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Ergosterol Attenuates Palmitate-Induced Tenocyte Apoptosis Through the Suppression of ER Stress and Myocyte-Derived IL-15/METRNL-Mediated Crosstalk

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ABSTRACT

Ergosterol (EG), the main bioactive ingredient in *Wolfiporia hoelen*, has been shown to suppress inflammation and oxidative stress in various disease models. However, the effects of EG on endoplasmic reticulum (ER) stress and apoptosis in tenocytes in *in vitro* obesity models have not been studied. Protein expression in tenocytes was assessed by Western blotting. Terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) staining was performed to distinguish apoptotic cells, and ELISA was conducted to quantify the protein content in the tenocyte culture medium. The autophagosomes were visualized as punctate structures via monodansylcadaverine (MDC) staining. In this study, we found that treatment with EG prevented apoptosis, collagen degradation, and ER stress and enhanced cell migration in tenocytes treated with palmitate. EG treatment augmented peroxisome proliferator-activated receptor delta (PPAR δ) expression and the expression of autophagy markers. PPAR δ small interfering RNA (siRNA) or 3-methyladenine, an autophagy inhibitor, abolished the effects of EG on palmitate-mediated tenocyte dysfunction. Interleukin 15 (IL-15) and meteorin-like protein (METRNL) from C2C12 myocytes treated with EG also ameliorated tenocyte injury caused by palmitate. Collectively, our findings suggest that EG exerts protective effects against palmitate-induced metabolic stress in tendinopathy by alleviating ER stress through the PPAR δ /autophagy axis and enhancing IL-15- and METRNL-mediated muscle–tendon crosstalk, supporting its potential as a natural compound-based therapeutic candidate.

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Ergosterol; tendinopathy; ER stress; apoptosis; obesity; myokine

1. Introduction

Tendinopathy is a chronic degenerative disease caused by repetitive mechanical overuse, leading to collagen breakdown and tenocyte dysfunction. Recent studies have shown that, in addition to mechanical damage, intracellular stress, particularly endoplasmic reticulum (ER) stress, plays a key role in its pathophysiology [1,2]. Excessive mechanical load, inflammatory cytokines, and metabolic stress can induce protein misfolding in the ER, triggering the unfolded protein response (UPR). While initially protective, prolonged ER stress leads to apoptosis and tendon degeneration. This process worsens with obesity, which is now recognized as a major risk factor for tendinopathy [1,2]. In addition to increased mechanical load, obesity induces metabolic inflammation and free fatty acid (FFA) accumulation, especially accumulation of saturated fatty acids such as palmitic acid, which aggravate ER stress and disrupt tenocyte

homeostasis. Therefore, treatment strategies must go beyond pain relief to address cellular mechanisms such as ER stress. Additionally, myokines – muscle-derived signaling molecules – are gaining attention for their potential to reduce ER stress [3], suppress inflammation [4], and mitigate oxidative stress [5]. The incorporation of myokine-based approaches, along with ER stress modulation and systemic obesity management, offers a promising direction for future tendinopathy therapies. Among these, interleukin 15 (IL-15) and meteorin-like protein (METRNL) have emerged as regulators of metabolism and tissue homeostasis.

Ergosterol (EG), a sterol component primarily found in *Wolfiporia hoelen*, has attracted attention for its various pharmacological activities, including anti-inflammatory, antioxidant, and antiobesity effects [6]. In particular, it has been reported to suppress the production of inflammatory cytokines such as TNF α and

Figure 1. EG prevents apoptosis in hyperlipidemic tenocytes. (A) Chemical structure of EG; cell viability in tenocytes treated with EG (0–100 μ M) for 24 h (B) or palmitate (0–400 μ M) for 24 h (C); (D) cell viability assay and caspase 3 activity assay in tenocytes treated with 400 μ M palmitate, EG (0–50 μ M), and/or 200 nM rapamycin for 24 h. TUNEL staining in tenocytes in the presence of palmitate and/or EG for 24 h. The data are presented as the means $\pm$ SDs from three independent biological replicates. Statistical analysis was performed via one-way ANOVA with Tukey's post hoc test (two-sided). * p < 0.05 vs. control; ! p < 0.05 vs. palmitate alone.

compound (purity $\geq 99\%$; MedChemExpress, Monmouth Junction, NJ), originally provided by Dr. Kyoung-Tae Lee. EG and 3-methyladenine (3-MA; Sigma, St. Louis, MO) and rapamycin (Sigma, St. Louis, MO) were dissolved in dimethyl sulfoxide (DMSO; Sigma, St. Louis, MO) to prepare stock solutions, and the final concentration of DMSO in all experiments did not exceed 0.1% (v/v). Corresponding DMSO vehicle controls were included in all experimental conditions. Cells were exposed to palmitate (200 or 400 μM), EG (0–100 μM), 3-MA (5 mM), and/or rapamycin (200 nM) for 24 h. Recombinant human IL-15 and METRNL proteins were purchased from R&D Systems (Minneapolis, MN) and administered according to the manufacturer's recommendations.

2.2. Apoptosis

2.2.1. Cell viability assay

Tenocytes were incubated with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; Sigma, St. Louis, MO) for 30 min at 37°C to assess cell viability. Cell viability, caspase-3 activity, and terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) assays were conducted following established protocols [10] with minor modifications. The resulting purple formazan crystals were dissolved in DMSO for 30 min at 26°C with gentle agitation, and absorbance was subsequently recorded at 560 nm using a microplate reader (Bio-Rad, Hercules, CA).

2.2.2. Caspase 3 activity assay

Caspase-3 enzymatic activity was determined using a commercial colorimetric assay kit (Abcam, Cambridge, MA) in accordance with the manufacturer's instructions.

2.2.3. DNA fragmentation

Apoptotic DNA fragmentation was analyzed via TUNEL using a commercial assay kit (Abcam, Cambridge, MA), following the protocol provided by the manufacturer.

2.3. Cell migration assay

Tenocytes (1×10^6 cells/well) were seeded into six-well plates. A scratched gap was introduced via a sterile pipette tip, and residual debris was removed by washing with PBS. After 24 h of treatment under the indicated conditions, cell migration was observed via phase-contrast microscopy.

2.4. Protein expression assay

2.4.1. Sample preparation

Tenocytes were washed with PBS, scraped, and lysed in extraction buffer (PROPREP; Intron Biotechnology, Seongnam, Republic of Korea) at 4°C for 1 h with intermittent vortexing. The lysates were subsequently centrifuged (15,000 rpm, 55 min, 4°C), and the supernatants were mixed with SDS sample buffer and boiled at 95°C for 7 min.

2.4.2. Procedure

Proteins (30–50 μg) were separated on 10–12% SDS-PAGE gels and transferred to nitrocellulose membranes (Sigma, St. Louis, MO). The membranes were blocked with 5% skim milk for 30 min and then incubated with primary antibodies (containing 5% BSA or skim milk), followed by incubation with secondary antibodies (1:2000) in skim milk.

The primary antibodies used included anti-CHOP (1:3000), anti-eIF2 α (1:2000), anti-p62 (1:2500), anti-peroxisome proliferator-activated receptor delta (PPAR δ) (1:2000), and anti- β -actin (1:4000) (Santa Cruz Biotechnology, Dallas, TX) antibodies. Additionally, anti-phospho eIF2 α (1:1000; Cell Signaling, Danvers, MA) and anti-LC3 (1:1000; Novus Biologicals, Centennial, CO) antibodies were used.

2.5. Suppression of gene expression

Tenocytes at ~80% confluence were transfected with 20 nM small interfering RNA (siRNA) targeting PPAR δ , IL-15, or METRNL (Santa Cruz Biotechnology, Dallas, TX) via Viafect (Promega, Madison, WI) in Opti-MEM (WELGENE, Kyungsan, Republic of Korea) according to the manufacturer's instructions. After 24 h, the medium was replaced, and the cells were analyzed for 24 h thereafter.

2.6. Determination of protein levels in the culture supernatants

Following experimental treatments, the culture media were collected from tenocyte plates and stored at -70°C until analysis. The concentrations of MMP1, MMP13, collagen type I, IL-15, and METRNL in the supernatants were measured using commercially available ELISA kits (Abcam, Cambridge, MA) according to the manufacturer's instructions. The procedure for ELISA-based quantification of proteins in the culture supernatant was adapted from a previously reported method [10].

2.7. Autophagosome visualization

Autophagic activity in tenocytes was assessed via monodansylcadaverine (MDC; Sigma, St. Louis, MO) staining. The cells were fixed with 1% formaldehyde for 15 min at room temperature, incubated with 100 μ M MDC at 37°C for 1 h, and then washed with PBS. The autophagosomes were visualized as fluorescent green puncta under a fluorescence microscope, and quantification was performed in randomly selected fields.

2.8. Statistical analyses

Statistical analyses were performed via GraphPad Prism software (version 8; GraphPad Software, San Diego, CA). The data are presented as the means $\pm$ standard deviations (SDs). Depending on the parameter assessed, the results are expressed in one of three formats: (i) normalized ratios (e.g., relative to the control or maximal response), (ii) arbitrary units (e.g., caspase-3 enzymatic activity), or (iii) absolute quantitative values (e.g., protein concentration in ng/mL or migration distance in μ m), as specified in the figure legends. Each experiment was independently repeated three times ($n = 3$). Group differences were analyzed via one-way repeated-measures analysis of variance (ANOVA) followed by Tukey's post hoc test for multiple comparisons. Quantitative assessments of protein expression levels and wound closure in migration assays were performed via ImageJ software (National Institutes of Health, Bethesda, MD).

3. Results

3.1. EG prevents apoptosis in hyperlipidemic tenocytes

In the current study, tenocytes were treated with various concentrations of EG to optimize the cell treatment conditions. There was no significant decrease in cell viability in tenocytes treated with EG (0–50 μ M) (Figure 1(B)). Moreover, 400 μ M palmitate reduced cell viability up to 50%. In addition, a reduction in cell viability was not detected in tenocytes exposed to 200 μ M palmitate for 24 h (Figure 1(C)). Thus, 50 μ M EG, which had no effect on cell viability, was used throughout the study. For the assessment of apoptosis, 400 μ M palmitate was administered, whereas 200 μ M palmitate was utilized to induce metabolic stress. Palmitate treatment, as an *in vitro* obesity model, reduced cell viability but increased the number of TUNEL-positive cells and caspase 3 activity. However, additional EG treatment reversed all these changes (Figure 1(D)). Furthermore,

rapamycin, an autophagy inducer, also abrogated the effects of palmitate on cell viability and caspase 3 activity (Figure 1(D)).

3.2. EG enhances cell migration and suppresses ECM degradation signaling in hyperlipidemic tenocytes

Inappropriate tenocyte migration and ECM degradation play key roles in the development of tendinopathy [11]. Impaired cell migration was observed in tenocytes in the presence of palmitate. However, treatment with EG reversed these effects in a dose-dependent manner (Figure 2(A)). Type I collagen provides tensile strength and limited elasticity to the tendon. During the pathogenesis of tendinopathy, it is degraded primarily by MMP1 and 13, leading to disruption of the ECM [12,13]. Palmitate treatment increased the MMP1 and MMP13 levels, whereas it reduced the level of type I collagen in the culture medium of tenocytes. However, these changes were reversed by EG administration (Figure 2(B)).

3.3. EG mitigates ER stress in hyperlipidemic tenocytes through the PPAR δ /autophagy axis

ER stress in tenocytes plays a causative role in the development of tendinopathy [1]. Treatment with palmitate increased the expression of ER stress markers, such as phosphorylated eIF2 α and CHOP, in tenocytes, which was reversed by EG (Figure 3(A)). We next investigated how EG ameliorates ER stress in tenocytes. PPAR δ and autophagy have been shown to mitigate ER stress in various cell types [14,15]. EG treatment increased PPAR δ expression and the expression of autophagy markers such as autophagic vesicles, LC3 conversion and p62 degradation in tenocytes (Figure 3(B)). siRNA-mediated suppression of PPAR δ abrogated the effects of EG on autophagy markers (Figure 3(C)). PPAR δ siRNA or 3-MA, an autophagy inhibitor, reduced the effects of EG on ER stress as well as apoptosis, cell migration, and ECM-mediated signaling in palmitate-treated tenocytes (Figure 3(D–G)).

3.4. EG enhances IL-15 and METRNL release from hyperlipidemic myocytes and attenuates tenocyte dysfunction

In addition to the direct effects of EG on tenocytes, we investigated the effects of IL-15 and METRNL, which have anti-ER stress properties [16,17], on tenocyte dysfunction caused by systemic obesity. Low IL-15 and METRNL levels have been reported in the serum of obese patients [18,19]. In addition,

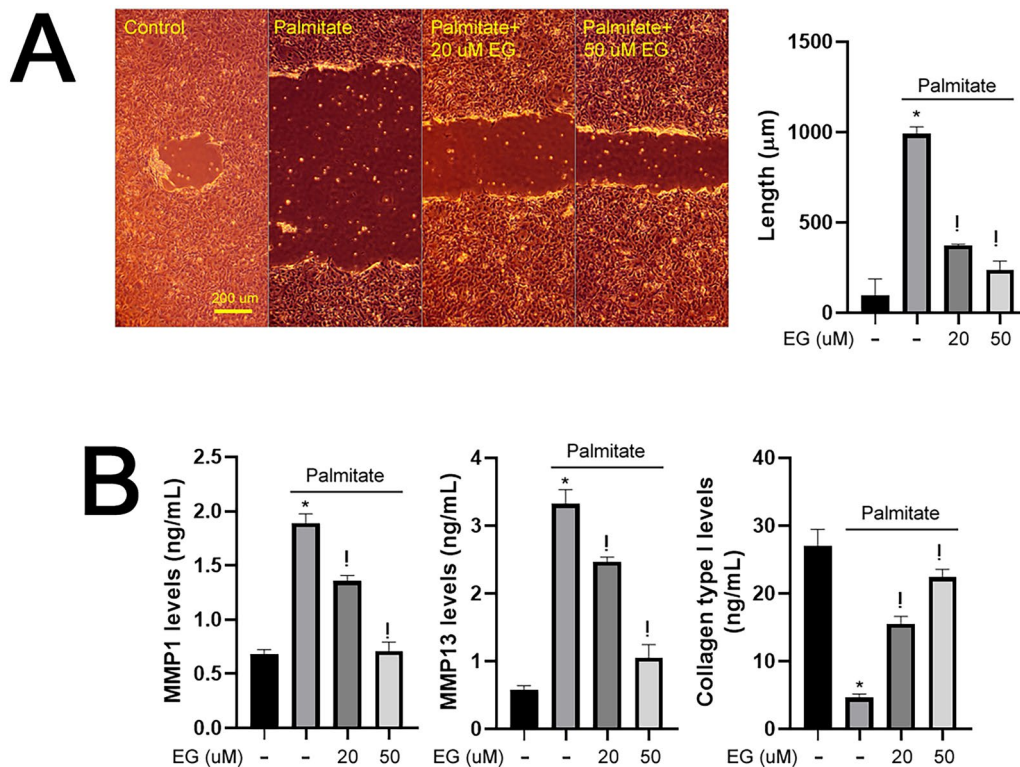


Figure 2. EG enhances cell migration and attenuates ECM degradation in tenocytes under hyperlipidemic conditions. (A) Cell migration assay in tenocytes exposed to 200 μM palmitate and/or EG (0–50 μM) for 24 h; (B) ELISAs for determining MMP1, MMP13, and collagen type I levels in culture medium of tenocytes treated with 200 μM palmitate and/or EG (0–50 μM) for 24 h. The data are presented as the means ± SDs from three independent biological replicates. Statistical analysis was performed via one-way ANOVA with Tukey's post hoc test (two-sided). * $p < 0.05$ vs. control; ! $p < 0.05$ vs. palmitate alone.

we confirmed that the secretion of IL-15 and METRNL, which was impaired by palmitate treatment in C2C12 myoblasts, was restored by EG (Figure 4(A)). To recapitulate skeletal muscle–tendon communication in an *in vitro* model, tenocytes were exposed to human recombinant IL-15 or METRNL. Treatment with IL-15 or METRNL mitigated ER stress, resulting in attenuated apoptosis, impaired cell migration, and normalization of EC degradation in palmitate-treated tenocytes (Figure 4(B–E)).

4. Discussion

Epidemiological evidence consistently demonstrates an increased incidence of tendinopathy in obese patients, attributed not only to increased mechanical loading but also to obesity-associated low-grade chronic inflammation and lipid deposition in various organs, which accelerate tendon degeneration. Current treatment strategies, such as weight loss [20] and rehabilitation exercises [21] to address the underlying cause and analgesics and steroid injections to improve symptoms [22], have demonstrated limited effectiveness. Weight loss and systematic rehabilitation require long-term commitment and compliance, and repeated use of analgesics or steroids carries the risk of serious side effects, making long-term

management difficult. Therefore, novel natural compound-based strategies that not only exert local protective effects on tendon tissue but also improve systemic metabolic dysfunction due to obesity without placing undue physical strain on the patient warrant investigation. In the present study, several novel findings are as follows: (1) EG prevents apoptosis and promotes cell migration in tenocytes under hyperlipidemic conditions. (2) EG is associated with increased PPARδ-regulated autophagy, accompanied by attenuation of ER stress. (3) IL-15 and METRNL secreted by myocytes partially rescued hyperlipidemic tenocyte dysfunction.

Tenocyte apoptosis and ECM degradation are widely recognized as central pathological events in the onset and progression of tendinopathy [23]. Excessive apoptosis reduces the population of functional tenocytes, impairing their ability to maintain and repair the ECM. Concurrently, upregulated matrix-degrading enzymes, such as MMP-1, accelerate the breakdown of type I collagen – the principal structural component conferring tensile strength to tendons – thereby compromising the mechanical integrity of the tissue. In parallel, aberrant cell migration further exacerbates disease progression by disrupting coordinated tendon remodeling and repair processes. Impaired migration limits the recruitment

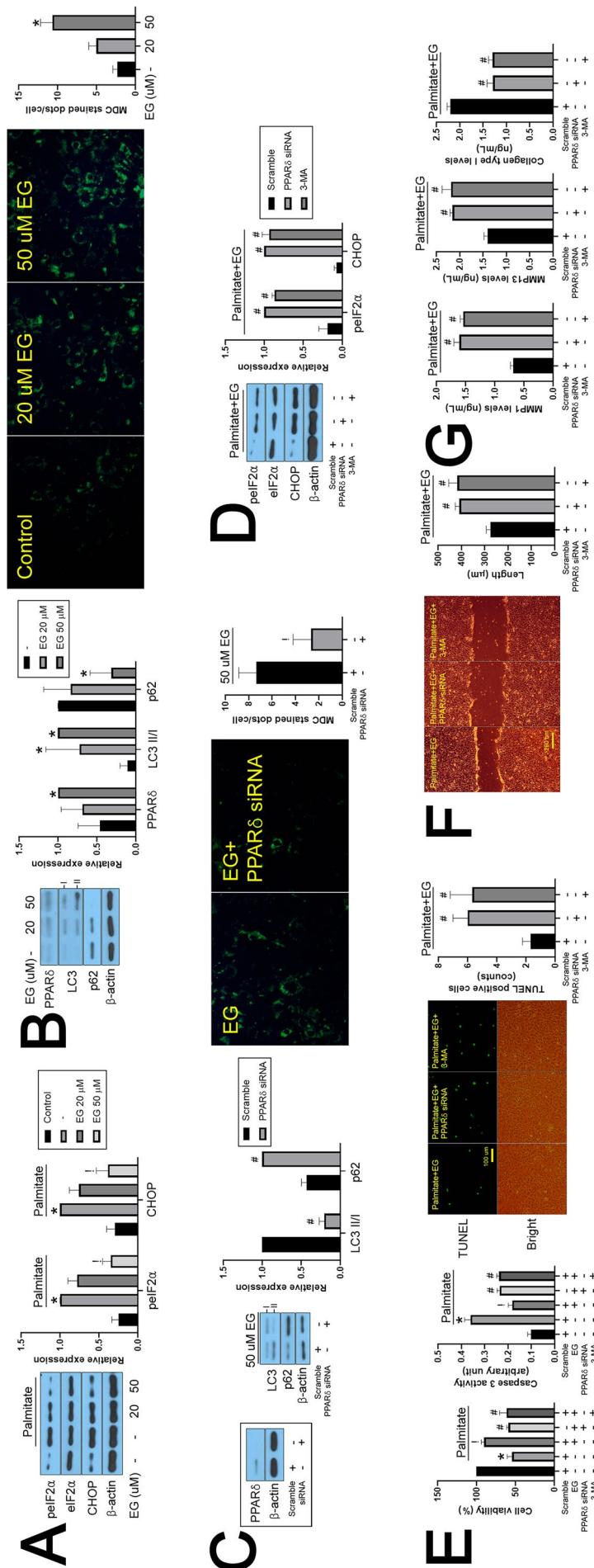


Figure 3. PPAR δ and autophagy-mediated suppression of ER stress contribute to the protective effects of EG on hyperlipidemic tenocytes. (A) Western blotting of phosphorylated eIF2 α and CHOP in tenocytes exposed to 200 μ M palmitate and/or EG (0–50 μ M) for 24 h; (B) Western blotting of LC3 I/II and p62 as well as MDC staining in tenocytes exposed to EG (0–50 μ M) for 24 h; (C) Western blotting of LC3 I/II and p62 as well as MDC staining in PPAR δ siRNA-transfected tenocytes exposed to EG (50 μ M) for 24 h; (D) Western blotting of phosphorylated eIF2 α and CHOP in PPAR δ siRNA-transfected tenocytes exposed to 200 μ M palmitate and EG (50 μ M), and/or 3-MA (5 mM) for 24 h; (E) cell viability and caspase 3 activity assays, and TUNEL staining in PPAR δ siRNA-transfected tenocytes exposed to 200 μ M palmitate and EG (50 μ M), and/or 3-MA (5 mM) for 24 h; (F) ELISAs for determining MMP1, MMP13, and collagen type I levels in culture medium of PPAR δ siRNA-transfected tenocytes exposed to 200 μ M palmitate and EG (50 μ M), and/or 3-MA (5 mM) for 24 h. The data are presented as the means $\pm$ SDs from three independent biological replicates. Statistical analysis was performed via one-way ANOVA with Tukey's post hoc test (two-sided). * p < 0.05 vs. control; # p < 0.05 vs. palmitate alone; $^{\dagger}p$ < 0.05 vs. palmitate + EG.

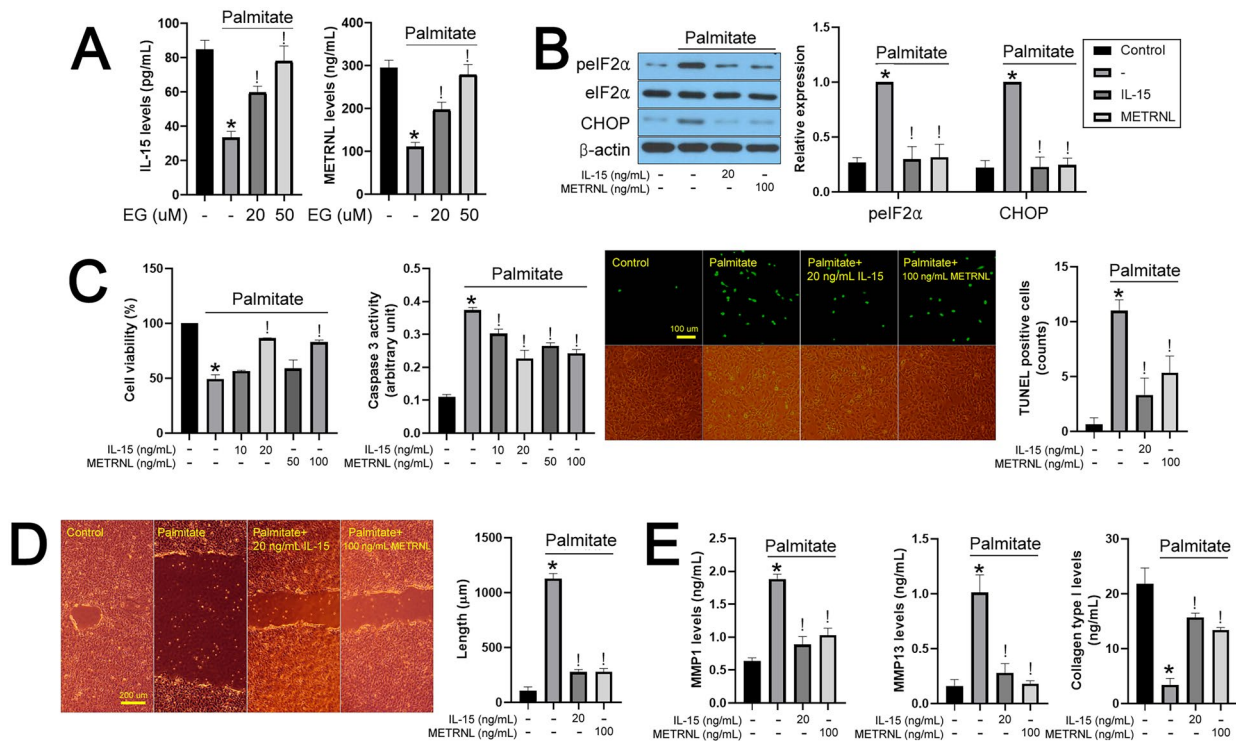


Figure 4. Skeletal muscle cell-derived IL-15 and METRNL attenuate FFA-induced ER stress and dysfunction in tenocytes. (A) ELISAs for determining IL-15 and METRNL levels in culture medium of C2C12 myocytes treated with EG (0–50 μM) for 24 h; (B) Western blotting of phosphorylated eIF2α and CHOP in tenocytes exposed to 200 μM palmitate, IL-15 (20 ng/mL), and/or METRNL (100 ng/mL) for 24 h; (C) cell viability and caspase 3 activity assays in tenocytes in the presence of 400 μM palmitate, IL-15 (0–20 ng/mL) and/or METRNL (0–100 ng/mL) for 24 h. TUNEL staining in tenocytes in the presence of 400 μM palmitate, IL-15 (20 ng/mL) and/or METRNL (100 ng/mL) for 24 h; (D) cell migration assay in tenocytes treated with 200 μM palmitate, IL-15 (20 ng/mL), and/or METRNL (100 ng/mL) for 24 h; (E) ELISAs for determining MMP1, MMP13, and collagen type I levels in culture medium of tenocytes treated with 200 μM palmitate, IL-15 (20 ng/mL), and/or METRNL (100 ng/mL) for 24 h. The data are presented as the means ± SDs from three independent biological replicates. Statistical analysis was performed via one-way ANOVA with Tukey's post hoc test (two-sided). * $p < 0.05$ vs. control; ! $p < 0.05$ vs. palmitate alone.

of tenocytes to sites of microdamage, delaying or preventing effective matrix restoration [24]. Collectively, the interplay of increased apoptosis, excessive ECM degradation, and dysregulated cell migration fosters a degenerative microenvironment that drives the transition from early tendon injury to chronic tendinopathy. Therefore, we first examined the effects of EG on apoptosis and cell motility, as well as on the release of MMP-1 and collagen type I by tenocytes, in an *in vitro* obesity model. Elevated apoptosis, impaired cell migration and collagen type I degradation were detected in palmitate-treated tenocytes. However, additional EG treatment reversed all these changes. These findings highlight the potential protective effects of EG as an obesity-associated metabolic stress-induced tenocyte dysfunction by simultaneously mitigating local tendon degeneration and addressing obesity-induced metabolic stress.

ER stress in tenocytes not only induces apoptosis but also impairs cell migration, both of which play pivotal roles in the progression of tendinopathy [24]. Prolonged activation of the UPR under metabolic or mechanical stress shifts signaling to proapoptotic pathways, such as CHOP induction and eIF2α

phosphorylation, leading to the loss of viable tenocytes [1]. This depletion of functional cells limits their ability to synthesize and repair the extracellular matrix. Simultaneously, ER stress disrupts cytoskeletal organization and focal adhesion dynamics, impeding the directionality of tenocyte migration toward microdamage sites. Consequently, tendon healing is delayed, and ongoing matrix degradation persists unchecked. The combination of excessive apoptosis and impaired cell migration creates a degenerative tendon microenvironment, accelerating the onset and chronic progression of tendinopathy. Therefore, appropriately regulating ER stress may be an important therapeutic strategy for managing obesity-related tendinopathy. In the presence of palmitate, EG administration significantly attenuated the expression of ER stress markers in tenocytes. This inhibition of ER stress may contribute to the protective effects of EG against FFA-induced tenocyte dysfunction.

PPARδ is a nuclear receptor transcription factor that plays a critical role in regulating fatty acid oxidation, glucose metabolism, anti-inflammatory responses, and cellular survival [25]. PPARδ not only modulates energy metabolism but also contributes to

the maintenance of cellular homeostasis, particularly under metabolic stress conditions such as hyperlipidemia [26]. The activation of PPAR δ can counteract these processes by increasing fatty acid oxidation [27], reducing reactive oxygen species (ROS) production [28], and promoting autophagy [29]. Previous studies have shown that PPAR δ activation suppresses CHOP expression, increases LC3-II conversion, and promotes p62 degradation, collectively inhibiting ER stress-mediated apoptosis in various cell types. For example, Ji et al. reported that GW0742, a PPAR δ agonist, prevents apoptosis in astrocytes by suppressing ER stress via UCP2-mediated mitophagy [30]. PPAR δ activation by GW501516 mitigates ER stress through autophagy-dependent signaling, thereby attenuating apoptosis in palmitate-treated human cardiac cells. Conversely, elevated ER stress levels are detected in the hearts of PPAR δ knockout mice [31]. Thus, we selected PPAR δ as a key molecule for defense against ER stress. We found that treatment with EG increased PPAR δ expression in tenocytes. Suppression of PPAR δ by siRNA reduced the effects of EG on apoptosis and cell migration as well as ER stress in tenocytes treated with palmitate. These findings suggest that EG mitigates tenocyte dysfunction under hyperlipidemic conditions in association with PPAR δ -associated signaling of ER stress. However, it should be noted that the present findings are based on associative evidence, and causality or pathway necessity was not directly established. Further studies employing genetic or pharmacological pathway-specific interventions will be required to delineate the causal roles of PPAR δ -associated signaling and autophagy in mediating the effects of EG.

Autophagy is a conserved intracellular degradation process that plays a crucial role in maintaining cellular homeostasis by removing damaged proteins and organelles, thereby alleviating ER stress and preventing excessive activation of the UPR [32]. Therefore, the activation of autophagy may be a potential regulatory mechanism for treating ER stress-related diseases. For example, Sun et al. demonstrated that IL-38 mitigates ER stress through an autophagy-mediated pathway, thereby ameliorating hepatic steatosis [33]. Rapamycin, an autophagy activator, prevents adipocyte apoptosis by suppressing ER stress [34]. Conversely, Munch et al. reported that autophagy deficiency results in the accumulation of ubiquitinated proteins, ER stress, and apoptosis in *Arabidopsis* [35]. Furthermore, autophagy is positively regulated by PPAR δ phosphorylation [29]. In this study, we demonstrated that EG treatment increased the expression of autophagy markers in tenocytes, whereas 3-MA abrogated the effects of EG on palmitate-mediated tenocyte injury. Additionally, PPAR δ siRNA reduced the EG-mediated induction of autophagy. These findings suggest that autophagy, which acts downstream of PPAR δ , contributes to the cytoprotective effects of EG on hyperlipidemic tenocytes. Autophagic flux was not directly assessed.

Myokines are bioactive peptides secreted by skeletal muscle that mediate communication between muscles and various organs. Recently, they have been suggested to influence the biological functions of tendons. Among them, IL-15 and METRNL exhibit anti-inflammatory, antioxidant, and ER stress-inhibitory effects in various tissues [16,36,37]. Specifically, IL-15 enhances mitochondrial function and inhibits apoptosis under metabolic stress [38],

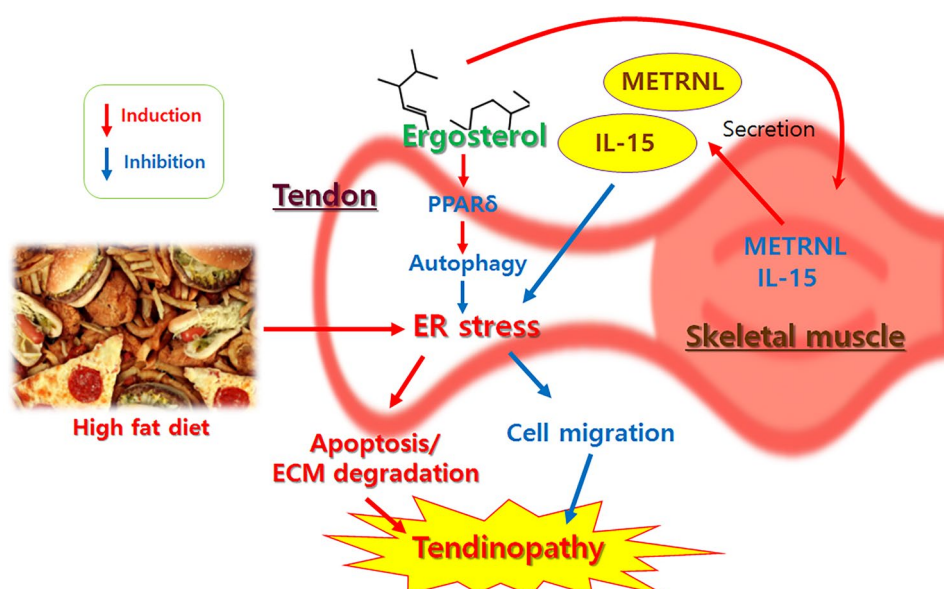


Figure 5. Schematic diagram of EG effects on tenocyte dysfunction under hyperlipidemic conditions.

whereas METRNL promotes tissue repair and attenuates inflammatory signaling [39]. In this study, the serum concentrations of IL-15 and METRNL were decreased in obese patients, suggesting that myokine supplementation may be beneficial for obesity-associated tendinopathy. Furthermore, we demonstrated that EG treatment increased IL-15 and METRNL secretion in C2C12 myocytes. CM from EG-treated myocytes attenuated apoptosis, enhanced cell migration, normalized extracellular matrix signaling, and reduced ER stress in palmitate-treated tenocytes. siRNA for IL-15 or METRNL reduced the effects of EG-stimulated CM on tenocytes in the presence of palmitate, except for tenocyte migration. These results suggest that systemic effects of EG can at least partially influence muscle-tendon communication under obesity-associated metabolic stress through skeletal muscle-derived IL-15 and METRNL. In addition to IL-15 and METRNL, additional EG-regulated myokines may play a role in promoting tenocyte migration.

In the present study, we demonstrated that EG mitigates ER stress via the PPAR δ /autophagy axis, thereby alleviating apoptosis, impaired cell migration, and collagen degradation in hyperlipidemic tenocytes. Moreover, we demonstrated that cross-signaling between myocytes and tenocytes, mediated by IL-15 and METRNL, contributes to the protective effects of EG (Figure 5). These findings suggest that EG may be a potential candidate compound modulating obesity-associated metabolic stress responses in tendon cells through both local and systemic mechanisms.

5. Limitations

This study has several limitations that should be acknowledged. First, the palmitate-treated tenocyte system used in this study does not aim to fully recapitulate chronic tendinopathy. Rather, it serves as an *in vitro* model of lipotoxic and metabolic stress conditions that are relevant to obesity-associated cellular dysfunction in tenocytes. Second, this study was conducted *in vitro* using palmitate-treated tenocytes. While this allowed for the analysis of cellular mechanisms under controlled conditions, it may not fully reflect the multifactorial *in vivo* environment of obesity-related tendinopathy. Third, while this study focused on two representative myokines, IL-15 and METRNL, other muscle-derived factors may also contribute to muscle-tendon signaling. Fourth, the pharmacokinetics, bioavailability, and long-term safety of EG after systemic administration remain to be elucidated. Nevertheless, by integrating the regulation of ER stress through the PPAR δ /autophagy

axis and myokine-mediated cross-communication, these findings provide novel mechanistic insights and strong rationales for future *in vivo* and clinical studies to develop EG as a therapeutic option for obesity-related tendinopathy.

Author contributions

MKP, JHK, and JHJ: conceptualization, investigation, and methodology. TWJ and KTL: conceptualization, data curation, formal analysis, funding acquisition, investigation, and role/writing – original draft. AMA: formal analysis; validation; writing – original draft, writing, review, and editing. All the authors approved the final version of the manuscript. All the authors are responsible for the overall integrity of the work.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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