOCS-3, IL-1β, and Keap-1, while enhancing Nrf-2 binding activity and the expression of NQO-1 and GST-P1 genes.

Conclusion: A supplement containing resveratrol and muscadine polyphenols suppresses the increase in oxidative stress, lipopolysaccharide and LBP concentrations, and expression of TLR-4, CD14, IL-1β and SOCS-3 in mononuclear cells after an HFHC meal. It also stimulates specific Nrf-2 activity and induces the expression of the related antioxidant genes, NQO-1 and GST-P1. These results demonstrate the acute antioxidant and antiinflammatory effects of resveratrol and polyphenolic compounds in humans in the postprandial state. **(*J Clin Endocrinol Metab* 96: 1409–1414, 2011)**

The intake of a high-fat, high-carbohydrate (HFHC) meal induces oxidative and inflammatory stress as reflected by increased reactive oxygen species (ROS) generation, increased expression of the subunits of reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, increased nuclear factor-κB binding in mononuclear cells (MNC), and increased expression and plasma concentrations of proinflammatory cytokines (1). Such meals also elicit an increase in plasma concentrations of endotoxin [lipopolysaccharide (LPS)] and lipoprotein binding protein (LBP) and the expression of its receptor, toll-like receptor-4 (TLR-4) (2). Our recent work has shown that HFHC meals also evoke an increase in the expression of the suppressor of cytokine signaling-3 (SOCS-3), a key protein responsible for interference with insulin and leptin signal transduction (3–5).

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Abbreviations: ARE, Antioxidant response element; EU, endotoxin unit(s); GST-1P, glutathione S-transferase-1P; HFHC, high-fat, high-carbohydrate; LBP, lipoprotein binding protein; LPS, lipopolysaccharide; MNC, mononuclear cells; NADPH, reduced nicotinamide adenine dinucleotide phosphate; NQO-1, NAD(P)H:quinone oxidoreductase-1; Nrf-2, NF-E2-related factor-2; RMANOVA, repeated measures ANOVA; ROS, reactive oxygen species; SOCS-3, suppressor of cytokine signaling-3; SOD, superoxide dismutase; TLR-4, toll-like receptor-4.

One potent antioxidant mechanism triggered by oxidative stress is that based on the induction of the transcription factor NF-E2-related factor-2 (Nrf-2), which activates the transcription of a series of genes that have an antioxidant response element (ARE) in their promoters (6). These genes include NAD(P)H:quinone oxidoreductase-1 (NQO-1), glutathione S-transferase-1P (GST-1P), superoxide dismutase (SOD)-2, thioredoxin, and glutamate cysteine ligase-2 (GCL-2). All of these proteins exert potent antioxidant effects by increasing the clearance or neutralization of ROS. The transcriptional activity of Nrf-2 is regulated by Keap-1, a cytosolic protein that binds to Nrf-2 and thus prevents its translocation into the nucleus. An increase in Keap-1 expression is therefore inhibitory to Nrf-2 activity, whereas a decrease enhances Nrf-2 activity. Nrf-2 has recently been shown to protect vascular cells from oxidative stress (7, 8). There are hitherto no data on the action or the modulation of Nrf-2 in humans *in vivo*.

We have recently shown that an extract of *Polygonum cuspidatum* containing resveratrol suppresses ROS generation, $p47^{phox}$ and SOCS-3 expression, and nuclear factor-κB binding when given to normal, healthy subjects (at 40 mg/d) over a period of 6 wk (9). In view of the above, we have now hypothesized that the intake of a preparation containing resveratrol and polyphenols from muscadine grapes reduces or prevents HFHC meal-induced oxidative and inflammatory stress and the induction of SOCS-3. In addition, it induces Nrf-2 activity and the transcription of its target antioxidant genes.

Subjects and Methods

Subjects

A group of 10 (six females) normal-weight, healthy, nonsmoking subjects (age, 37 ± 4 yr; body mass index, 22.6 ± 0.5 kg/m^2; blood pressure, 117 ± 4 and 74 ± 3 mm Hg; fasting glucose concentration, 86 ± 9 mg/dl; fasting insulin, 6.5 ± 9 μU/ml; triglycerides, 68 ± 9 mg/dl; total cholesterol, 138 ± 11 mg/dl) were recruited for this crossover and placebo-controlled study. After an overnight fast, subjects were given, on two separate visits 1 wk apart, a 930-calorie HFHC meal (2) with either a single dose of a nutraceutical supplement or a placebo 10 min before the meal. The supplement consisted of 100 mg of resveratrol from *P. cuspidatum* extract plus 75 mg of total polyphenols from a muscadine grape extract (Shaklee Corporation, Pleasanton, CA). Blood samples were collected at baseline and at 1, 3, and 5 h after meal intake. The experimental protocol was approved by the Human Research Committee of the State University of New York at Buffalo, and each subject signed an informed consent.

MNC isolation, Western blotting, and RT-PCR

MNC and polymorph nuclear cells were isolated (1), and protein and mRNA expression (2, 10) was determined as previously described. Antibodies against TLR-4, SOCS-3, Keap-1 (Abcam Inc., Cambridge, MA), CD14, and actin (Santa Cruz Biotechnology, Santa Cruz, CA) were used. Expression of Nrf-2, NQO1, GST, SOD, and IL-1β mRNA was measured using specific primers (Invitrogen, Carlsbad, CA).

Nrf-2 DNA binding activity

Nuclear Nrf-2 DNA binding activity was measured by EMSA as described previously (1, 11). Oligonucleotide corresponding to the Nrf-2 binding sites on NQO1 promoter (12) (sense, *GTCAGTGTCACTGAGTCGTCTTAGA*; and antisense, *CAGTCACAGTGACTCAGCAGAATCT*) was used. The specificity of the band was confirmed by supershifting the band with specific antibody against Nrf-2 (Santa Cruz Biotechnology) and by competition with cold oligonucleotides.

Plasma endotoxin and LBP concentrations were measured as previously described (10).

Statistical analysis

Statistical analysis was conducted using SigmaStat software version 3.1 (SPSS Inc., Chicago, IL). Data are expressed as mean ± SE. Percentage change from baseline was calculated, and statistical analysis for change from baseline was carried out using one-way repeated measures ANOVA (RMANOVA) followed by Holm-Sidak *post hoc* test. Two-factor RMANOVA analysis followed by Tukey's *post hoc* test was used for all multiple comparisons between groups.

Results

Supplement effect on meal-induced oxidative stress

After consumption of the meal, MNC protein levels of $p47^{phox}$ (NADPH subunit) increased by 148 ± 38% over baseline ($P < 0.05$) in the placebo group, whereas no significant changes were observed in the supplement group (Fig. 1, A and B). The DNA binding activity of the antioxidant transcription factor Nrf-2 was significantly increased by 150 ± 39% over baseline ($P < 0.05$) at 3 h after meal and supplement intake, whereas meal consumption in the placebo group resulted in a significant reduction in Nrf-2 binding activity at 5 h (Fig. 1, C and D). This was associated with a significant reduction by 48 ± 6% ($P < 0.05$) in the supplement group and a significant increase by 66 ± 10% ($P < 0.05$) in the supplement group in Nrf-2 inhibitor, Keap-1, protein levels in MNC (Fig. 1, A and E). Additionally, significant increases ($P < 0.05$) in the mRNA expression of Nrf-2 target genes, NQO-1 and GST-P1 (data not shown), and in NQO-1 protein levels (Fig. 1, A and F) were observed after the meal plus supplement, whereas there was no change in the expression of NQO-1 or GST-P1 after the meal plus placebo. There was no change in the expression of other Nrf-2 target genes including SOD-2, thioredoxin, or GCL in either group.

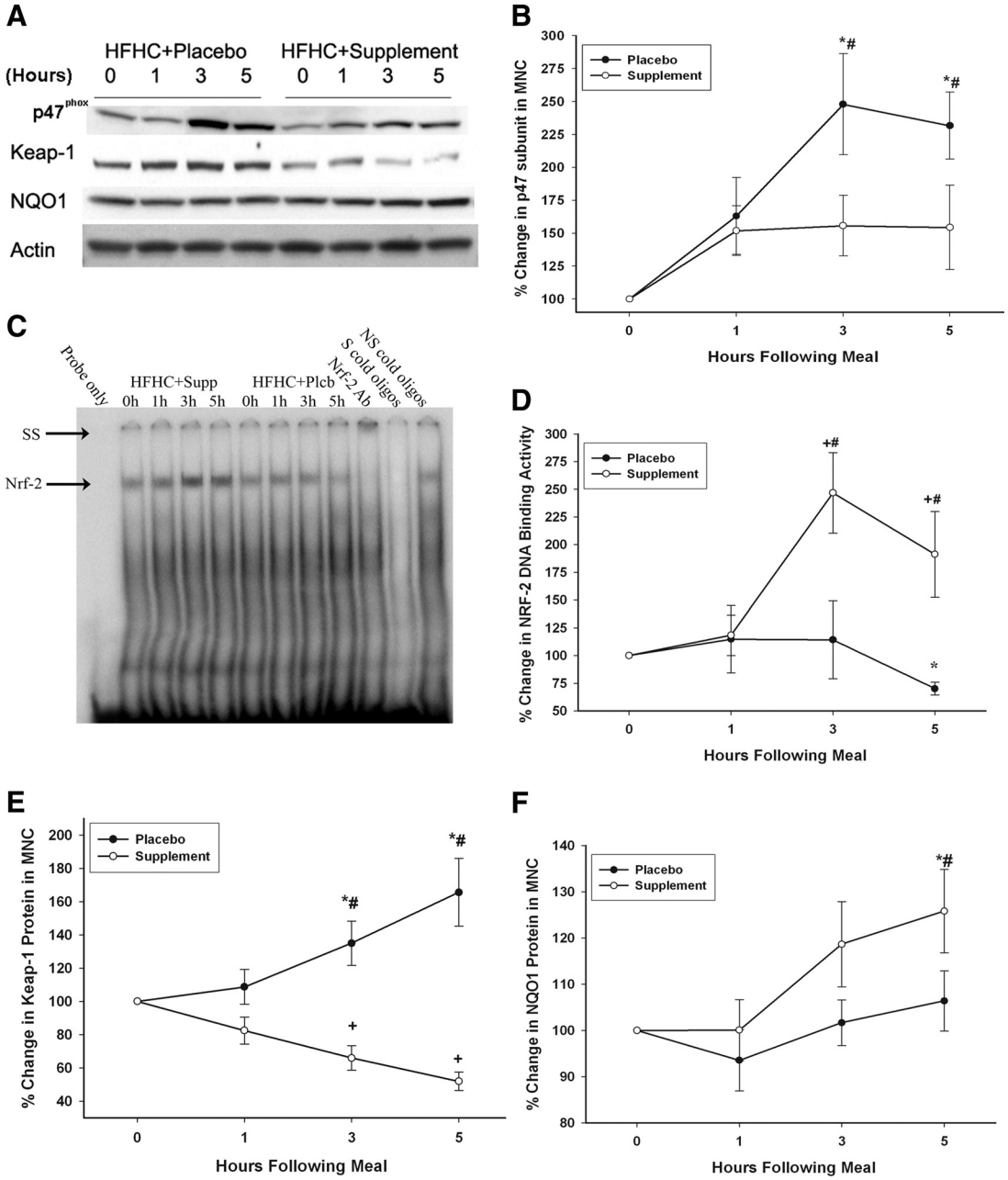

FIG. 1. Change from baseline (%) in oxidative stress markers. A, Representative Western blot and protein content of $p47^{phox}$ (B), Keap-1 (E) and NQO1 (F) in total MNC lysates. C and D, Representative shifted and supershifted (SS) band of Nrf-2 binding to specific ARE site in NQO-1 promoter using EMSA in MNC nuclear extracts before and after intake of 930-calorie HFHC meal plus placebo (HFHC + plcb) or a resveratrol and polyphenols supplement (HFHC + Supp) in 10 normal, healthy subjects in a crossover design. Quality controls for EMSA include incubating a 5-h sample from HFHC + Plcb (Plcb) with Nrf-2 antibody (Nrf-2 Ab), cold Nrf-2 oligonucleotide (S cold oligos), or nonspecific oct-1 oligonucleotide (NS cold oligos). Samples were collected before and 1, 3, and 5 h after intake. Data are expressed as mean ± SE. *, Placebo + supplement, $P < 0.05$ compared with baseline by RMANOVA; #, $P < 0.05$ between groups by two-way RMANOVA.

Supplement effect on meal-induced inflammation

There was a significant, steady increase in plasma concentrations of LPS in the placebo group from 0.23 ± 0.02 endotoxin units (EU)/ml to 0.29 ± 0.03, 0.33 ± 0.04, and 0.36 ± 0.03 EU/ml at 1, 3, and 5 h, respectively, after the meal (60 ± 16% over baseline at 5 h; $P < 0.05$). In contrast, plasma LPS concentrations decreased significantly by 28 ± 7% below baseline at 1 h (from 0.30 ± 0.04 EU/ml to 0.22 ± 0.04, 0.33 ± 0.04, and 0.34 ± 0.05 EU/ml at 1, 3, and 5 h, respectively) in the supplement group. This was associated with an increase in plasma levels of the LBP by 38 ± 16% over the baseline at 3 h (from 10.78 ± 1.2 to 15.6 ± 2.4 μg/ml; $P < 0.05$; Fig. 2A) in the placebo group; this increase was prevented by the intake of the nutraceutical supplement. The intake of the HFHC meal in the placebo group induced a significant increase in mRNA expression of IL-1β by 91 ± 28% (from 0.34 ± 0.08 to 0.61 ± 0.14 relative units compared with control sample;

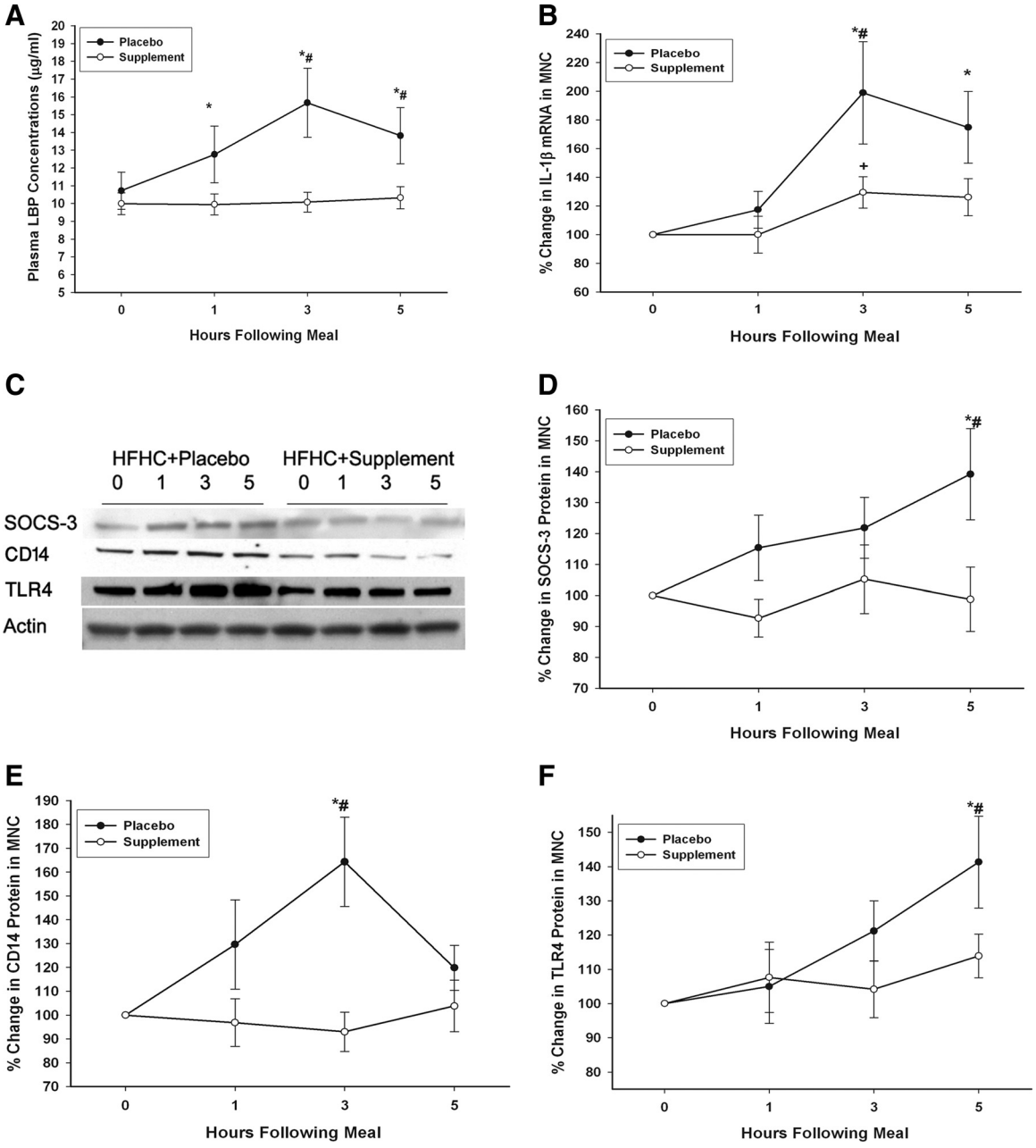

FIG. 2. Change in LBP (A) concentrations by immunolinked assay, mRNA expression of IL-1β (B) by real time PCR and immunoblotting representative gel (C) for the protein content of SOCS-3 (D), CD14 (E), and TLR4 (F) in MNC before and after intake of 930-calorie HFHC meal plus placebo or a resveratrol and polyphenols supplement in 10 normal healthy subjects in a crossover design. Samples were collected before and at 1, 3, and 5 h after meal intake. Data are represented as mean ± SE. *, Placebo + supplement, $P < 0.05$ compared with baseline by RMANOVA; #, $P < 0.05$ between groups by two-way RMANOVA.

$P < 0.05$), whereas this increase was significantly reduced in the supplement group to 29 ± 11% over the baseline (from 0.38 ± 0.1 to 0.47 ± 0.11 relative units compared with control sample; $P < 0.05$) (Fig. 2B).

Expression of SOCS-3, TLR-4, and CD14 proteins

As shown in Fig. 2, C and D, SOCS-3 protein levels in MNC increased by 39 ± 13% ($P < 0.05$) over baseline in the placebo group, whereas no significant changes in SOCS-3 protein levels were measured in the supplement group. Similarly, there was a significant increase in the expression of CD14 and TLR-4 protein by 67 ± 19 and 40 ± 15%, respectively ($P < 0.05$), over baseline after the HFHC meal, which was prevented by the concomitant intake of the supplement but not the placebo (Fig. 2, C, E, and F).

Discussion

As demonstrated previously (1, 2), the consumption of the HFHC meal with the placebo produced an increase in the

expression of $p47^{phox}$, an essential subunit of NADPH oxidase that generates the $O\dot{2}$ radical and mediates oxidative stress. The HFHC meal also induced an increase in LPS and LBP concentration and the expression of TLR-4, CD14, and SOCS-3, all of which were either reduced or totally prevented by the supplement. The prevention of an increase in LPS is intriguing but is consistent with the action of orange juice, which we described recently (9). It is possible that resveratrol, the muscadine polyphenols, and orange juice may exert a direct protective antiinflammatory effect on the intestinal epithelium, including any change in intestinal permeability. This issue requires further investigation.

The induction of CD14 and TLR-4 along with an increase in LPS and LBP by the meal has implications for the pathogenesis of both atherogenesis and insulin resistance. The deletion of TLR-4 protects against both insulin resistance in mice fed a high-fat diet (13) and atherosclerosis in mice with apolipoprotein E deletion. In this context, there is a significant expression of TLR-4 in cells of the atherosclerotic plaque (14).

Our other major novel observation is that there was a significant increase in the intranuclear binding activity of Nrf-2 at 3 and 5 h after consumption of the HFHC meal with the supplement. In contrast, Nrf-2 binding activity actually decreased (at 5 h) in the placebo group. Nrf-2 is an important transcription factor that mediates protective responses to oxidative stress by binding to the ARE of several genes, including those responsible for glutathione synthesis and phase II drug metabolism (6). Consistent with this, the expression of Keap-1, the protein to which Nrf-2 is bound in the cytosol and which also causes its ubiquitination and proteasomal degradation, was reduced markedly in the group receiving the supplement, whereas it increased significantly in the placebo group. The decline in Keap-1 levels would potentially stabilize Nrf-2, allow its translocation into the nucleus, and facilitate the initiation of transcription of multiple protective antioxidant genes including NQO-1 and GST-1P, two major enzymes with antioxidant activity. Nrf-2 has also been shown to reverse biochemical dysfunction in endothelial cells induced by high glucose concentrations (8). Nrf-2 expression and activity have recently been shown to be diminished with aging (15).

The prevention of the increase in SOCS-3, a protein that interferes with insulin and leptin signal transductions, after the HFHC meal in the supplement group suggests that the supplement may have a role in preventing insulin and leptin resistance in long-term studies (2). The expression of SOCS-3 has been shown to be elevated in obese humans in whom it is inversely related to insulin receptor phosphorylation (16). The prevention of the induction of IL-1β by the intake of the supplement is also potentially insulin sensitizing because IL-1β in turn induces the expression of SOCS-3.

In conclusion, the intake of a resveratrol and polyphenol-based nutritional supplement before an HFHC meal significantly reduced multiple indices of oxidative and inflammatory stress: $p47^{phox}$, IL-1β, CD14, and TLR-4 expression and LPS and LBP concentrations. The intake of the supplement also induced the key antioxidant transcription factor, Nrf-2, and its gene targets, NQO-1 and GST-1P, while suppressing Keap-1, the antagonist of Nrf-2.

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