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Effects of quercetin nanoemulsion on SIRT1 activation and mitochondrial biogenesis in the skeletal muscle of high-fat diet-fed mice

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ABSTRACT

BACKGROUND/OBJECTIVES: Quercetin (QT) is a plant flavonoid that offers health benefits owing to its various bioactive properties; however, as a hydrophobic substance, it has considerably low bioavailability. We previously demonstrated that QT nanoemulsion (QT+NE) formulated via oil-in-water nanoemulsification exhibited more effective cholesterol-lowering activity than ordinary QT in high cholesterol-fed rats. In this study, we investigated the effects of QT+NE on the regulation of skeletal muscle mitochondrial function in high-fat diet (HD)-fed mice.
MATERIALS/METHODS: C57BL/6J mice were fed a normal chow diet (ND), HD (45% of calories from fat), or HD with 0.05% QT+NE or QT for 11 weeks. We analyzed sirtuin 1 (SIRT1) activation, mitochondrial changes, and the expression of genes involved in mitochondrial biogenesis in skeletal muscle.
RESULTS: Body weight and body weight gain decreased in the QT+NE group compared with that in the HD group ($P < 0.05$), but not in the QT group. Epididymal adipose tissue weight decreased in both the QT and QT+NE groups ($P < 0.05$). Plasma lipid levels also improved in both the QT and QT+NE groups ($P < 0.05$). QT+NE intake upregulated the messenger RNA levels of SIRT1, peroxisome proliferator-activated receptor-γ coactivator 1-α, nuclear respiratory factor 1, and mitochondrial transcription factor A in skeletal muscle compared with HD intake alone ($P < 0.05$), whereas QT did not. In particular, SIRT1 activity was significantly increased in the QT+NE group compared with that in the QT group ($P < 0.05$). HD intake reduced mitochondrial DNA content compared with ND intake; nevertheless, QT+NE intake retained it ($P < 0.05$).
CONCLUSION: Collectively, our findings suggest that QT+NE may be beneficial in enhancing mitochondrial biogenesis in skeletal muscle of HD-fed mice, which may be associated with SIRT1 activation.

Keywords: Quercetin; sirtuin 1; mitochondrial biogenesis; skeletal muscle; obesity

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Conflict of Interest
The authors declare no potential conflicts of interests.

Author Contributions
Conceptualization: Kim Y; Formal analysis: Lee MS; Funding acquisition: Doo M; Investigation: Kim Y; Methodology: Lee MS; Supervision: Kim Y; Writing - original draft: Lee MS; Writing - review & editing: Lee MS, Doo M, Kim Y.

INTRODUCTION

Obesity refers to the excessive accumulation of fat in the body associated with an imbalance between energy intake and expenditure owing to excessive calorie intake, endocrine disorders, and lack of exercise. Obesity contributes to risk factors for metabolic diseases, such as cancer, cardiovascular disease, and diabetes, and causes mitochondrial changes that elicit mitochondrial dysfunction [1,2]. In particular, increased body fat owing to a high-calorie diet and lack of exercise causes sarcopenic obesity, which diminishes muscle function, and is accompanied by the dysfunction of mitochondria, which play an important role in maintaining muscle function [2,3]. In fact, mitochondrial lipid catabolic processes in the skeletal muscle of individuals with obesity are reduced compared with those in individuals without obesity, indicating that mitochondrial dysfunction leads to reduced mitochondrial content and impaired oxidative capacity [4].

Skeletal muscle, which accounts for 40–50% of the body weight in humans, serves an essential role in maintaining bioenergy homeostasis and is a tissue with high mitochondrial density [3]. Mitochondria are intracellular organelles that contribute to energy production and possess a unique genome of mitochondrial DNA (mtDNA) [5]. Maintaining mitochondrial function requires the removal of damaged mitochondria and biogenesis of new mitochondria. In skeletal muscle, sirtuin 1 (SIRT1) activates peroxisome proliferator-activated receptor-γ coactivator 1-α (PGC-1α), a key regulator of mitochondrial biogenesis [6]. The activated PGC-1α interacts with the transcription factor nuclear respiratory factor 1 (NRF1) to promote mitochondrial transcription factor A (TFAM) expression, leading to increased mtDNA transcription and replication [6].

Quercetin (QT) is a major polyphenolic flavonoid found in nature, including various vegetables and fruits, and it possesses certain health benefits, such as antioxidant, anti-inflammatory, anti-cancer, anti-diabetic, and anti-obesity properties [7,8]. For QT to exhibit health benefits, it needs to be absorbed into the body at concentrations that allow it to participate in physiological activity [9]. However, because QT is a hydrophobic molecule with low solubility, it is not metabolized or absorbed in the human intestine, resulting in relatively low bioavailability [10]. The bioavailability of lipophilic polyphenolic compounds, such as QT, can be enhanced by improving solubility and absorbability using oil-in-water (O/W) nanoemulsion technology [11,12]. QT nanoemulsion (QT+NE) formulated via O/W nanoemulsification exhibits enhanced bioavailability compared with pure QT and exerts anti-diabetic, anti-obesity, anti-hepatotoxic, and anti-neurotoxic efficacy [13-15]. We previously reported that QT+NE displayed more effective hypocholesterolemic activity than QT in high-cholesterol-fed rats [16]. In addition, QT+NE has been shown to improve the bioavailability of QT and is effective against obesity [14]. However, it remains to be determined whether the anti-obesity effect of QT+NE affects skeletal muscle function. In this study, we investigated the underlying mechanisms, focusing on whether QT+NE regulates SIRT1 activation and mitochondrial biogenesis in the skeletal muscle of high-fat diet (HD)-fed mice.

MATERIALS AND METHODS

Reagents

QT and the bicinchoninic acid (BCA) Protein Assay Kit were purchased from Thermo Fisher Scientific (Pittsburgh, PA, USA). Tween 80 and sodium alginate were procured from Sigma-

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Aldrich (St. Louis, MO, USA). Caprylic/capric triglyceride (Captex® 355) was acquired from Abitec (Janesville, WI, USA). Soybean lecithin was obtained from IFC Solutions (Linden, NJ, USA). Tiletamine–zolazepam (Zoletil™ 50) was secured from Virbac Laboratories (Carròs, France). Xylazine (Rompun®) was purchased from Bayer (Leverkusen, Germany). Aspartate aminotransferase (AST), alanine aminotransferase (ALT), triglyceride (TG), total cholesterol (TC), and high-density lipoprotein–cholesterol (HDL-C) assay kits were acquired from the Asan Pharmaceutical (Seoul, Korea). The hematoxylin and eosin (H&E) Stain Kit was procured from ScyTek Laboratories (Logan, UT, USA). SIRT1 Activity Assay and Nuclear Extraction kits were obtained from Abcam (Cambridge, UK). RiboEx™ reagent was secured from GeneAll Biotechnology (Seoul, Korea). Moloney murine leukemia virus (M-MLV) Reverse Transcriptase and Universal SYBR Green PCR Master Mix were purchased from Bioneer (Daejeon, Korea). The Genomic DNA Purification Kit was procured from Gentra Systems (Minneapolis, MN, USA).

QT+NE preparation

QT+NE was kindly supplied by the Korea Food Research Institute (Wanju, Korea) and used as an oil-in-water nanoemulsion containing QT formed via complexation and self-assembly with Tween 80, Captex 355, sodium alginate, and soybean lecithin, as described in our previous study [16].

Animals

This study was approved by the Institutional Animal Care and Use Committee (IACUC) of Ewha Womans University (approval number: IACUC No. 19-013; approval date: March 27, 2019). Three-to-four week-old male C57BL/6J mice were purchased from DooYeol Biotech (Seoul, Korea). They were individually housed in separate cages and kept under a 12-h light–dark cycle, constant temperature of 22 ± 2°C, and humidity of 55 ± 5%. A total of 24 mice were subjected to the dietary intervention, with 6 mice in each group.

Experimental diet

After a week of acclimation, the mice were randomly assigned to 4 groups and fed an experimental diet for 11 continuous weeks as follows: (1) normal chow diet (ND; 2018S Teklad Global 18% Protein, 6% Fat, Rodent Diet; Harlan Teklad, Madison, WI, USA), (2) 45% HD, (3) HD supplemented with 0.05% QT+NE, and (4) HD supplemented with 0.05% QT. Mice were fed HD to induce obesity. ND was included as a control group to confirm HD-induced obesity. The diet compositions are shown in **Table 1**, and HD, QT+NE, and QT group mice received their respective diets *ad libitum*. Individual body weight and food intake were weighed once a week during the experiment. At the end of the experiment, the mice were anesthetized with an intraperitoneal injection of a 5:2 (v/v) mixture of Zoletil 50 and Rompun after a 16-h overnight fast. Whole blood samples were collected via cardiac puncture and left at room temperature for 2 h. Subsequently, the blood samples were centrifuged at 4°C and 3,500 rpm for 15 min to obtain serum. The collected serum, epididymal adipose tissue (EAT), and skeletal muscle tissue were stored at −70°C until further analysis.

Serum metabolite assay

Serum AST and ALT levels were measured using commercial kits. After measuring absorbance at 505 nm via the enzymatic colorimetric reaction, each value was calculated by substituting it into the standard curve. Serum TG, TC, and HDL-C concentrations were measured using commercial kits according to the manufacturers' instructions. Low-density lipoprotein-cholesterol (LDL-C) content was analyzed using the Friedewald WT formula [17].

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Table 1. Composition of experimental diets (g/kg)

Components	HD	QT+NE	QT
Casein	170.73	170.73	170.73
Sucrose	121.95	121.95	121.95
Dextrose	154.00	154.00	154.00
Corn starch	201.71	201.21	201.21
Cellulose	60.98	60.98	60.98
Lard	230.50	230.50	230.50
Mineral mix (AIN-93G)	42.68	42.68	42.68
Vitamin mix (AIN-93)	12.20	12.20	12.20
L-cystein	2.20	2.20	2.20
Choline bitartrate	3.05	3.05	3.05
QT+NE	-	0.50	-
QT	-	-	0.50
Total	1,000	1,000	1,000
Energy density (kcal)	4,605	4,605	4,605
Carbohydrate (% as kcal)	42	42	42
Protein (% as kcal)	13	13	13
Fat (% as kcal)	45	45	45

HD, high-fat diet; QT, quercetin; QT+NE, quercetin nanoemulsion.

H&E staining

H&E staining of EAT was performed as described in a previous study [18]. EAT was fixed in 10% neutral buffered formalin at room temperature overnight. After being embedded with paraffin, the EAT was cut into 6-μm thick sections and subsequently stained with H&E according to the manufacturer's instructions. Digital images of tissue sections were observed using an Olympus IX51 inverted microscope (magnification, 400×; Olympus, Tokyo, Japan). Adipocyte size was determined using ImageJ software (National Institutes of Health, Bethesda, MD, USA).

Transmission electron microscopy (TEM)

TEM images of skeletal muscle mitochondria were observed as previously described [18]. Skeletal muscle tissue was sliced into ≤ 2-mm sections and post-fixed with 2% glutaraldehyde and 1% osmium tetroxide. The fixed tissue samples were embedded in epoxy resin (Epon 812) and sectioned to 1-μm thickness using an ultramicrotome (Reicher-Jung, Deerfield, IL, USA). Tissue sections were stained with 1% toluidine blue and observed under an accelerating voltage of 80 kV using an H-7650 transmission electron microscope (magnification 20,000×; Hitachi, Tokyo, Japan).

SIRT1 activity

SIRT1 activity in skeletal muscle tissue was assayed using SIRT1 activity kits according to the manufacturer's instructions, as previously described [18]. The nuclear fraction was extracted from skeletal muscle tissue using a nuclear extraction kit according to the manufacturer's instructions. Fluorescence intensity was measured at 340 nm/460 nm (excitation/emission) using a microplate reader (Thermo Fisher Scientific) and normalized to protein content. Protein content was measured using the BCA Protein Assay Kit according to the manufacturer's instructions. SIRT1 activity was expressed as fold change compared to that of the HD group.

Reverse-transcription quantitative polymerase chain reaction (RT-qPCR)

Total RNA from skeletal muscle tissue was isolated using RiboEx Reagent, and the RNA concentration was quantified using a NanoDrop™ spectrophotometer (Thermo Fisher Scientific). To determine the messenger RNA (mRNA) level, complementary DNA was

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synthesized using M-MLV reverse transcriptase. RT-qPCR amplification was performed on a Rotor-Gene® Q Thermocycler (QIAGEN, Hilden, Germany) using 2X SYBR™ Green PCR Master Mix. The sequences of each primer are shown in **Table 2**. Relative quantification of amplification products was normalized to β-actin and analyzed using the delta–delta Ct method [19].

mtDNA content

Total DNA in skeletal muscle tissue was extracted using a Puregene® DNA isolation kit according to the manufacturer's instructions. DNA was extracted from 50 mg of skeletal muscle tissue using lysis buffer containing proteinase K. Relative mtDNA content was quantified by measuring mitochondrial (*Cox1*, subunit 1 of cytochrome oxidase) and nuclear (*Gapdh*, glyceraldehyde 3-phosphate dehydrogenase) genes using RT-qPCR [18]. The nucleotide sequences of each primer are listed in **Table 2**.

Statistical analysis

Statistical analyses were performed using SPSS (version 25; IBM Corp., Armonk, NY, USA), and values are expressed as the mean ± standard error of the mean (SEM). Significant differences among the 3 groups (HD, QT+NE, and QT groups) were analyzed using one-way analysis of variance followed by Tukey's multiple comparison test, and significant differences with $P < 0.05$ are expressed with different letters (a–c). Significant differences between the ND and HD groups were determined using the 2-tailed Student's *t*-test, and statistical significance was set at $P < 0.05$ and $P < 0.01$.

RESULTS

Body weight, food intake, and EAT weight

Changes in body weight and food intake after 11 weeks of supplementation are shown in **Table 3**. Food intake did not significantly differ among all groups (ND, HD, QT+NE, and QT). Energy intake was significantly higher in the HD group than in the ND group ($P < 0.05$), but there was no significant difference among the experimental diet groups (HD, QT+NE, and QT). The final body weight of mice fed HD showed a significant increase of 33.4% compared with that in the ND group ($P < 0.05$). In contrast, the final body weights of the QT+NE and

Table 2. Primer sequences used for RT-qPCR

Gene	GeneBank No.	Primer sequence (5′–3′)
β-actin	NM_007393	Forward: GGA CCT GAC AGA CTA CCT CA
		Reverse: GTT GCC AAT AGT GAT GAC CT
Cox1	NC_005089	Forward: TAG CCG GAA ATC TAG CCC AT
		Reverse: GTT ATG GCT GGG GGT TTC AT
Nrf1	NM_010938	Forward: AAG TAT TCC ACA GGT CGG GG
		Reverse: TGG TGG CCT GAG TTT GTG TT
Gapdh	NC_000072	Forward: GGA GCC AAA AGG GTC ATC AT
		Reverse: TAC CAT GAG CCC TTC CAC AA
Pgc-1α	NM_008904	Forward: GGG CCA AAC AGA GAG AGA GG
		Reverse: GTT TCG TTC GAC CTG CGT AA
Sirt1	NM_019812	Forward: TAC CCC ATG AAG TGC CTC AA
		Reverse: AAC CAA TTC CTT TTG TGG GC
Tfam	NM_009360	Forward: GAG GCC AGT GTG AAC CAG TG
		Reverse: GTA GTG CCT GCT GCT CCT GA

RT-qPCR, reverse-transcription quantitative polymerase chain reaction; *Cox1*, subunit 1 of cytochrome oxidase; *Nrf1*, nuclear respiratory factor 1; *Gapdh*, glyceraldehyde 3-phosphate dehydrogenase; *Pgc-1α*, peroxisome proliferator-activated receptor-γ coactivator 1-α; *Sirt1*, sirtuin 1; *Tfam*, mitochondrial transcription factor A.

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QT groups decreased by 14.9% ($P < 0.05$) and 7.9%, respectively, compared with that in the HD group. Body weight gain increased 2.0-fold in the HD group compared with that in the ND group but decreased by 25.9% ($P < 0.05$) and 15.0% in the QT+NE and QT groups, respectively, compared with that in the HD group (**Fig. 1A**). EAT weight decreased by 51.0% and 28.3% in the QT+NE and QT groups, respectively, compared with that in the HD group ($P < 0.05$; **Fig. 1B**). Adipocyte size in EAT was observed using H&E staining (**Fig. 1C**) and was found to decrease in the QT+NE and QT groups ($P < 0.05$; **Fig. 1D**).

Serum lipids, AST, and ALT levels

Serum lipid, AST, and ALT levels are shown in **Table 4**. Serum TG, TC, and LDL-C levels were higher in the HD group than in the ND group ($P < 0.05$) and lower in the QT+NE and QT groups than in the HD group ($P < 0.05$). Serum HDL-C was significantly reduced in the HD

Table 3. Effect of QT+NE and QT on body weight and food intake

Variables	ND	HD	QT+NE	QT
Initial body weight (g)	17.40 ± 0.35	17.38 ± 0.47	17.36 ± 0.38	17.36 ± 0.54
Final body weight (g)	26.15 ± 0.34**	34.89 ± 0.78[a]	29.70 ± 0.52[b]	32.15 ± 1.59[ab]
Food intake (g/day)	3.91 ± 0.01	3.79 ± 0.09	3.95 ± 0.08	3.86 ± 0.15
Energy intake (kcal/day)	12.10 ± 0.05*	17.59 ± 0.43	18.35 ± 0.37	17.92 ± 0.69

Values are expressed as mean ± standard error of the mean (n = 6).
ND, normal chow diet; HD, high-fat diet; QT, quercetin; QT+NE, quercetin nanoemulsion.
[a,b]Different letters in a row indicate significant differences among the 3 groups (HD, QT+NE, and QT groups) at $P < 0.05$. Significant differences between the ND and HD groups are indicated: *$P < 0.05$, **$P < 0.01$.

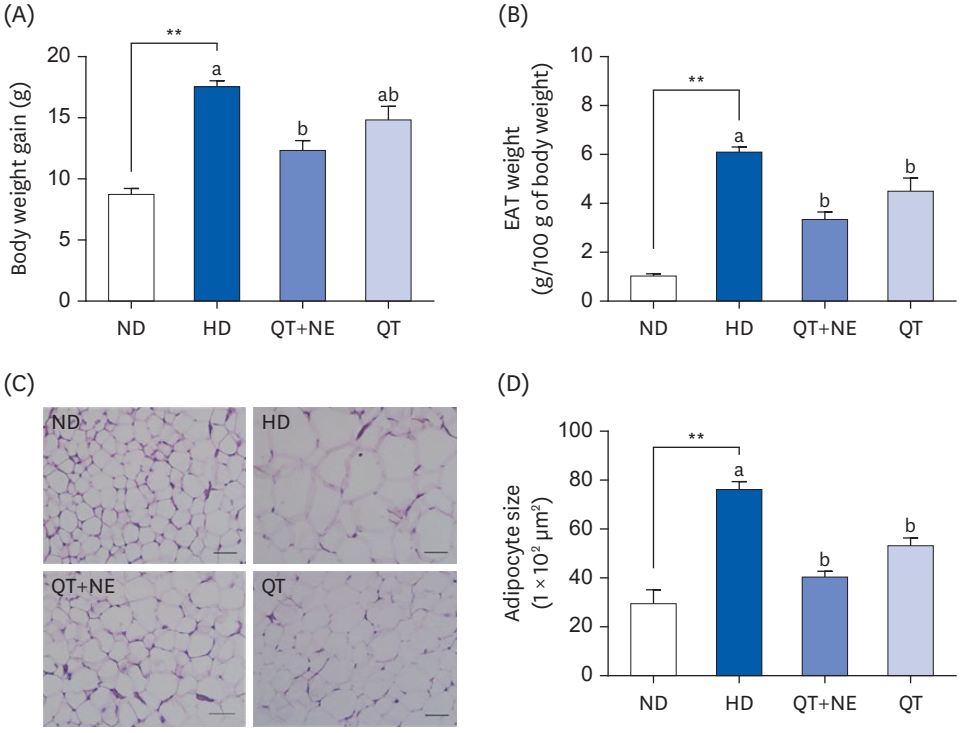

Fig. 1. Effects of QT+NE and QT on body weight and epididymal fat accumulation. (A) Body weight gain. (B) EAT weight. (C) EAT H&E staining (scale bar = 50 μm; magnification of 400×). (D) EAT adipocyte size (area per adipocyte, μm^2). Values are expressed as the mean ± standard error of the mean (n = 6).
ND, normal chow diet; HD, high-fat diet; QT, quercetin; QT+NE, quercetin nanoemulsion; EAT, epididymal adipose tissue; H&E, hematoxylin and eosin.
[a,b]Different letters indicate significant differences among the 3 groups (HD, QT+NE, and QT groups) at $P < 0.05$. Significant differences between the ND and HD groups are indicated: **$P < 0.01$.

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Table 4. Effects of QT+NE and QT on serum lipids, AST and ALT levels

Metabolites	ND	HD	QT+NE	QT
TG (mmol/L)	1.12 ± 0.05*	1.31 ± 0.03[a]	1.16 ± 0.04[b]	1.15 ± 0.04[b]
TC (mmol/L)	4.76 ± 0.05*	5.94 ± 0.33[a]	4.73 ± 0.18[b]	5.04 ± 0.13[b]
HDL-C (mmol/L)	3.15 ± 0.09*	2.49 ± 0.04	2.69 ± 0.09	2.67 ± 0.05
LDL-C (mmol/L)	1.10 ± 0.09**	2.85 ± 0.35[a]	1.59 ± 0.20[b]	1.86 ± 0.16[b]
AST (IU/L)	53.23 ± 3.81	51.93 ± 3.87	51.92 ± 2.43	52.55 ± 7.57
ALT (IU/L)	17.13 ± 0.62	16.67 ± 0.60	16.52 ± 0.85	16.75 ± 0.87

Values are expressed as mean ± standard error of the mean (n = 6).
ND, normal chow diet; HD, high-fat diet; QT, quercetin; QT+NE, quercetin nanoemulsion; TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; AST, aspartate aminotransferase; ALT, alanine aminotransaminase.
[a,b]Different letters in a row indicate significant differences among the 3 groups (HD, QT+NE, and QT groups) at $P < 0.05$. Significant differences between the ND and HD groups are indicated: $^{*}P < 0.05$, $^{**}P < 0.01$.

group compared with that in the ND group ($P < 0.01$); however, no significant difference was noted among experimental diet groups (HD, QT+NE, and QT). AST and ALT are enzymes synthesized in the liver, and when liver cell damage occurs, excessive quantities of these enzymes are released from the liver into the blood [20]. Serum AST and ALT levels, known indicators of liver damage, exhibited no significant differences among all groups (ND, HD, QT+NE, and QT), suggesting that the experimental diet did not cause hepatotoxicity.

Effects of QT+NE on SIRT1 activation in skeletal muscle

We investigated whether SIRT1, which acts as a central regulator of skeletal muscle mitochondrial biogenesis [21], is regulated by QT+NE and QT. SIRT1 in skeletal muscle was evidently suppressed by 11-week HD intake. In the QT+NE group, *Sirt1* mRNA expression levels increased 1.64-fold compared with those in the HD group ($P < 0.05$; **Fig. 2A**), and SIRT1 activity also increased 1.46-fold ($P < 0.05$; **Fig. 2B**). Moreover, SIRT1 activity in the QT+NE group was significantly higher than that in the QT group ($P < 0.05$). In contrast, SIRT1 mRNA and activity levels in the QT group tended to increase compared with those in the HD group; nevertheless, no significant difference was observed.

Effects of QT+NE on the mRNA expression levels of genes related to mitochondrial biogenesis in skeletal muscle

To elucidate the regulatory mechanism underlying SIRT1's interaction with mitochondrial biogenesis, we analyzed the effect of QT+NE and QT on the mRNA expression levels of *Pgc-1α*,

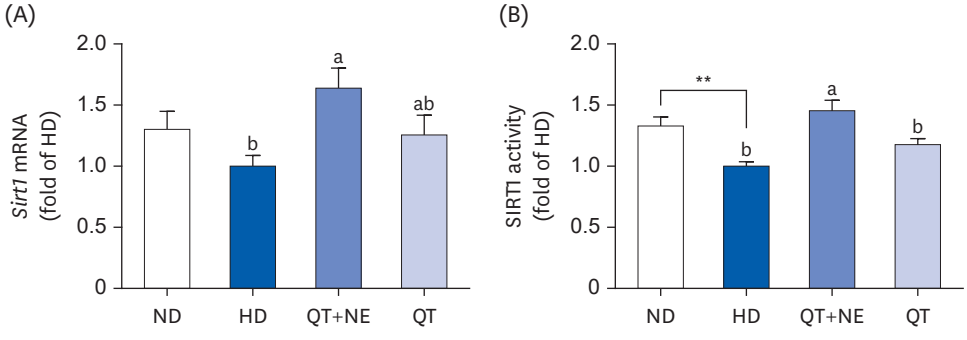

Fig. 2. Effects of QT+NE and QT on SIRT1 regulation in skeletal muscle. SIRT1 mRNA expression (A) and activity (B). Values are expressed as the mean ± standard error of the mean (n = 6).
ND, normal chow diet; HD, high-fat diet; QT, quercetin; QT+NE, quercetin nanoemulsion; SIRT1, sirtuin 1; mRNA, messenger RNA.
[a,b]Different letters indicate significant differences among the 3 groups (HD, QT+NE, and QT groups) at $P < 0.05$. Significant differences between the ND and HD groups are indicated: $^{**}P < 0.01$.

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Nrf1, and *Tfam*. These levels increased 1.60-, 2.00-, and 1.61-fold, respectively, in the QT+NE group compared with those in the HD group ($P < 0.05$; **Fig. 3A**) but tended to increase in the QT group.

Effects of QT+NE on skeletal muscle mitochondrial changes

The copy number of the mitochondrial genome, mtDNA, is regulated by TFAM and reflects the abundance of mitochondria in a cell [5]. To confirm mitochondrial changes in skeletal muscle, we quantified mtDNA levels and observed mitochondrial morphology using TEM. The mtDNA content decreased by 48.3% in the HD group compared with that in the ND group but significantly increased 1.85-fold in the QT+NE group compared with that in the HD group ($P < 0.05$; **Fig. 3B**). Mitochondrial images are shown in **Fig. 3C**. The HD group displayed a decrease in the number of mitochondria compared with the ND group, whereas the QT+NE and QT groups exhibited an increase in the same (**Fig. 3C**).

DISCUSSION

Our findings support the hypothesis that QT+NE plays a role in preventing obesity and regulating mitochondrial biogenesis in skeletal muscle. Dietary QT exerts anti-obesity effects, reducing body weight and inhibiting body fat accumulation in HD-fed mice [22-24]. Interestingly, QT reportedly prevents obesity-induced skeletal muscle atrophy and increases

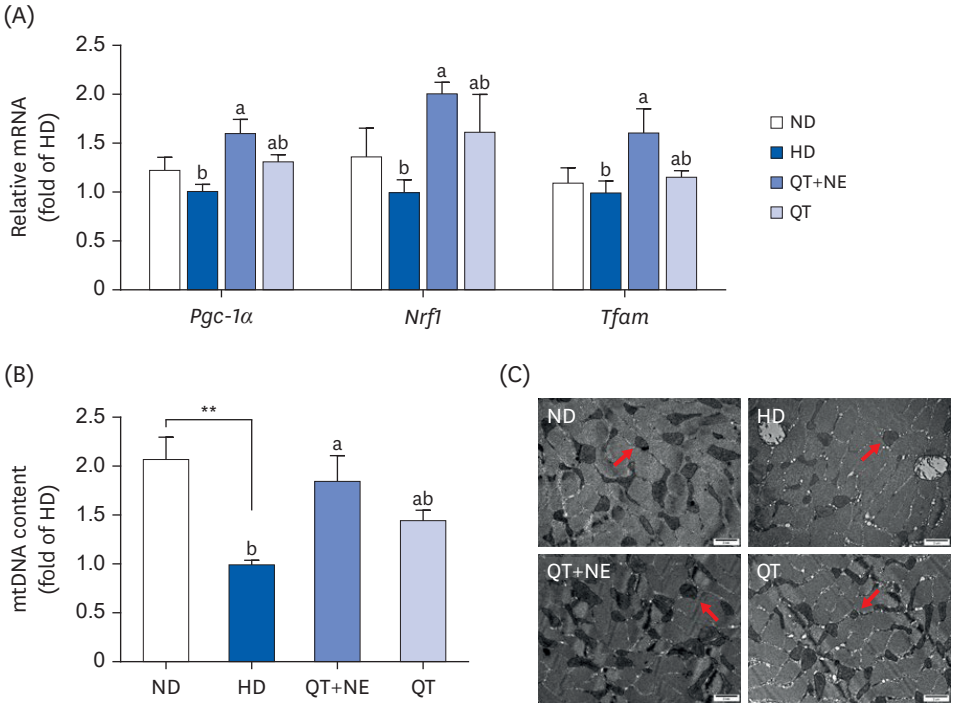

Fig. 3. Effects of QT+NE and QT on mitochondrial biogenesis in skeletal muscle. (A) mRNA expression levels of *Pgc-1α*, *Nrf1*, and *Tfam*. (B) mtDNA content. (C) TEM imaging (scale bar = 2 μm; magnification of 20,000×). Red arrows indicate mitochondria. Values are expressed as the mean ± standard error of the mean (n = 6).
ND, normal chow diet; HD, high-fat diet; QT, quercetin; QT+NE, quercetin nanoemulsion; mRNA, messenger RNA; *Pgc-1α*, peroxisome proliferator-activated receptor-γ coactivator 1-α; *Nrf1*, nuclear respiratory factor 1; *Tfam*, mitochondrial transcription factor A; TEM, transmission electron microscope; mtDNA, mitochondrial DNA.
[a,b]Different letters indicate significant differences among the 3 groups (HD, QT+NE, and QT groups) at $P < 0.05$. Significant differences between the ND and HD groups are indicated: $^{**}P < 0.01$.

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mitochondrial biogenesis in the brain and muscles [25,26]. Furthermore, it was recently found to prevent muscle loss induced by cancer and chemotherapy by improving muscle mass and mitochondrial content [27]. This suggests that QT influences body weight reduction and the regulation of muscle mitochondrial function despite its low bioavailability. Pangeni *et al.* [14] reported that orally administered QT+NE (150 mg/kg, leading to a dietary QT dose of approximately 0.09%) not only increased in bioavailability 33.5-fold but also significantly reduced body weight gain and fat mass compared with QT. QT needs to be administered at doses of 0.025–0.1% (in the diet) over a period of time (8–12 weeks) to significantly reduce body weight in animal models [22-24]. Therefore, this study used QT or QT+NE at a dose of 0.05% for 11 weeks. In this study, the EAT weight and adipocyte size in HD-fed mice were significantly reduced in both QT+NE and QT groups. Body weight gain was significantly suppressed in the QT+NE group compared to the HD group, but tended to be lower in the QT group.

SIRTs are nicotinamide adenine dinucleotide-dependent histone deacetylases that regulate numerous biological processes and comprise 7 types (SIRT1 to SIRT7) [28]. SIRT1, which is mainly found in the cell nucleus, has been shown to be a longevity gene that extends lifespan via calorie restriction [29]. In mammalian metabolism, SIRT1 is a nutrient-sensing deacetylase that regulates fatty acid, glucose, and energy metabolism and serves a critical role in pathological processes, such as aging, cancer, type 2 diabetes, obesity, inflammation, and mitochondrial dysfunction [28,30,31]. In particular, as a regulator of energy metabolism, SIRT1 regulates PGC-1α-mediated mitochondrial biogenesis [6,21]. Several studies have reported that QT promotes SIRT1 activity and mitochondrial biogenesis [32-34]. QT has been found to increase SIRT1 protein expression in L6 myotubes and rat skeletal muscle and improve mitochondrial dysfunction and biogenesis via the AMPK/SIRT1 signaling pathway in osteoarthritis rat chondrocytes [32,33]. QT was recently shown to protect neurons by activating SIRT1 and mitochondrial biogenesis in neurons and mitigating oxidative stress-induced damage [34]. In this study, the mRNA and activity of SIRT1 were increased by QT+NE, and its activity was significantly higher in QT+NE than in QT. This suggests that QT+NE may be more effective than QT in activating SIRT1 in skeletal muscle of HD-fed rats.

PGC-1α, which is instrumental in mitochondrial biogenesis, promotes its role via SIRT1 deacetylation [6]. It induces NRF1/2 expression, and in turn, NRF1 activates TFAM, which is directly involved in mtDNA replication [6]. mtDNA is required for the synthesis of new mitochondria and regulation of mitochondrial function [5]. Genetic or diet-induced obesity reduces skeletal muscle *Pgc*-1α/β expression, and caloric restriction improves skeletal muscle mitochondrial function by increasing *Sirt1*, *Pgc-1α*, and *Tfam* expression as well as mtDNA content [35,36]. Henagan *et al.* [37] reported that dietary QT supplementation for 8 weeks in HD-fed mice upregulated skeletal muscle *Pgc-1α* expression and improved skeletal muscle mitochondrial function. A recent study demonstrated that SH-SY5Y cell exposure to QT not only stimulated the expression of mitochondria-related proteins (SIRT1, PGC-1α, and TFAM) but also protected neurons by alleviating oxidative stress-induced damage [38]. In this study, QT+NE supplementation increased mtDNA content and gene expression of *Pgc-1α*, *Nrf1*, and *Tfam* in skeletal muscle, whereas QT did not. This suggests that QT+NE helps restore muscle mitochondrial biogenesis.

In summary, supplementation of QT+NE significantly reduced final body weight compared with the HD group, whereas QT showed no significant difference. Both QT+NE and QT improved plasma lipid levels and decreased EAT weight and adipocyte size. In skeletal muscle, QT+NE increased SIRT1 activity, mtDNA content, and expression of mitochondrial

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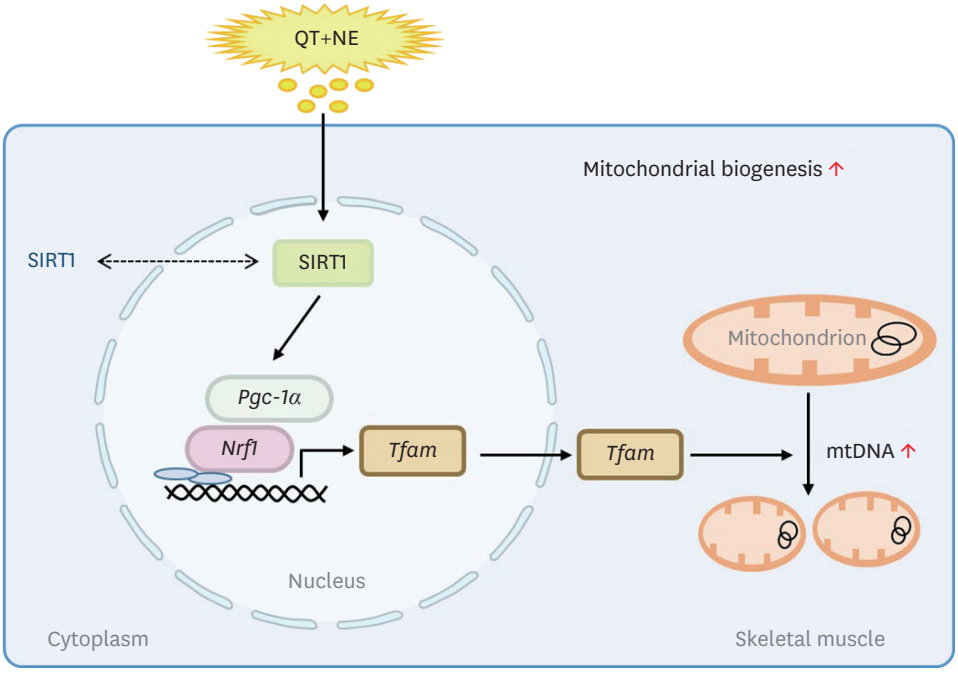

Fig. 4. Mechanistic schematic showing the potential effects of QT+NE on SIRT1 activation and mitochondrial biogenesis in skeletal muscle.
QT+NE, quercetin nanoemulsion; SIRT1, sirtuin 1; *Pgc-1α*, peroxisome proliferator-activated receptor-γ coactivator 1-α; *Nrf1*, nuclear respiratory factor 1; *Tfam*, mitochondrial transcription factor A; mtDNA, mitochondrial DNA.

biogenesis-related genes such as *Sirt1*, *Pgc-1α*, *Nrf1*, and *Tfam* compared with the HD group (**Fig. 4**). In addition, SIRT1 activity was higher in QT+NE than in QT. These results suggest that QT+NE may be partially related to SIRT1 activation to improve mitochondrial function in the muscles of HD-fed mice. However, this study has certain important limitations. Our findings provide some evidence of QT+NE's protective role against sarcopenic obesity. However, because this study was limited in scope in terms of identifying the mechanism underlying the QT+NE-induced improvement in muscle function, future studies should investigate its *in vivo* safety and bioavailability to expand its potential applications.

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