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Grape powder extract attenuates tumor necrosis factor α-mediated inflammation and insulin resistance in primary cultures of human adipocytes☆

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Abstract

Grapes are rich in phenolic phytochemicals that possess anti-oxidant and anti-inflammatory properties. However, the ability of grape powder extract (GPE) to prevent inflammation and insulin resistance in human adipocytes caused by tumor necrosis factor α (TNFα), a cytokine elevated in plasma and white adipose tissue (WAT) of obese, diabetic individuals, is unknown. Therefore, we examined the effects of GPE on markers of inflammation and insulin resistance in primary cultures of newly differentiated human adipocytes treated with TNFα. We found that GPE attenuated TNFα-induced expression of inflammatory genes including interleukin (IL)-6, IL-1β, IL-8, monocyte chemoattractant protein (MCP)-1, cyclooxygenase (COX)-2 and Toll-like receptor (TLR)-2. GPE attenuated TNFα-mediated activation of extracellular signal-related kinase (ERK) and c-Jun NH_2-terminal kinase (JNK) and activator protein-1 (AP-1, i.e., c-Jun). GPE also attenuated TNFα-mediated IκBα degradation and nuclear factor-kappa B (NF-κB) activity. Finally, GPE prevented TNFα-induced expression of protein tyrosine phosphatase (PTP)-1B and phosphorylation of serine residue 307 of insulin receptor substrate-1 (IRS-1), which are negative regulators of insulin sensitivity, and suppression of insulin-stimulated glucose uptake. Taken together, these data demonstrate that GPE attenuates TNFα-mediated inflammation and insulin resistance in human adipocytes, possibly by suppressing the activation of ERK, JNK, c-Jun and NF-κB.

Keywords: Grapes; Grape powder extract; Adipocytes; Inflammation; Insulin resistance

1. Introduction

Obesity is associated with low-grade, chronic inflammation of white adipose tissue (WAT) characterized by increased production of pro-inflammatory cytokines such as tumor necrosis factor α (TNFα) [1]. The inflammatory effects of TNFα involve the activation of nuclear factor-kappa B (NF-κB) and activator protein-1 (AP-1) [2–4]. Several studies have shown that activation of these transcription factors was responsible for increasing the expression of inflammatory cytokines such as interleukin (IL)-6, IL-1β, IL-8, and monocyte chemoattractant protein (MCP)-1 [3,5] and inflammatory proteins such as Toll-like receptor (TLR)-2 [6] and cyclooxygenase (COX)-2, which contributes to inflammatory prostaglandin production [7].

Obesity-associated inflammation plays an important role in the development of insulin resistance. Several studies reported that deletions of inflammatory cytokine genes including TNFα, IL-6 or MCP-1 protect against the development of insulin resistance and hyperglycemia in obese mice [8–11]. Moreover, inhibition of COX-2 activation in rats [12] and disruption of TLR expression in mice [13] protect against obesity-induced inflammation and insulin resistance. Furthermore, TNFα-activated mitogen-activated protein kinase (MAPK) signaling pathways, including extracellular signal-related kinase (ERK) and c-Jun-NH_2 terminal kinase (JNK), contribute to the development of insulin resistance [14–17]. Insulin receptor substrate 1 (IRS-1) is one of the targets for ERK and JNK that phosphorylate serine residues of IRS-1, which impairs the ability of the insulin receptor to phosphorylate tyrosine residues of IRS-1 [18], thereby reducing insulin action [17]. Finally, TNFα activates NF-κB, which in turn enhances the gene expression of protein tyrosine phosphatase (PTP)-1B, a negative regulator of insulin signaling, by dephosphorylating tyrosine residues on IRS-1 [19–21].

Grapes are rich in phenolic phytochemicals and are one of the most widely consumed fruits in the world. Human [22] and animal [23–25] studies have shown that lyophilized grape powder has cardioprotective effects due to its abundant content of polyphenols that possess anti-oxidative and anti-inflammatory properties. Furthermore, several polyphenols in grapes such as resveratrol [26] and quercetin [27,28] reduce inflammation or insulin resistance in rodent models. However, the protective effects of grapes or their byproducts

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on inflammation and insulin resistance in WAT are unclear, and the mechanisms by which grape products protect against inflammation and insulin resistance in human adipocytes are unknown.

Therefore, we investigated the extent to which grape powder extract (GPE) attenuated TNFα-mediated inflammation and insulin resistance in primary cultures of human adipocytes. We hypothesized that GPE attenuated TNFα-mediated induction of inflammatory genes and insulin resistance by suppressing the activation of MAPK, AP-1, or NF-κB.

2. Materials and methods

2.1. Preparation of GPE

Lyophilized grape powder, obtained from red, green and blue-purple seeded and seedless California grapes, was acquired from the California Table Grape Commission (CTGC). The CTGC grape powder has been reported to contain several types of polyphenols including anthocyanins, monomeric flavanols, flavonols and stilbenes [25]. The powder was extracted to remove the sugars (90% w/w) at the University of North Carolina at Greensboro (UNCG) using a Diaion HP-20 anion resin column and eluted from the column using methanol and subsequently lyophilized to become GPE. GPE was dissolved in dimethyl sulfoxide (DMSO) to make the concentration of 100 mg/ml as the stock solutions stored at −20°C. Stock solutions were diluted immediately before use.

2.2. Materials

All cell culture ware were purchased from Fisher Scientific (Norcross, GA, USA). Adipocyte medium was purchased from Zen Bio, Inc. (Research Triangle Park, NC, USA). Tri-Reagent was purchased from Molecular Research Center (Cincinnati, OH, USA). Gene-specific primers were purchased from Applied Biosystems (Foster City, CA, USA). Goat polyclonal antibody for anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Anti-phospho (Thr183/Tyr185) and -total JNK, anti-phospho (Thr202/Tyr204) and -total ERK, anti-phospho (Ser63) and -total c-Jun, anti-phospho (Ser307) and -total IRS-1, and anti-IκBα rabbit polyclonal antibodies were purchased from Cell Signaling Technologies (Beverly, MA, USA). Immunoblotting buffers and precast gels were purchased from Invitrogen (Carlsbad, CA, USA). Western Lightning Plus Chemiluminescence Substrate was purchased from Perkin Elmer Life Science (Boston, MA, USA). The Nucleofector and Dual Glo luciferase kits were obtained from Amaxa (Cologne, Germany) and Promega (Madison, WI, USA), respectively. All other reagents and chemicals were purchased from Sigma (St. Louis, MO, USA) unless otherwise stated.

2.3. Primary cultures of human adipocytes

Abdominal WAT was obtained from nondiabetic, Caucasian and African-American females, between the ages of 20 and 50 years old with a body mass index less than 32.0 during abdominoplasty. Approval was obtained from the Institutional Review Board at UNCG and the Moses Cone Memorial Hospital in Greensboro, NC, USA. Tissue was digested using collagenase, and stromal vascular (SV) cells were isolated, proliferated and induced to differentiate in adipocyte medium (AM-1, Zen Bio, Inc., RTP, NC, USA) plus 250 μmol/L isobutylmethylxanthine and 1 μmol/L of the thiazolidinedione rosiglitazone (BRL 49653, a gift from Dr. Per Sauerberg at Nova Nordisk A/S, Copenhagen, Denmark) for 3 days. Cultures were then grown in AM-1 only for 3–9 days as previously described [29]. Cultures containing ~50% preadipocytes and ~50% adipocytes, based on visual observations, were treated between Days 6 and 12 of differentiation. Each experiment was repeated at least twice at different times using a mixture of cells from two to three subjects unless otherwise indicated.

2.4. RNA isolation and real-time qPCR

Primary human SV cells were seeded in 35-mm dishes at 0.4×10^6 per dish and differentiated for 6 days. On Day 6, media was changed. Twenty-four hours later, cultures were pretreated with DMSO vehicle (0) or with 10, 30 or 60 μg/ml GPE for 1 h and subsequently treated with 0.5 ng/ml TNFα for 3 h. This 3-h treatment with TNFα was chosen based on the results of a pilot dose and time-course study (data not shown). Following treatment, cultures were harvested and total RNA was isolated using TRI-Reagent according to the manufacturer's protocol. For real-time qPCR, 2.0 μg total RNA was converted into first-strand cDNA using Applied Biosystems High-Capacity cDNA Archive Kit. Real-time qPCR was performed in an Applied Biosystems 7500 FAST Real Time PCR System using Taqman Gene Expression Assays. To account for possible variation related to cDNA input or the presence of PCR inhibitors, the endogenous reference gene GAPDH was simultaneously quantified for each sample, and data were normalized accordingly.

2.5. Immunoblotting

Primary human SV cells were seeded in 35-mm dishes at 0.4×10^6 per dish and differentiated for 6 days. On Day 6, media was changed. Twenty-four hours later, cultures were pretreated with DMSO vehicle (0) or with 10, 30 or 60 μg/ml GPE for 1 h and then treated with 0.5 ng/ml TNFα for 15 min. This 15-min treatment with TNFα was chosen based on the results of a pilot dose and time-course study (data not shown). Immunoblotting was conducted as previously described [30].

2.6. Transient transfections of human adipocytes

For measuring NF-κB activity, primary cultures of human adipocytes were transiently transfected with the NF-κB responsive luciferase (luc) reporter construct pNF-κB (Strategene, La Jolla, CA, USA) using the Amaxa Nucleofector system from Lonza Inc. (Walkersville, MD, USA). Briefly, on Day 6 of differentiation, 1.2×10^6 cells from a 60-mm plate were trypsinized and resuspended in 100 μl of Amaxa Nucleofector solution (cell line nucleofector kit V) and mixed with 1 μg of pNF-κB and 25 ng pRL-CMV for each sample. Electroporation was performed using the Amaxa Nucleofector Device (V-33 Nucleofector program). Cells were replated in 96-well plates after 10 min of recovery in calcium-free DMEM media. Following 24 h, transfected cells were pretreated with DMSO vehicle (0) or with 10, 30 or 60 μg/ml GPE for 1 h and then treated with 100 ng/ml TNFα for 24 h. This 24-h treatment with TNFα was chosen based on the results of a pilot dose and time-course study (data not shown). Firefly luciferase activity was measured using the Promega Dual-Glo luciferase kit and normalized to Renilla luciferase activity from the co-transfected control pRL-CMV vector. All luciferase data will be presented in relative light units as the ratio of firefly luciferase to renilla luciferase activity.

2.7. 2-[^{3}H]Deoxy-glucose uptake

Primary human SV cells were seeded in 12-well plates at 1.6×10^5 per well and differentiated for 12 days. On Day 12, media was changed to serum-free low glucose (5 mmol/L) and insulin (20 pmol/L)-containing media. Twenty-four hours later, cultures were pretreated with DMSO vehicle (0) or with 10, 30 or 60 μg/ml GPE for 1 h and then treated with 5 ng/ml TNFα for 24 h. This 24-h treatment with TNFα was chosen based on the results of a pilot dose and time-course study (data not shown). Culture media was removed and replaced with 0.5 ml of HBSS buffer containing 100 nmol/L human insulin for 10 min. After insulin preincubation, 2-[^{3}H]deoxy-glucose (2-DOG) was added to a final concentration of 0.5 μCi per well and incubated at 37°C for 90 min. Basal and insulin-stimulated 2-DOG uptake were measured as described previously [30].

2.8. Statistical analysis

Statistical analyses were performed for data in Figs. 1, 3B and 4A by testing the main effects of TNFα (− or +), GPE alone (0, 10, 30 or 60 μg/ml) and their interaction (TNFα×GPE) using two-way ANOVA (SPSS version 16.0 for Windows, SAS Institute, Cary, NC, USA). Statistical analyses were performed for data in Fig. 4C by using one-way ANOVA (SPSS version 16.0 for Windows). Tukey's HSD tests were used to compute individual pairwise comparisons of means ($P<.05$). Data are expressed as means±S.E.M.

3. Results

3.1. GPE blocks TNFα-induced inflammatory gene expression

To investigate the protective effects of GPE on TNFα-induced inflammation in human adipocytes, we pretreated primary cultures of newly differentiated human adipocytes with varying doses of GPE for 1 h followed by 3 h of TNFα treatment to induce inflammatory gene expression. GPE decreased TNFα-induced expression of IL-6, IL-1β, IL-8, MCP-1, COX-2 and TLR-2 in a dose-dependent manner (Fig. 1). No visible signs of GPE cytotoxicity were noted (e.g., no floating cells, no abnormal changes in cell morphology of the monolayer). These data demonstrate that GPE blocks TNFα-induced inflammatory gene expression in primary cultures of newly differentiated human adipocytes.

3.2. GPE prevents TNFα-mediated ERK and JNK activation

Because the phosphorylation of MAPK plays an important role in activating transcription factors that induce inflammatory gene expression, we investigated the impact of GPE on MAPK phosphorylation. We found that 1-h pretreatment of cultures of human

adipocytes with GPE blocked basal and TNFα-mediated phosphorylation of ERK and JNK in a dose-dependent manner (Fig. 2). These data demonstrate that GPE prevents TNFα activation of ERK and JNK in primary cultures of newly differentiated human adipocytes.

3.3. GPE decreases TNFα-mediated c-Jun and NF-κB activation

Given the important role that AP-1 and NF-κB play in the transcriptional activation of inflammatory genes, we examined the preventive effects of GPE on AP-1 and NF-κB activation. Pretreatment of the cultures with GPE for 1 h decreased TNFα-mediated phosphorylation of c-Jun, a component of AP-1 and a downstream target of JNK (Fig. 3A). Similarly, GPE blocked basal and TNFα-induced IκBα degradation (Fig. 3A). Consistent with these data, GPE attenuated TNFα-stimulated NF-κB reporter activity in a dose-dependent manner (Fig. 3B). Taken together, these data demonstrate that GPE decreases TNFα-mediated c-Jun phosphorylation and NF-κB activity in primary cultures of newly differentiated human adipocytes.

3.4. GPE prevents TNFα-mediated insulin resistance

Increased activation of MAPK, AP-1 and NF-κB, and inflammatory gene expression cause insulin resistance. This causal relationship has been linked to increased TNFα levels in plasma and WAT of obese individuals [1]. Thus, we examined the ability of GPE to prevent TNFα-mediated insulin resistance in adipocytes, using increased PTP-1B expression and phosphorylation serine residue 307 of IRS-1 (p-Ser307-IRS-1), which are negative regulators of insulin sensitivity, and decreased insulin-stimulated 2-DOG uptake as indicators of insulin resistance. GPE decreased TNFα-mediated PTP-1B expression (Fig. 4A) and p-Ser307-IRS-1 (Fig. 4B), and increased insulin-stimulated 2-DOG uptake (Fig. 4C) in a dose-dependent manner. Collectively, these data demonstrate that GPE prevents TNFα-mediated insulin resistance in primary cultures of newly differentiated human adipocytes, possibly by inhibiting upstream mediators of inflammation and their downstream negative regulators of insulin sensitivity.

4. Discussion

In this study, we demonstrated the protective effects of GPE on TNFα activation of upstream proteins and induction of inflammatory genes and negative regulators of insulin sensitivity in primary cultures of newly differentiated human adipocytes. We demonstrated that (1) GPE attenuated TNFα-induced expression of IL-6, IL-1β, IL-8, MCP-1, COX-2 and TLR-2 (Fig. 1); (2) GPE attenuated TNFα activation of ERK and JNK (Fig. 2), MAPK linked to the activation of NF-κB and c-Jun, a component of AP-1; (3) GPE prevented TNFα activation of c-Jun and attenuated TNFα-mediated IκBα degradation and NF-κB activity

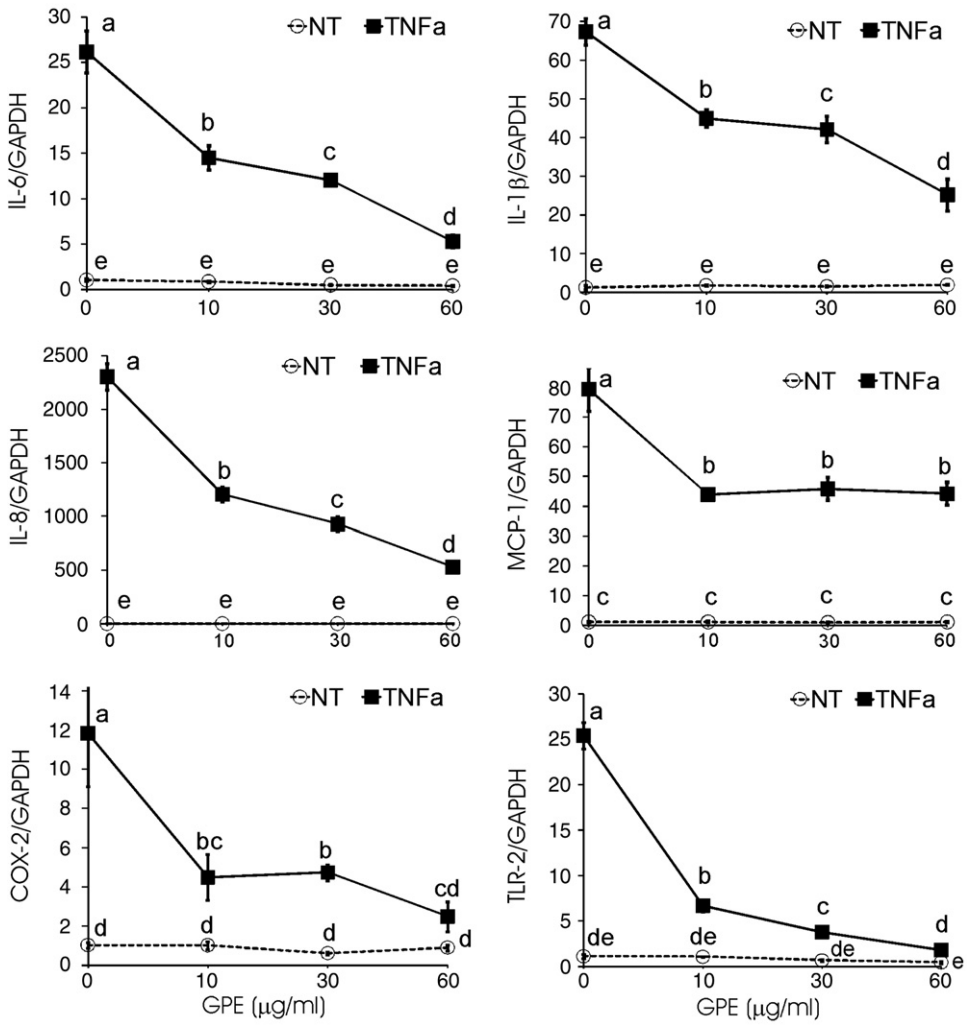

Fig. 1. GPE blocks TNFα-induced inflammatory gene expression. Cultures of newly differentiated human adipocytes were pretreated with DMSO vehicle (0) or with 10, 30 or 60 μg/ml GPE for 1 h and then treated with 0.5 ng/ml TNFα for 3 h. Subsequently, cultures were harvested for the determination of mRNA levels of IL-6, IL-1β, IL-8, MCP-1, COX-2 and TLR-2 by real-time qPCR. Data are representative of three independent experiments. Values are means±S.E.M., $n=3$. Means without a common letter differ, $P<.05$.

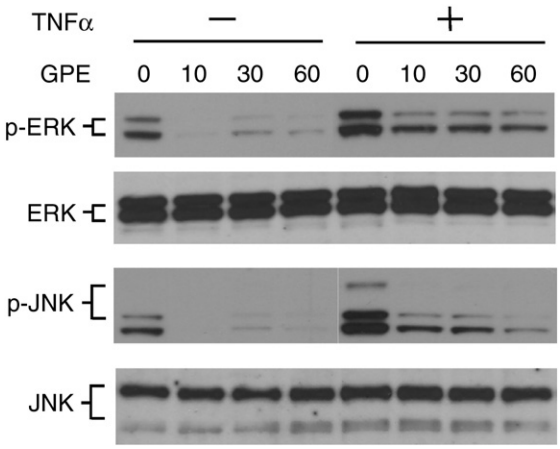

Fig. 2. GPE prevents TNFα activation of ERK and JNK. Cultures of newly differentiated human adipocytes were pretreated with DMSO vehicle (0) or with 10, 30 or 60 μg/ml GPE for 1 h and then treated with 0.5 ng/ml TNFα for 15 min. Subsequently, cultures were harvested for the determination of the protein levels of p-ERK, ERK, p-JNK and JNK by immunoblotting. Data are representative of three independent experiments.

(Fig. z3); and (4) GPE prevented TNFα-induced expression of PTP-1B and phosphorylation of serine residue 307 of IRS-1, which are negative regulators of insulin sensitivity, and suppression of insulin-stimulated glucose uptake (Fig. 4). Taken together, these findings are the first to demonstrate that GPE inhibits TNFα-mediated activation of ERK and

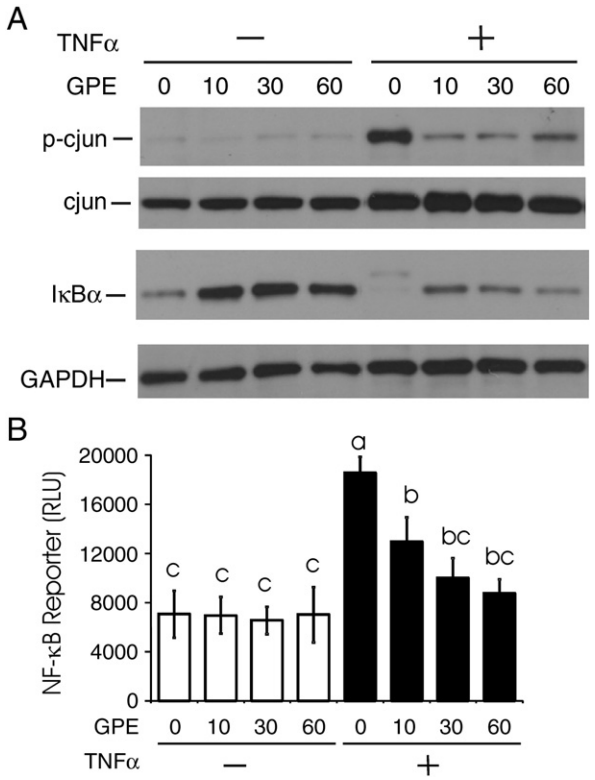

Fig. 3. GPE decreases TNFα-mediated c-Jun activation, IκBα degradation and NF-κB transcriptional activity. (A) Cultures of newly differentiated human adipocytes were pretreated with DMSO vehicle (0) or with 10, 30 or 60 μg/ml GPE for 1 h and then treated with 0.5 ng/ml TNFα for 15 min. Subsequently, cultures were harvested for the determination of the protein levels of p-c-Jun, c-Jun, IκBα and GAPDH by immunoblotting. (B) Cultures were transfected on Day 6 with pNF-κB luc and pRL-CMV. Twenty-four hours later, transfected cells were pretreated with DMSO vehicle (0) or with 10, 30 or 60 μg/ml GPE for 1 h and then treated with 100 ng/ml TNFα for 24 h. (A) Data are representative of three independent experiments. (B) Values are means±S.E.M., $n=6$. Means without a common letter differ, $P<.05$.

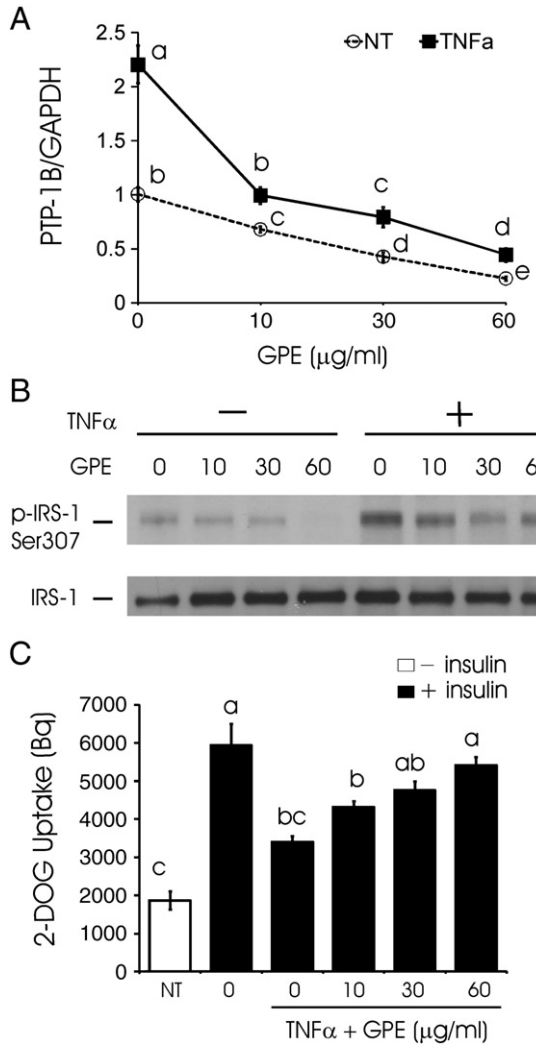

Fig. 4. GPE prevents TNFα-mediated insulin resistance. (A) Cultures of newly differentiated human adipocytes were pretreated with DMSO vehicle (0) or with 10, 30 or 60 μg/ml GPE for 1 h and then treated with 0.5 ng/ml TNFα for 3 h to determine the mRNA level of PTP-1B by real-time qPCR, or (B) for 15 min to determine the protein level of p-Ser307-IRS-1 by immunoblotting. (C) Cultures were incubated with serum-free low glucose media for 24 h and then pretreated with DMSO vehicle (0) or with 10, 30 or 60 μg/ml GPE for 1 h and then treated with 5 ng/ml TNFα for 24 h. Insulin-stimulated 2-DOG uptake was measured after a 100-min incubation with 100 nmol/L insulin. (A,B) Data are representative of two independent experiments. (A) Values are means±S.E.M., $n=3$. (C) Data are representative of two independent experiments. Values are means±S.E.M., $n=4$. Means without a common letter differ, $P<.05$.

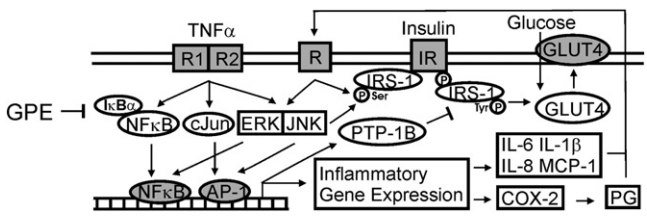

Fig. 5. Working model on how GPE prevents TNFα-mediated inflammation and insulin resistance. We propose that GPE initially blocks the activation of MAPK, AP-1 and NF-κB. This prevents them from inducing inflammatory gene expression, thereby blocking autocrine/paracrine signals associated with propagating the inflammatory cascade. By preventing this inflammatory cascade, GPE decreases TNF-α-mediated IRS-1-serine phosphorylation and increases IRS-1-tyrosine phosphorylation. Increased IRS-1 tyrosine phosphorylation increases GLUT4 translocation to the plasma membrane, thereby facilitating glucose uptake.

JNK and transcription factors AP-1 and NF-κB that induce inflammatory genes known to cause insulin resistance in human adipocytes.

Based on these data, we propose the following scenario by which GPE reduces TNFα-mediated inflammation and insulin resistance (Fig 5). We speculate that GPE initially attenuates TNFα-mediated activation of MAPK, AP-1 and NF-κB at 15 min. This GPE-mediated decrease in MAPK, AP-1 and NF-κB activation, in turn, attenuates TNFα-induced expression of inflammatory genes (i.e., IL-6, IL-1β, IL-8, MCP-1, COX-2 and TLR-2) at 3 h. This results in decreased autocrine/paracrine activation of the inflammatory cascade, including attenuation of negative regulators of insulin sensitivity (i.e., PTP-1B and p-Ser307-IRS-1).

TNFα was identified as one of the first links between obesity-associated inflammation and insulin resistance [8]. Elevated production of TNFα by WAT was reported in obese humans and is positively correlated with impaired glucose disposal [1]. However, mechanism(s) by which TNFα signals to its downstream targets in human adipocytes is (are) unclear. The activation of MAPK, AP-1 and NF-κB signaling by TNFα has been reported to cause inflammation and insulin resistance in several cell lines [2,16,18]. Consistent with these studies, we found that TNFα activates ERK, JNK, c-Jun and NF-κB, which together increase inflammatory gene expression and induce insulin resistance in primary cultures of newly differentiated human adipocytes.

Several studies have examined the anti-inflammatory properties of grape byproducts rich in phytochemicals such as wine, grape juice and grape seeds [31]. For example, Sakurai et al. [32] demonstrated that oligomerized grape seed polyphenols attenuated inflammation in cocultures of adipocytes and macrophages via their anti-oxidative properties. Lee et al. [33] demonstrated that red wine extract (RWE) inhibited 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced transformation of JB6 promotion-sensitive mouse skin epidermal (JB6 P+) cells by blocking the activation of Raf/MEK/ERK/p90RSK signaling pathway and subsequently suppressing the activation of AP-1 and NF-κB transcription factors. Similarly, our data showed that GPE attenuated TNFα-mediated inflammation and insulin resistance, in part, by suppressing the activation of ERK, JNK, c-Jun and NF-κB.

We also demonstrate that GPE effectively prevents TNFα-mediated insulin resistance in human adipocytes. Notably, GPE attenuated TNFα-induced expression of PTP-1B and phosphorylation of serine residue 307 of IRS-1, which are negative regulators of insulin sensitivity. Phosphorylation of serine residues of IRS-1 is one of the most common mechanisms by which MAPK and IκB kinase-β, a central coordinator of inflammatory responses through activation of NF-κB, decrease insulin-stimulated glucose uptake [34]. The activation of MAPK by TNFα increased the phosphorylation of serine residues on IRS-1 [18], which impairs the ability of the insulin receptor to phosphorylate tyrosine residues of IRS-1, thereby reducing insulin action [17]. PTP-1B, an NF-κB target gene [19,20], negatively regulates insulin sensitivity by dephosphorylating tyrosine residues of IRS-1 [21]. Thus, TNFα-induced PTP-1B gene expression [35] contributes to insulin resistance. Here, we demonstrate that GPE prevents TNFα-mediated insulin resistance in primary cultures of newly differentiated human adipocytes, possibly by inhibiting the activation of upstream mediators of inflammation such as ERK, JNK, c-Jun or NF-κB and their downstream negative regulators of insulin sensitivity such as PTP-1B expression and phosphorylation of serine residue 307 of IRS-1.

Lee et al. [33] found that the anticarcinogenic effects of RWE may be due to the higher content of the flavonol quercetin, as compared to the phytoalexin resveratrol. It has been reported that quercetin has anti-inflammatory properties. For example, quercetin supplementation of high fat-fed mice [27] or obese Zucker rats [28] reduced circulating markers of inflammation. *In vitro*, quercetin attenuated differentiation and markers of inflammation in murine 3T3-L1 adipocytes [36] and suppressed TPA-mediated activation of MEK/ERK, AP-1 and NF-κB in murine skin epidermal (JB6 P+) cells [33]. Notably, we found using reverse-phase HPLC that one of the major components in GPE was quercetin glucosides (9.2%) (unpublished data). However, quercetin glucosides may not exist in circulation, because β-glucosidase hydrolysis of quercetin glucosides occurs when GPE polyphenols are absorbed across the intestine and pass through the liver [37,38]. Also, the concentrations of quercetin found in circulation vary markedly, ranging from 0.1 to 10 μM [39,40]. Therefore, the doses of 10, 30 and 60 μg/ml GPE will contain ~3, 9 and 18 μM quercetin (molecular weight, 302.2), respectively. Thus, the 10 and 30 μg/ml GPE may provide physiological levels of quercetin for those consuming a diet rich in grape products.

The other components we found in GPE were catechins (4.2%), gallic acid (1.4%) and resveratrol (0.53%) (unpublished data). Resveratrol is a potential anti-inflammatory polyphenol in GPE. Several animal studies reported that resveratrol prevents insulin resistance and adiposity in mice fed a high-fat diet [26,41,42]. These studies suggest that resveratrol shifts excess calories away from storage in WAT and towards oxidation in muscle and brown adipose tissue. *In vitro*, resveratrol has been shown to reduce inflammation in murine 3T3-L1 adipocytes [43], enhance glucose transport in muscle [44], reduce oxidative stress in human lung epithelial cells [45,46], reduce ER stress in mouse macrophages [47] and decrease TNFα-mediated NF-κB activation in hepatocytes [48] and coronary arterial endothelial cells [49]. Studies are underway in our laboratory to determine the extent to which quercetin, resveratrol and other polyphenol candidates in GPE prevent inflammation and insulin resistance in primary cultures of human adipocytes.

Collectively, these data demonstrate that GPE attenuates TNFα-mediated inflammation and insulin resistance by suppressing the activation of ERK, JNK, c-Jun and NF-κB that induce the expression of inflammatory genes and negative regulators of insulin sensitivity in primary cultures of newly differentiated human adipocytes. *In vivo* studies are needed to determine the ability of GPE to recapitulate these *in vitro* findings in WAT, because of the extensive metabolism of polyphenols *in vivo* [50].

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