Dietary Flavonoids Differentially Reduce Oxidized LDL-Induced Apoptosis in Human Endothelial Cells: Role of MAPK- and JAK/STAT-Signaling

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Abstract

Endothelial apoptosis is a driving force in atherosclerosis development. Oxidized LDL promotes inflammatory and thrombotic processes and is highly atherogenic, as it stimulates macrophage cholesterol accumulation and foam cell formation. This study investigated multiple mitogen-activated protein kinase (MAPK)-responsive death/survival signaling pathways, through which flavonoids of (−)-epigallocatechin gallate (EGCG) and hesperetin exerted antiapoptosis in endothelial cells exposed to oxidized LDL. EGCG and hesperetin substantially diminished the oxidized LDL-induced 2',7'-dichlorofluorescein staining, suggesting that these flavonoids inhibited intracellular accumulation of oxidized LDL-triggered reactive oxygen species and consequent apoptosis. The Western-blot data revealed that oxidized LDL upregulated c-Jun N-terminal kinase (JNK) phosphorylation, which was rapidly reversed by EGCG and hesperetin. They mitigated the consequent activation of the JNK downstream on p53 and c-Jun. Moreover, oxidized LDL increased luciferase activity of p53 in endothelial cells transfected with a p53 promoter construct, the increase of which was strikingly downregulated by EGCG and hesperetin. Surprisingly, hesperetin but not EGCG attenuated phosphorylation of p38MAPK and its downstream c-myc and signal transducers and activators of transcription (STAT)1 evoked by oxidized LDL. This study also attempted to explore a linkage of Janus kinase (JAK)2/STAT3 activation to MAPK signaling in oxidized LDL-induced endothelial apoptosis. Notably, we found that the JAK2 inhibitor substantially blocked the JNK activation. Our findings suggest that EGCG and hesperetin may act as antiatherogenic agents blocking oxidized LDL-induced endothelial apoptosis via differential cellular apoptotic machinery. These data provide evidence that the interplay between p38MAPK and JAK-STAT pathways is involved in dietary flavonoid protection against oxidized LDL through hampering MAPK-dependent pathways involving the activation of JAK2. J. Nutr. 138: 983–990, 2008.

Introduction

Oxidative modification of LDL in the endothelial space of the arterial wall causes atherosclerosis irreverently to plasma cholesterol levels (1,2). Oxidized LDL can be generated from native LDL by transition metal ions such as copper ion (Cu2⁺) and by inorganic oxidants such as H2O2 (3). It was suggested that oxidized LDL might be a local mediator promoting thrombosis (4). In addition, vascular cells in the atherosclerotic lesions undergo apoptosis upon a treatment with oxidized LDL (5). The cytotoxic components of oxidized LDL are one or more oxysterols, which may induce apoptosis in a range of submicromolar doses (6). We reported that oxidized LDL induces endothelial apoptosis through enhancing the production of lipid peroxidative products (3,7). It has been proposed that antioxidants such as vitamin C and N-acetylcysteine inhibit oxidant production and prevent apoptosis (8,9).

Epidemiologic studies show that a high consumption of polyphenolic flavonoids is inversely related to the risk of cardiovascular diseases (10) and this phenomenon may be associated with their antioxidant capacity to scavenge various types of radicals in aqueous and organic environments (11,12). Flavonoids such as (−)-epigallocatechin gallate (EGCG)6 and hesperetin exert differ-

6 Abbreviations used: EGCG, (−)- epigallocatechin gallate; FBS, fetal bovine serum; HUVEC, human umbilical vein endothelial cell; JAK, Janus kinase; JNK, c-Jun N-terminal kinase; MAPK, mitogen-activated protein kinase; NF-κB, nuclear factor-κB; ROS, reactive oxygen species; STAT, signal transducers and activators of transcription; TBS-T, Tris-buffered saline-Tween 20.

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ential inhibition of oxidized LDL-induced apoptosis in human endothelial cells by dampening production of lipid peroxidative products (7). Kaempferol diminished the apoptosis induced by oxidized LDL components by being partially mediated by the estrogen receptors (13). In addition, resveratrol attenuated oxidized LDL-provoked apoptotic features, generation of reactive oxygen species (ROS), and accumulation of intracellular calcium, indicating that red wine intake may protect against oxidized LDL-induced endothelial dysfunction (14). However, the underlying molecular mechanisms by which these flavonoids protect from apoptosis triggered by diverse stimulators, including oxidized LDLs, remain to be investigated.

Numerous reports showed that ROS may instigate cell death via the activation of mitogen-activated protein kinase (MAPK) under oxidative circumstances (14). Oxidant-triggered neuronal apoptosis was observed via an inhibition of extracellular signal-regulated kinase 1/2 phosphorylation and activation of p38 MAPK, concomitantly with nuclear factor κB (NF-κB) transactivation (15). However, N-acetylcysteine inhibits the activation of phospho-c-Jun N-terminal kinase (JNK) and p38MAPK and suppresses the activity of redox-sensitive activating protein-1 and NF-κB regulating expression of apoptotic genes (16). EGCG regulates multiple downstream signaling pathways and inhibits activating protein-1-dependent transcriptional activity of in cancer cell lines (17,18). In addition, other signal transducers and transcription activators participate in the transduction of death/survival signals (19). It has been recently shown that the Janus kinase (JAK)-signal transducers and activators of transcription (STAT) pathway is an integral part in the myocardial response to various cardiac insults, including myocardial infarction, and plays a prominent role in the cardioprotection against oxidative damage (20). Oxidized LDL induces the activation of JAK2, STAT1, and STAT3 through an intracellular oxidative stress by means of its lipid peroxidation products, implying that the STAT activation might be related to their pro-inflammatory and fibro-proliferative effect in the atherosclerotic plaque (21).

When constitutively different EGCG and hesperetin were applied in micromolar concentrations to human umbilical vein endothelial cells (HUVEC) exposed to 5 μmol/L Cu²⁺-oxidized LDL, it tested the hypothesis that these flavonoids may hamper oxidized LDL-induced apoptosis through modulating JAK-STAT pathways as well as redox-sensitive MAPK-signaling cascades. In addition, this study also investigated whether the MAPK pathway activated by oxidized LDL was dependent on JAK-STAT activation.

**Materials and Methods**

**Materials.** Polyphenolic flavonoids (flavanol EGCG and flavanone hesperetin), M199 medium chemicals, human epidermal growth factor, hydrocortisone, and 2',7'-dichlorodihydrofluorecein diacetate (DCFH) were obtained from Sigma-Aldrich Chemical, as were all other reagents, unless specifically stated elsewhere. Collagenase was purchased from Worthington Biochemicals. Fetal bovine serum (FBS), penicillin-streptomycin, and trypsin-EDTA were provided by Cambrex. Antibodies of Jun, human phospho-p53, human phospho-c-myc, human phospho-JNK, human phospho-p38 MAPK, human phospho-c-Jun, human phospho-p33, human phospho-c-myc, human phospho-STAT1, human phospho-JAK, and human phospho-STAT3 were obtained from Cell Signaling Technology. Horseradish peroxidase-conjugated goat anti-rabbit IgG and cyanine 3-conjugated goat anti-rabbit IgG were provided by Jackson ImmunoResearch Lab. EGCG and hesperetin were dissolved in dimethyl sulfoxide for live culture with cells (22); the final concentration of dimethyl sulfoxide was ≤0.05%.

**Plasma LDL preparation.** Human plasma LDL was prepared by discontinuous density gradient ultracentrifugation as previously described (7). Human normolipidemic pooled plasma LDL fraction was dialyzed overnight against 0.154 mol/L NaCl and 0.01% EDTA (pH 7.4) at 4°C and used within 4 wk. Protein concentration of the plasma LDL fraction was determined by the Lowry method (23) and concentrations of triacylglycerol (Triglyceride assay kit, Asan Pharmaceutical), total cholesterol (Total cholesterol assay kit, Asan Pharmaceutical), and phospholipids (Phospholipid B, Wako Pure Chemical) were measured using diagnostic kits. The contents of total protein, triacylglycerol, total cholesterol, and phospholipid in the prepared LDL fraction were all within the appropriate ranges.

LDL oxidation was confirmed by an electrophoretic mobility test. Aliquots of prepared LDL fraction were run on a 0.8% agarose gel in barbitral buffer (pH 8.6). The gel was immediately fixed in a 5% trichloroacetic acid solution and rinsed in 70% ethanol (3). In addition, measurements of lipid peroxidation were performed to validate LDL oxidation (3,7).

**Primary culture of endothelial cells.** HUVEC were isolated using collagenase as described elsewhere (22) and cultured in 25 μmol/L HEPES-buffered M199 containing 10% FBS, 2 mmol/L glutamine, 0.75 g/L human epidermal growth factor, and 75 mg/L hydrocortisone at 37°C in a humidified atmosphere of 5% CO₂ in air. Cells were identified by verifying their cobblestone morphology and uptake of acetylated LDL fluorescently labeled with 1,1'-dioctadecyl-3,3,3',3'-tetramethyl-indocarbocyanine perchlorate.

Cells (7 × 10⁶) were pretreated with 25 μmol/L EGCG and hesperetin for 30 min and exposed to 0.1 g/L LDL cholesterol in the absence and presence of Cu²⁺ for 24 h to induce endothelial apoptosis. After the incubation with Cu²⁺-oxidized LDL, the 3-(4,5-dimethylthiazol-yl)-2,5-diphenyl tetrazolium bromide assay was performed to quantitate cellular viability (7,22). The purple formazan product was dissolved in 0.5 mL isopropanol with gentle shaking. Absorbance of formazan dye was measured at λ = 570 nm with background subtraction using λ = 690 nm.

**Intracellular ROS production.** Oxidant generation of HUVEC was measured as a previously described method with a minor modification (22). This method was based on an oxidant conversion of DCFH to the fluorescent 2',7'-dichlorofluorescein for measuring the cellular ability to produce ROS. Cells (7 × 10⁶) challenged with Cu²⁺-oxidized LDL were washed twice with PBS and incubated for 30 min with 10 μmol/L DCFH in prewarmed M199 (†2% FBS). Fluorescent images were taken using a fluorescence microscopy (Olympus BX 51, Olympus Optical).

**Western-blot analysis.** Western-blot analysis was performed using whole cell extracts from HUVEC as previously described (24). Cell lysates containing equal amounts of total protein were fractionated by electrophoresis on 10% SDS-PAGE gels and transferred onto a nitrocellulose membrane. Nonspecific binding was blocked by soaking the membrane in TBS-T buffer [0.5 mol/L Tris-HCl (pH 7.5), 1.5 mol/L NaCl, and 1% Tween 20] containing 5% nonfat dry milk for 3 h. The membrane was incubated overnight at 4°C with a primary antibody (polyclonal rabbit anti-phospho-JNK, polyclonal rabbit anti-phospho-p38 MAPK, polyclonal rabbit anti-phospho-p53, polyclonal rabbit anti-phospho-c-Jun, polyclonal rabbit anti-phospho-c-myc, polyclonal rabbit anti-phospho-STAT1, polyclonal rabbit anti-phospho-STAT3, and polyclonal rabbit anti-phospho-JAK2). After 3 washes with TBS-T, the membrane was then incubated for 1 h with a goat anti-rabbit IgG conjugated to horseradish peroxidase. The protein levels were determined using SuperSignal West Pico chemiluminescence detection reagents (Pierce Biotechnology) and Konica X-ray film (Konica).

**Caspase-3-like protease activity.** The cell extracts were suspended in 100 mmol/L HEPES buffer (pH 7.4) containing 0.5 mmol/L phenylmethylsulfonyl fluoride, 10 mg/L leupeptin, and 5 mg/L aprotinin and pepstatin (24). DEVDase activity was measured proteolytic cleavage of a chromogenic substrate for caspase-3-like protease, Asp-Glu-Val-Asp-p-nitroanilide.

**Immunocytochemistry.** After HUVEC challenged with Cu²⁺-LDL were thoroughly washed with TBS and fixed with 4% formaldehyde for
15 min, cells were incubated for 1 h with 4% FBS in TBS to block any nonspecific binding. After washing with TBS, polyclonal rabbit anti-phospho-p53 was added to cells and incubated overnight at 4°C. Cells were washed with TBS and incubated with a cyanine 3-conjugated goat anti-rabbit IgG as a secondary antibody. Fluorescent images were obtained by a fluorescence microscope.

**Transcriptional reporter gene assays.** To study the p53 promoter activity, we used a promoter-reporter construct with the luciferase gene driven by the transactivant p53 promoter vector (Panomics). Nucleofection of HUVEC was performed for gene delivery according to the optimized protocols provided by the manufacturer (Axamaxis Biosystem). Briefly, cells were pelleted and gently resuspended in 100 μL of Nucleofector solution (Axamaxis Biosystem), mixed with 3 μg p53 reporter vector in the Axamaxis cuvette, and pulsed in the nucleofector device (the program A-34). Immediately after, cells were transferred into prewarmed fresh medium in 12-well plates. After 24-h nucleofection, transfected cells were treated for 24 h with oxidized LDL in the absence and presence of EGCG or hesperetin. Luciferase activity was measured using the Dual-Luciferase Reporter kit (Promega Biosciences) and the relative luciferase activity was calculated according to the manufacturer’s instructions.

**Results**

**Inhibition of oxidized LDL-induced endothelial apoptosis.** In the previous study (7), we found that 5 μmol/L Cu²⁺ per se maintained HUVEC viable without an induction of peroxidation. LDL underwent considerable oxidative modification by 5 μmol/L Cu²⁺, evidenced by LDL electrophoretic mobility test. During 24-h incubations, LDL in the presence of Cu²⁺ decreased cell viability by ~30% (Fig. 1A). This massive cell death was associated with a marked increase in formation of thiobarbituric acid-reactive substances (7). EGCG and hesperetin at a nontoxic dose of 25 μmol/L attenuated the rate of oxidized LDL-induced cell death; the inhibition by hesperetin tended to be greater (P > 0.05) than that by EGCG. Oxidized LDL-exposed cells revealed a substantial disappearance of 2,7'-dichlorofluorescein staining in the presence of EGCG and hesperetin (Fig. 1B), indicating that this ROS inhibition was responsible for blocking the lipid peroxidation. Culture with EGCG and hesperetin fully prevented oxidized LDL-induced HUVEC apoptosis through inhibiting caspase-3 cleavage (Fig. 1C). In addition, EGCG and hesperetin blocked the caspase-3-like activity elevated by oxidized LDL (Fig. 1D).

**Differential MAPK inhibition.** This study attempted to determine whether EGCG and hesperetin inhibit apoptosis in oxidized LDL-exposed cells through blocking MAPK signaling cascades. The inhibitory effects of EGCG and hesperetin on oxidized LDL-elicited activation of JNK and p38MAPK were examined in HUVEC. The treatment of cells with oxidized LDL instigated phosphorylation of JNK immediately within 5 min, which was attenuated by EGCG (Fig. 2A). In addition, hesperetin also blocked oxidized LDL-induced activation of JNK rapidly within 15 min. The level of phosphorylated p38MAPK was rapidly increased following the exposure of HUVEC to oxidized LDL (Fig. 2B). The oxidized LDL-activated phosphorylation of p38MAPK remained high even at 60 min in EGCG-treated cells, whereas hesperetin almost completely abolished p38MAPK phosphorylation induced by oxidized LDL (Fig. 2B).

**Differential inhibition of MAPK downstream signaling.** This study investigated the involvement of the kinase cascades in endothelial apoptosis induced by oxidized LDL. Western-blot analysis revealed a marked increase in the active phosphorylated form of c-Jun, one of the downstream effectors of JNK, in oxidized LDL-treated endothelial cells (Fig. 3). As expected, both EGCG and hesperetin elicited a marked reduction of oxidized LDL-induced activation of c-Jun within 15–30 min. We also investigated the contributions of c-myc and STAT1, downstream effectors of p38MAPK, to protective effects of EGCG and hesperetin on oxidized LDL-caused apoptosis. A delayed activa-

![FIGURE 1](https://example.com/figure1.png)
tion in c-myc was observed in oxidized LDL-treated HUVEC (Fig. 4A). Hesperetin exhibited a marked inhibition of c-myc phosphorylation, whereas EGCG did not have such an effect. In addition, STAT1 activation substantially increased 1 h after exposure to oxidized LDL (Fig. 4B). When cells were pretreated with EGCG, STAT1 remained active. In contrast, hesperetin substantially suppressed the STAT1 activation.

**Inhibition of p53 translocation.** It was investigated that EGCG and hesperetin attenuated activation of the apoptotic factor p53 induced by oxidized LDL. The phosphorylated form of p53 was upregulated in cells injured by oxidized LDL relative to undamaged cells (Fig. 5A). In contrast, both antiapoptotic EGCG and hesperetin dampened oxidized LDL-triggered p53 phosphorylation within 2 h. The inhibitory effects of EGCG and hesperetin on oxidized LDL-induced p53 phosphorylation were also immunocytochemically determined using specific phospho-p53 antibody (Fig. 5B). There was relatively weak activation of p53 in untreated cells. Heavy nuclear staining in cells exposed to oxidized LDL was observed, indicating that p53 was activated and translocated into the nucleus. With EGCG and hesperetin, oxidized LDL-exposed cells diminished fluorescent staining of nuclear p53 (Fig. 5B). In addition, transfection experiments using a construct containing the p53 promoter and luciferase reporter gene demonstrated that p53 promoter activity was induced after treatment with oxidized LDL (Fig. 5C). When EGCG and hesperetin were added, the induction was substantially downregulated.

**Blockade of JAK2/STAT3 pathway.** We investigated whether oxidized LDL influence the JAK/STAT pathway and whether the blockade of this pathway was responsible for the cytoprotection of EGCG and hesperetin against oxidized LDL toxicity. Oxidized LDL immediately induced JAK2, one of the Janus tyrosine kinases involved in the JAK/STAT pathway (Fig. 6A). Likewise, the downstream STAT3 was activated in oxidized LDL-exposed cells through the activation of the upstream receptor-associated JAK, which occurred rapidly within 15 min (Fig. 6B). EGCG and hesperetin at 25 μmol/L blocked activation of JAK2 and prevented STAT3, indicating that EGCG and hesperetin may be antagonists to oxidized LDL-elicited induction of JAK2/STAT3. This study attempted to next investigate whether the activation of JNK and p38MAPK by oxidized LDL entailed the induction of JAK2. The MAPK pathway has been shown to play an important role in the regulation of JAK/STAT signaling (25). The JAK2 inhibitor substantially attenuated oxidized LDL-stimulated JNK activation within 30 min and the p38MAPK activation was slightly inhibited (Fig. 7), suggesting that oxidized LDL activated the JNK-responsive pathways via switching on the JAK2 signaling. Thus, we concluded that the inhibition of JAK2 signaling by EGCG and hesperetin interfered with the JNK signaling.
Discussion

An elevated level of LDL is one of the most important risk factors for atherosclerosis and cardiovascular morbidity (1,2,5). Oxidized LDL elicit endothelial activation that is assumed to be the key step in the initiation of atherosclerosis and is likely to be prevalent in earlier stages of atherosclerotic lesions (26). In our previous studies, oxidized LDL caused endothelial apoptosis with enhancing thiobarbituric acid-reactive substances, a biomarker of lipid peroxidation (3,7). This study showed that oxidized LDL increased intracellular ROS accumulation, which could be responsible for the production of lipid peroxidative products. Oxidized LDL is capable of inducing apoptosis in human endothelial cells by activating multiple ROS-sensitive signaling pathways (27). This study provides an extensive investigation of the effects of oxidized LDL on MAPK signaling pathway leading to apoptosis.

Antioxidants such as vitamin C and N-acetylcysteine that can inhibit production of ROS have been proposed to prevent apoptosis (8,9). We have previously shown that polyphenolic flavonoids may differentially prevent Cu$^{2+}$-oxidized LDL-induced apoptosis and promote cell survival as potent antioxidants eliminating lipid peroxidative products (7). In particular, the flavanol EGCG and the flavanone hesperetin prevented oxidized LDL injury and prolonged endothelial survival as antiapoptotic agents in human vascular endothelium, in which these survival potentials appeared to be linked to their disparate chemical structure. This study showed that EGCG and hesperetin mitigated the caspase-3 activation induced by LDL oxidized in the presence of Cu$^{2+}$, proving that these flavonoids blocked endothelial apoptosis. However, the underlying molecular mechanisms by which EGCG and hesperetin prevent apoptosis along with atherosclerosis development triggered by Cu$^{2+}$-oxidized LDL remains to be investigated. It was assumed that the cellular and molecular antiapoptotic features of EGCG might be attributed to their antioxidant capacity via both cytosolic and mitochondrial mechanisms (24).

The natural compounds of EGCG and hesperetin exhibited a more powerful antioxidant capacity in the cell-free systems (7). Intracellular ROS loading may directly entail the depletion of intrinsic antioxidant potentials and the activation of the transduction pathways leading to apoptosis. Overexpression of Cu, Zn-superoxide dismutase, or catalase reduced oxidized LDL-induced cell proliferation through a reduction of JNK and extracellular signal-regulated kinase 1/2 phosphorylation (28). In the current study, there was a substantial ROS loading instigated during culturing oxidized LDL, whereas this stress burden was alleviated in the EGCG- or hesperetin-treated cells. Accordingly, one plausible mechanism is interference of oxidized LDL-elicited ROS-mediated intracellular signaling pathway. The phosphorylated status of MAPK and the downstream effectors play important roles in the survival, proliferation, and cell cycle transition triggered by various stimuli or under oxidative stress conditions (28,29).

The boosted redox status by EGCG and hesperetin may attenuate ROS-dependent death signaling cascades. In this study,
EGCG and hesperetin diminished Cu^{2+}-oxidized LDL-triggered death signaling by modulating MAPK-responsive cellular apoptotic machinery. However, it was shown that EGCG and hesperetin differentially operated MAPK-dependent signaling pathways. The inhibitory effect of EGCG on oxidized LDL-induced apoptosis was mediated mainly through blunting JNK-dependent signaling pathways, whereas hesperetin blocked the apoptosis by dampening both JNK-dependent and p38 MAPK-responsive signaling cascades. Accordingly, oxidized LDL-triggered activation of c-Jun and p53, the downstream effectors of JNK, was downregulated in EGCG- or hesperetin-treated HUVEC. In contrast, the upregulated levels of phospho-STAT1 and phospho-c-myc, the downstream effectors of p38 MAPK, were lessened only by hesperetin treatment, indicating that its antiapoptotic feature was at least mediated by interfering with p38 MAPK-responsive signaling pathway.

It has recently been argued that the JAK-STAT pathway orchestrates the response to cellular damage, along with the potential benefits and challenges in manipulating this pathway in cardiovascular therapy. Oxidative stress has been implicated to activate JAK-STAT signaling pathway (30,31). Oxidized LDL induced the activation of JAK2, STAT1, and STAT3 by generation of lipid peroxidative products (21). This study also showed that oxidized LDL fired the JAK-STAT signaling pathway and that EGCG and hesperetin were antagonists to the oxidized LDL induction of JAK2-STAT3. Furthermore, JAK2 signaling shut off by oxidized LDL was at least in part involved in the activation of JNK that was suppressed by hesperetin. It has been suggested that...
MAPK and JAK/STAT pathways interplay in vascular injury and atherosclerosis (32). Together, oxidized LDL elicited endothelial apoptosis through differential signaling pathways involving JAK-STAT and MAPK that were hampered by antioxidant EGCG and hesperetin.

We cannot determine from these data how mechanistic signals for inhibiting oxidized LDL-induced apoptosis differ between EGCG and hesperetin. EGCG and hesperetin might be acting on oxidized LDL-responsive membrane receptors. The flavonoid kaempferol diminishes apoptosis in vascular smooth muscle induced by a component of oxidized LDL, which effect was partially mediated by the estrogen receptor-α (13). Isoheminattor afforded cytoprotection against oxidized LDL via an inhibition of lectin-like oxidized LDL receptor-1 upregulation, in which p38MAPK activation and NF-κB nuclear translocation were impaired (33). In our previous study, we found that luteolin attenuated oxidized LDL uptake with suppressing lectin-like oxidized LDL receptor-1 expression in human endothelial cells exposed to oxidized LDL (26). In addition, baicalein blocked oxidized LDL-induced downregulation of insulin-like growth factor-1 receptor and apoptosis via impairing redox-sensitive pathways distinct from oxidized LDL signaling through MAPK- and peroxisome proliferator-activated receptor-γ-involved pathways (34). Nobletiure, a citrus polymethoxylated flavone, suppressed mRNA expression of scavenger receptors of CD36 and CD68 leading to the blockade of modified LDL uptake in THP-1 human monocyte-like cells (35). Unfortunately, this study did not investigate redox-sensitive candidate receptors, possibly responding to antiapoptotic effects on the activity of oxidized LDL-exposed HUVEC.

In summary, our results provide new insights into the relative contributions of JNK, p38 MAPK, and their downstream transcription factors responsible for effects of EGCG and hesperetin on cell survival after oxidized LDL injury (Fig. 8). Oxidized LDL-induced endothelial apoptosis was abolished by EGCG through blunting ROS-triggered activation of JNK, whereas the antiapoptotic feature of hesperetin was mediated by interrupting both JNK- and p38 MAPK-responsive death pathways. In addition, EGCG and hesperetin blocked oxidized LDL-activated JAK2/STAT3-dependent signaling pathway(s). Accordingly, EGCG and hesperetin appear to differentially switch off apoptotic death cascades and hence dampened activation of caspase-3 against the apoptotic trigger. Consequently, dietary interventions with antioxidant components such as EGCG and hesperetin might limit cellular oxidative damage.

**Literature Cited**


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