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Effects and mechanisms of a prototype goji-derived nanovesicles-enriched beverage for conflicting cellular senescence



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ABSTRACT

Goji (*Lycium barbarum* L.) berries, a traditional Chinese herbal remedy, have long been used as both medicine and food due to their numerous health benefits. Today, the most popular form of goji consumption is commercial beverages, particularly goji not-from-concentrate (NFC) juice. However, the bioactive substances in goji berries are often lost during production, transportation, and storage, limiting the juice's potential health benefits. In this study, we introduce goji-derived nanovesicles (GqDNVs) into goji NFC juice to enhance its health effects. These GqDNVs, which help prevent senescence in skeletal muscles, are added to create a composite beverage that we have named "QiNeng". The targeted metabolomic profiling and small RNA sequencing analyses reveal that both QiNeng and goji NFC juice contain amino acids, miRNAs, and other bioactive substances. Notably, functional miRNAs, amino acids, and lipids were detected in C2C12 muscle cells after co-culturing with QiNeng and goji NFC juice. Compared to goji NFC juice alone, QiNeng not only retains similar antioxidant properties but also shows greater efficacy in inhibiting the cellular senescence, preventing myotube atrophy, promoting mitochondrial function, and improving energy-related metabolism. QiNeng enhances energy metabolism, supports cell growth, and inhibits muscle cell senescence, making up for the deficiencies of standard goji NFC juice.

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1. Introduction

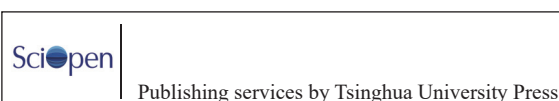
Goji (*Lycium barbarum* L.) berries, called "gou qi", have been used as traditional herbal medicine in China for centuries. A chapter

of *Sheng Ji Zong Lu* (Vol. 198), a medical text of the Imperial Academy of Medicine in the Song Dynasty (published in 1117 AD), describes the creation of the "Erjing Pill", which was made from goji and huangjing (*Polygonatum sibiricum* Red). This pill was believed to enhance "qi" and inhibit aging. Modern pharmacological studies have also confirmed that goji berries and their polysaccharides possess a range of biological activities, including antioxidant effects^[1], hypoglycemic and hypolipidemic benefits^[2], immune modulation, and skeletal muscle improvement^[3-4], making goji a potential health-promoting food.

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With the upward trend in global life expectancy, the population is increasingly aging^[5]. Aging is a crucial risk factor for various diseases such as metabolic disorders, cardiovascular diseases, cognitive decline, cancer, and sarcopenia^[6]. In parallel with the advancement of the economy and society, there is a growing demand for healthier lifestyles and nutritionally valuable foods. Due to its natural appearance, flavor, and nutrient content^[7-8], not-from-concentrate (NFC) juice is becoming more popular among consumers than reconstituted-from-concentrate (RFC) juice. Various goji NFC juice products are already available in the Chinese market.

Recent research has extracted plant-derived exosome-like nanoparticles from fruits and vegetables, which contain components and functions similar to those of the original plants^[9]. These nanoparticles hold great promise for clinical application in the treatment of different diseases^[10] and can pass through the intestinal barrier^[11]. Our previous study isolated goji-derived nanovesicles (GqDNVs) from goji berries and found that they improved skeletal muscle quality and function in muscle atrophy models^[12]. This suggests that GqDNVs could be developed as a material for both pharmaceuticals and food products.

Skeletal muscle accounts for approximately 40% of adult human body mass and plays a vital role in posture, physical activity, and glucose and lipid metabolism^[13-14]. However, sarcopenia (age-related muscle wasting) is a significant contributor to the development of various diseases^[15], and muscle wasting has been linked to increased mortality risk in our previous publication^[16]. Previous studies have shown that goji extract and polysaccharides can promote muscle function in mice^[4] and rabbits^[17], as well as enhance glucose uptake and energy metabolism^[3,18]. Our earlier research demonstrated that GqDNVs, when injected intramuscularly, could reduce muscle wasting and improve energy metabolism in mice with dexamethasone-induced atrophy^[12].

However, taking GqDNVs or goji extract through capsules or injections is inconvenient for everyday use. The challenge of preserving fresh goji berries and quickly transporting them to consumers results in a loss of bioactive components in NFC juice during production and storage, including vitamin C, antioxidants, and polyphenolic compounds^[19-20]. To overcome these challenges,

we hypothesize that “QiNeng”, a prototype juice made from goji NFC juice and GqDNVs, could address these issues in real-world applications.

In this study, we used the C2C12 cell line (mouse myoblasts) to investigate the effects and mechanisms of QiNeng in combating the expression of aging markers and other aging-related cellular changes. Our goal is not only to enhance the current NFC beverages but also to provide evidence for future animal studies, clinical trials, and practical applications of QiNeng in everyday life.

2. Materials and methods

2.1 Preparation of GqDNVs

The preparation of GqDNVs was based on our previous publication^[12]. Briefly, the fresh goji berries (*Lycium barbarum* L., sourced from Zhongwei, Ningxia, China, by cold-chain transportation) were rinsed with deionized water and then juiced with a high-speed blender. The goji berry juice was then centrifuged at progressively higher speeds: first at $1\,000 \times g$ for 10 min, followed by $3\,000 \times g$ for 20 min, and finally at $10\,000 \times g$ for 40 min. The sediment was discarded after each step. Next, the liquid was subjected to ultracentrifugation at $150\,000 \times g$ for 1.5 h. The resulting pellet was suspended in phosphate-buffered saline (PBS) and transferred to a discontinuous sucrose gradient (8%, 30%, 45%, 60%) for further ultracentrifugation at $150\,000 \times g$ for 1.5 h^[21]. A visible band between the 30% and 45% sucrose layers was collected and ultracentrifuged once again at $150\,000 \times g$ for 1.5 h. The final pellet was resuspended in PBS and filtered sequentially through 0.44 and 0.22 μm filters.

Goji NFC juice was commercially purchased from Ke Yi Kang, a brand under COFCO Corp., Beijing, China. To avoid hindering C2C12 cell growth, the solid components of the NFC juice, including fruit residues, were removed. To achieve this, the juice was centrifuged at $2\,000 \times g$ for 20 min and filtered through a 0.22 μm membrane. The filtered juice was then combined with GqDNVs to create QiNeng. Further details regarding the preparation process of QiNeng can be found in the following section (Fig. 1).

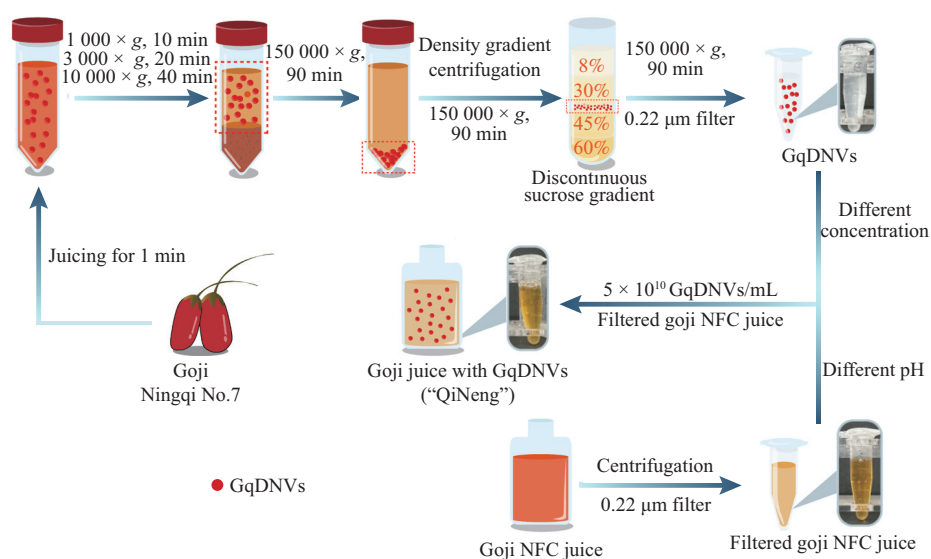


Fig. 1 The extraction process of GqDNVs and preparation of goji NFC juice and QiNeng.

2.2 Cell culture conditions

C2C12 cells were cultured in Dulbecco's modified Eagle's medium (DMEM; C11995500BT, Gibco, Waltham, MA, USA) supplemented with 10% fetal bovine serum, 100 IU/mL penicillin and 0.1 mg/mL streptomycin in an incubator at 37 °C in a 5% of CO₂ atmosphere. C2C12 cells were inoculated with a differentiation medium containing DMEM, 2% horse serum, 100 IU/mL penicillin, and 0.1 mg/mL streptomycin for five days to differentiate into myotubes. The primary culture methods and details are referred to in our published study^[12].

2.3 QiNeng's preparation, cell treatment, and cell viability

Cell counting kit-8 (CCK-8; C0038, Beyotime, Shanghai, China) was used to determine cell viability. Cells were seeded in 96-well plates at a 5×10^3 per well in 100 μ L medium for 24 h. To first find the appropriate concentrations of H₂O₂, the C2C12 cells were treated with different concentrations of H₂O₂ (200, 400, 600, 700, 800, 900, 1 000, 1 100, and 1 200 μ mol/L) for 24 h, respectively. Finally, 600 μ mol/L H₂O₂ was finally chosen to treat C2C12 cells. Next, regarding the preparation of QiNeng, we used 5×10^9 , 2×10^{10} , 5×10^{10} , 1×10^{11} , and 5×10^{11} particles/mL of GqDNVs to mix with filtered goji NFC juice^[22-23] to culture with C2C12 cells for 24 h to find a suitable GqDNVs concentration. Then, the C2C12 cells were also treated with filtered goji NFC juice or QiNeng (5×10^{10} particles/mL GqDNVs) without H₂O₂ for 48 h to prove they have no obvious toxic effect. The co-culture of juice and C2C12 cells mentioned in this study was conducted by adding goji NFC juice or QiNeng to DMEM medium at a ratio of 1:19 (V/V). CCK-8 reagent (10 μ L) was added to each well (100 μ L medium) and cultured for 2 h. The absorbance at 450 nm was measured by a microplate reader (Infinite M Nano, Tecan, Switzerland). All data are shown in the supplementary data (Fig. S1). Following the above procedure, in the following section, we set group names as C (PBS), H (600 μ mol/L H₂O₂ + PBS), HG (600 μ mol/L H₂O₂ + goji NFC juice), and HGD groups (600 μ mol/L H₂O₂ + QiNeng, containing 5×10^{10} particles/mL GqDNVs).

2.4 Characterization of GqDNVs in filtered goji NFC juice and QiNeng

GqDNVs suspension, the filtered goji NFC juice, and QiNeng were visualized using transmission electron microscopy (HT-7800, Hitachi, Tokyo, Japan), and characterizations (size and concentration) were determined by nanoparticle tracking analysis (NTA) using the N30E Flow NanoAnalyzer (NanoFCM, Xiamen, China)^[12,24].

2.5 Measurement of β -galactosidase staining in C2C12 cells

We used a β -galactosidase staining kit (C0602, Beyotime, Shanghai, China) to stain the C2C12 cells after treatment. In this section, C2C12 cells were seeded in 6-well plates and divided into four groups ($n = 3$). The C2C12 cells were incubated with the intervention for 24 h in the C, H, HG, and HGD groups after five days of differentiation. The inverted fluorescent microscope (MF53-N, Mshot, Guangzhou, China) was used to observe the areas. We measured and calculated the positive area of

β -galactosidase staining in 10 fields per well (30 fields in each group) through ImageJ software.

2.6 Measurement of the diameter of C2C12 cells

We used Jenner-Giemsa staining (C0133, Beyotime, Shanghai, China) to stain the tubes of C2C12 cells after differentiation. The C2C12 cells were inoculated in 6-well plates with the treatment for 24 h in the C, H, HG, and HGD groups after five days of differentiation ($n = 3$). The myotubes of C2C12 cells were observed by the inverted fluorescence microscope (Axio Observer 7, Zeiss, Oberkochen, Germany). The diameters of about 100 tubes per well (300 tubes in each group) were measured with ImageJ software, and each myotube was measured three times to obtain its average value^[24].

2.7 The autophagy staining in C2C12 cells

The autophagosomes in C2C12 cells were estimated by an autophagy staining assay kit with monodansylcadaverine (MDC) and propidium iodide (PI; C3019M, Beyotime, Shanghai, China). Monodansylcadaverine (green) labeled the autophagosomes, and PI (red) stained the necrotic cells or cells that lost cell membrane integrity in late apoptosis. The C2C12 cells were incubated with 6-well plates and divided into four groups: C, H, HG, and HGD ($n = 3$). The fluorescence staining (9 fields in each well) was detected by an inverted fluorescence microscope (Axio Observer 7, Zeiss, Oberkochen, Germany) and measured by ImageJ software.

2.8 Measurement of reactive oxygen species (ROS) in C2C12 cells

The ROS levels detection used a ROS assay kit (S0033S, Beyotime, Shanghai, China). The C2C12 cells were incubated in 6-well plates and divided into four groups, including the C, H, HG, and HGD groups ($n = 4$). An inverted fluorescence microscope (Axio Observer 7, Zeiss, Oberkochen, Germany) was used to detect 12 fields in each well. The fluorescence signaling of ROS in C2C12 cells was measured by ImageJ software.

2.9 The staining of mitochondria and lysosomes in C2C12 cells

Mito-Tracker Green (C1048, Beyotime, Shanghai, China), carbocyanine with a fluorescent probe, was used to mark the mitochondria. Lyso-Tracker Red (C1046, Beyotime, Shanghai, China), with a red fluorescent label, was used to mark the lysosomes. The nucleus was marked by the Hoechst 33342 (C1027, Beyotime, China). The C2C12 cells were incubated in 6-well plates and divided into four groups (C, H, HG, and HGD; $n = 3$). After the 24 h-intervention, C2C12 cells were cocultured with the two fluorescent probes for 45 min at 37 °C. Then, the C2C12 cells were cocultured with Hoechst 33342 for 10 min. The fluorescent signaling was detected with the inverted fluorescence microscope (Axio Observer 7, Zeiss, Oberkochen, Germany). ImageJ measured the fluorescence staining of lysosomes and mitochondria with nine fields per well (27 fields in each group).

2.10 Measurement of mitochondrial membrane potential in C2C12 cells

The enhanced mitochondrial membrane potential assay kit with 5,5',6,6'-tetrachloro-1,1',3,3'-tetraethyl-imidacarbocyanine iodide (JC-1, C2003S, Beyotime, Shanghai, China) was utilized to estimate the mitochondrial membrane potential. The C2C12 cells were seeded in 6-well plates and divided into four groups (C, H, HG, and HGD; $n = 5$). C2C12 cells were incubated with the intervention for 24 h and then cocultured with JC-1 solution for 20 min in the incubator. The fluorescent signaling was detected with the inverted fluorescence microscope (Axio Observer 7, Zeiss, Oberkochen, Germany). We measured the fluorescence intensity of JC-1 monomers and aggregates in 5 fields per well (25 fields in each group) through ImageJ.

2.11 Biochemical indicators in C2C12 cells

The C2C12 cells were seeded in 6-well plates and divided into four groups (C, H, HG, and HGD; $n = 5$). After treatment, the concentrations of lactate dehydrogenase (LDH) and creatine kinase (CK) in cells were measured by commercial kits (A020-2-1 and A032-1-1, Nanjing Jiancheng Bioengineering Institute, Nanjing, China). The antioxidants (superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px)), lipid peroxidation indicators (malondialdehyde (MDA)), and the content of ATP were measured by commercial kits (S0101S, S0059S, S0131S, and S0026, Beyotime, Shanghai, China). The illumination value of ATP was measured with a multifunctional microplate detector (Spark, Tecan Austria GmbH, Grödig, Austria). The absorbance values of other indicators were measured with a microplate reader (Infinite M Nano, Tecan, Männedorf, Switzerland).

2.12 Quantitative real-time polymerase chain reaction (qPCR) analysis

We extracted RNA from the four groups (C, H, HG, and HGD) using the RNA Easy Fast Tissue/Cell Kit (DP451, TIANGEN, Beijing, China). FastKing gDNA Dispelling RT SuperMix (KR118-02, TIANGEN, China) was used to synthesize cDNA. qPCR was performed with FastReal qPCR PreMix (SYBR Green) (FP217-02, TIANGEN, China) using Quant Studio™ 7 Flex (Applied Biosystems, Foster City, CA, USA). Reaction conditions: 95 °C for 2 min; 40 cycles of 95 °C for 5 s and 60 °C for 15 s. Each sample has three duplicates, and the results were processed by the $2^{-\Delta\Delta Ct}$ method.

2.13 Measurement of mitochondrial DNA (mtDNA)

C2C12 cells from four groups (C, H, HG, and HGD) were treated with the TIANamp Genomic DNA kit (DP304-03, TIANGEN, Beijing, China). The number of mitochondria was estimated as the relative copy number of mtDNA by qPCR. The measurement of qPCR was introduced above. The relative mtDNA copy number was determined by measuring the amplification of cytochrome C oxidase I (*COI*), a mitochondrial-encoded gene, normalized to β 2-microglobulin (β 2-MG)^[25].

2.14 Small RNA sequencing

Small RNA sequencing was performed by the Illumina platform (Illumina NovaSeq X Plus, Personalbio, Shanghai, China) using RNA

samples from GqDNVs, goji NFC juice, and QiNeng via the Illumina platform in Hiseq Single-End mode. The raw data were filtered and reprocessed (the identical sequences were merged, and the abundance was recorded). Then, the sequences, which were removed duplicates, were compared with Rfam^[26] and miRbase^[27] databases to obtain the annotation information of RNA. The miRNA read counts were calculated by the number of sequences matching conserved miRNA. The exon model count per million mapped reads (CPM) was used to examine the overall gene expression patterns of the samples. The CPM value was calculated as follows: CPM = number of reads matching the gene/number of total reads matching the gene $\times 10^6$. The density distribution of CPM indicated the gene expression pattern of the entire sample.

2.15 Targeted metabolome analysis

The amino acids and organic acids group, oxylipins, and energy-related metabolites were detected by MetWare platform (<http://www.metware.cn/>) based on the AB Sciex QTRAP 6500 LC-MS/MS platform^[28]. We detected the amino acids and organic acids group in GqDNVs suspension, goji NFC juice, QiNeng, and C2C12 cells after treatment. Oxylipins and energy-related metabolites were measured in C2C12 cell samples after treatment. Specific methods and conditions are documented in the Supplementary.

2.15.1 Targeted metabolome analysis of the amino acids and organic acids group

Significant differences in metabolites between groups were determined by the absolute rate of change. The change in metabolites that were more than 2-fold or less than 0.5-fold between groups was considered a difference in metabolites.

2.15.2 Targeted metabolome analysis of the oxylipins and energy-related metabolites

Significantly regulated metabolites between groups were determined by variable importance in projection (VIP) and absolute change rate. VIP values were extracted from orthogonal partial least squares discriminant analysis (OPLS-DA) results. Data were mean-centered before OPLS-DA. To avoid overfitting, a permutation test (200 permutations) was performed. Metabolites that met the following conditions were considered as different metabolites between the two comparison groups: VIP > 1; fold change ≥ 2 , or fold change ≤ 0.5 .

Identified metabolites were annotated using the Kyoto Encyclopedia of Genes and Genomes (KEGG) compound database (<http://www.kegg.jp/kegg/compound/>), and annotated metabolites were then mapped to the KEGG pathway database (<http://www.kegg.jp/kegg/pathway.html>).

2.16 Statistical analysis

GraphPad Prism for Windows (version 9.0.0, GraphPad Software, San Diego, CA, USA) was used for data analysis and graph creation. In this study, multiple groups (≥ 3 groups) were compared and analyzed using one-way analysis of variance (ANOVA), and the

pairwise comparisons in multiple groups were analyzed using Šidák's multiple comparison analysis. The bilateral *t*-test was used to compare the values between the two groups. *P*-values below 0.05 were considered statistically significant.

3. Results and discussion

3.1 Characterizations of GqDNVs suspension, goji NFC juice, and QiNeng

At room temperature, filtered goji NFC juice appeared as an orange, transparent solution with a sweet aroma and a pH of 4.13 ± 0.03 . In contrast, the GqDNVs suspension was a colorless, transparent solution with no noticeable scent and a pH of 7.38 ± 0.03 . In this study, the QiNeng solution consisted of goji NFC juice and 5×10^{10} GqDNVs/mL. Its appearance and smell were similar to goji NFC juice, while its pH was slightly higher at 4.24 ± 0.02 , likely due to the addition of GqDNVs suspended in PBS. We utilized the CCK8 assay to determine the optimal concentration of GqDNVs in QiNeng (Fig. S1). The specific results are detailed in the subsequent subsection.

The concentrations of extracellular vesicles in GqDNVs suspension, filtered goji NFC juice, and QiNeng were measured using NTA and transmission electron microscopy (Figs. 2a–b). Under transmission electron microscopy, the GqDNVs in PBS exhibited the typical “cup-disk” shape of extracellular vesicles. Goji NFC juice contained a few extracellular vesicles, and their quantity was significantly lower than in the GqDNVs suspension ($P < 0.05$). After adding GqDNVs, the concentration of extracellular vesicles in QiNeng increased, with a size distribution similar to that of the GqDNVs suspension.

Extracellular vesicles are prone to degradation after storage at 4, -20 , and -80 °C, especially after freeze-thaw cycles^[29]. Over a 7-day storage period, GqDNVs stored in PBS at 4 °C were more stable than those stored at -20 °C ($P < 0.05$). This difference might be attributed to the freeze-thaw cycle. Furthermore, the degradation rate of GqDNVs in QiNeng was lower than in PBS ($P < 0.05$) despite higher concentrations of GqDNVs in both the PBS suspension and QiNeng compared to goji NFC juice ($P < 0.05$) (Fig. S2). This suggests that QiNeng remains relatively stable for up to seven days when stored at 4 °C. NFC juice products in China are typically sold at this temperature in supermarkets, shops, or snack bars, and their production and cold chain transportation comply with Chinese agricultural industry standards (NY/T 3909–2021 and NY/T 3910–2021).

3.2 Results from targeted metabolome analysis showed that QiNeng contains representative amino acids for skeletal muscle health

The base peak chromatogram revealed differences among the three groups, with the targeted metabolome analysis of amino acids and organic acids proving reliable (Fig. S3). Principal component analysis (PCA) demonstrated that goji NFC juice and QiNeng (goji NFC juice with 5×10^{10} particles/mL GqDNVs) exhibited similar profiles, while the GqDNVs suspension (2×10^{11} particles/mL) showed distinct characteristics (Fig. 2c and Table S1). QiNeng was particularly rich in amino acids and their derivatives (Fig. 2d), such as betaine (2321.24 ± 443.64 ng/mL) and leucine ($39770.88 \pm$

2935.29 ng/mL) (Table S1). Both of these compounds are beneficial for skeletal muscle health^[30–31]. In contrast, the GqDNVs suspension had the lowest concentration of metabolites among the three groups. However, some amino acids, including arginine (13.53 ± 16.29 ng/mL) and leucine (18.28 ± 3.06 ng/mL), were still detected in the GqDNVs suspension. Despite GqDNVs containing fewer metabolites than goji NFC juice, GqDNVs may offer several advantages that metabolites alone do not provide. Previous research has shown that exosomes can cross the blood-brain barrier^[32], enhance the phagocytic activity of cells (particularly tumor cells)^[33–34], and maintain long circulation *in vivo* even in an inflammatory environment^[35]. These indicate that exosomes exhibit strong stability, bioavailability, and biocompatibility. Similarly, GqDNVs, as a type of exosome, may possess comparable properties. Further experiments were performed to assess the effects of goji NFC juice and QiNeng on a cellular aging model, ultimately determining whether QiNeng provided superior benefits compared to goji NFC juice.

3.3 The QiNeng could improve the cell viability of C2C12 cells

Sarcopenia, an aging-related pathological syndrome characterized by progressive loss of skeletal muscle mass and strength, arises from multisystem failures, encompassing mitochondrial dysfunction, anabolic resistance, and neuromuscular junction destabilization^[15,36]. Oxidative stress mediated by H_2O_2 was linked to skeletal muscle atrophy in previous studies^[37–39]. Therefore, H_2O_2 administration has become a method to establish *in vitro* models of oxidative stress-induced muscle wasting^[40–42]. In this study, 600 μ mol/L H_2O_2 was selected to induce oxidative stress-related aging in C2C12 cells for further experiments based on CCK8 assay results (Fig. 3a). Preliminary experiments indicated that supplementing DMEM with juice at 1:19 or 1:49 (*V/V*) ratios exerted no significant cytotoxicity on C2C12 cells (Fig. S4a). Consequently, the primary experimental design utilized a 1:19 (*V/V*) dilution of goji NFC juice or QiNeng in DMEM to establish a co-culture system with C2C12 cells, thereby investigating the biological effects of goji juice. Despite the acidity of goji NFC juice (pH 4.13 ± 0.03) and QiNeng (pH 4.24 ± 0.02), a 1:19 (*V/V*) dilution with DMEM effectively buffered the system to maintain physiological pH (7.0–7.6), creating an optimal microenvironment for C2C12 cells proliferation^[43].

Both goji NFC juice and QiNeng (without pH alterations) demonstrated the best ability to mitigate the toxic effects of H_2O_2 (Figs. S4b–c). Next, different concentrations of GqDNVs in QiNeng were tested to assess their impact on cell viability (Figs. S4d–e). These results demonstrated that 2×10^{10} , 5×10^{10} , and 1×10^{11} particles/mL of GqDNVs exhibit attenuation of H_2O_2 -induced cytotoxicity in C2C12 cells. Notably, the 5×10^{10} particles/mL of GqDNVs showed statistically superior cytoprotective efficacy compared to other concentrations. Therefore, QiNeng with 5×10^{10} particles/mL of GqDNVs in goji NFC juice (without pH changes) was thus confirmed for further analysis.

After 48 h of co-culturing with goji NFC juice, no adverse effects on cell viability were observed, whereas QiNeng notably increased cell viability (Fig. 3b). Compared to goji NFC juice alone, QiNeng significantly mitigated the cell toxicity induced by 600 μ mol/L H_2O_2 over 24 h (Fig. 3c). These results suggest that QiNeng holds promising potential in alleviating H_2O_2 -induced toxicity in C₂C₁₂ cells.

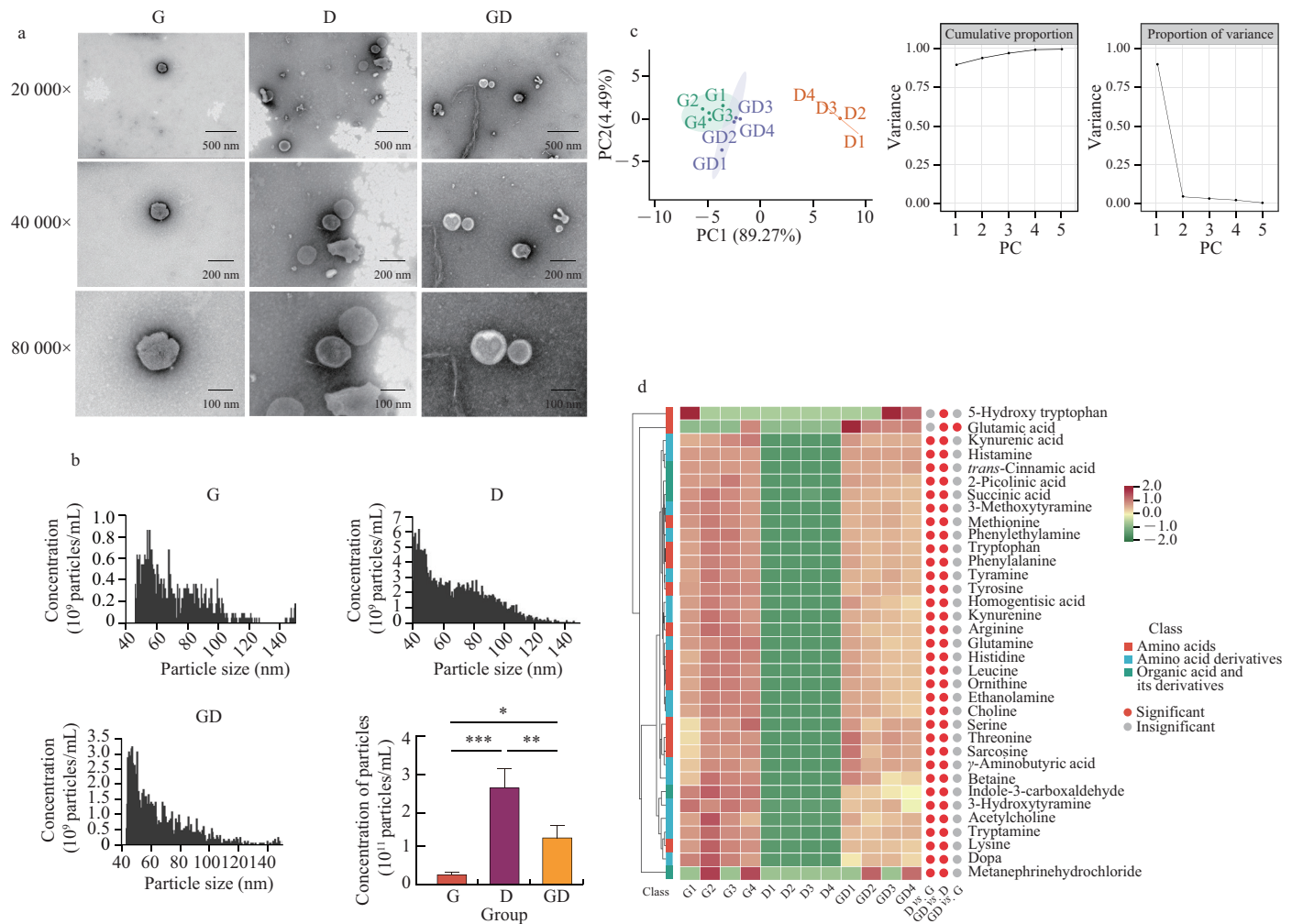


Fig. 2 The illustration of transmission electron microscopy and NTA of GqDNVs suspension, goji NFC juice, and QiNeng. (a) Picture of GqDNVs suspension (D), goji NFC juice (G), and QiNeng (GD) under transmission electron microscopy. (b) The average particle concentration and particle size of GqDNVs, goji NFC juice, and QiNeng obtained by NTA. One-way ANOVA analysis and Šidák's multiple comparisons analysis were used in the between-group comparisons. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. (c) The PCA of the metabolites in GqDNVs suspension, goji NFC juice, and QiNeng. (d) The relative concentration of the metabolites. D, G, and GD represent GqDNVs suspension, goji NFC juice, and QiNeng, respectively.

3.4 The QiNeng could inhibit myotube atrophy and cellular senescence in C2C12 cells

The activity of β -galactosidase is a key marker of cellular senescence^[44–46]. Using Jenner-Giemsa staining (Figs. 3d–e) and β -galactosidase staining (Figs. 3f–g), we observed that exposure to 600 $\mu\text{mol/L}$ H_2O_2 induced muscle cell atrophy and senescence in differentiated C2C12 myotubes. However, the β -galactosidase-stained area and myotube diameters revealed that QiNeng significantly alleviated the cellular senescence and muscle atrophy caused by 600 $\mu\text{mol/L}$ H_2O_2 . Goji NFC juice also showed improvements in these parameters, thus indicating some protective effects.

3.5 QiNeng could reduce the amounts of autophagosomes under the H_2O_2 condition

Maintaining functional autophagy is crucial for overall health, as an imbalance in autophagy can accelerate aging and contribute to disease^[47]. Transmission electron microscopy images showed numerous well-defined, elliptical mitochondria and a few autophagosomes in the

cytoplasm of differentiated C2C12 myotubes (Fig. 4a). After treatment with 600 $\mu\text{mol/L}$ H_2O_2 , some mitochondria appeared vacuolated, and the number of autophagosomes increased, indicating hyper-enhanced autophagy. In contrast, QiNeng reduced the number of autophagosomes, though some vacuolated mitochondria were still present.

To quantify the extent of autophagy, we used an autophagy staining assay to measure autophagosome numbers (Figs. 4b–d). Quantitative analysis of the fluorescence intensity showed that 600 $\mu\text{mol/L}$ H_2O_2 significantly increased the number of autophagosomes, demonstrating typical cellular damage in C2C12 cells. Goji NFC juice (HG) did not alter autophagy, which was already upregulated after H_2O_2 treatment. However, QiNeng (HGD) significantly alleviated the H_2O_2 -induced increase in autophagosomes, suggesting a protective effect against excessive autophagy.

3.6 QiNeng and goji NFC juice reduced the oxidative stress induced by H_2O_2 in C2C12 cells

The administration of H_2O_2 can induce oxidative stress^[48], which is closely linked to autophagy^[49] and is considered one of the key mechanisms of cellular senescence^[50]. In our study, H_2O_2 significantly

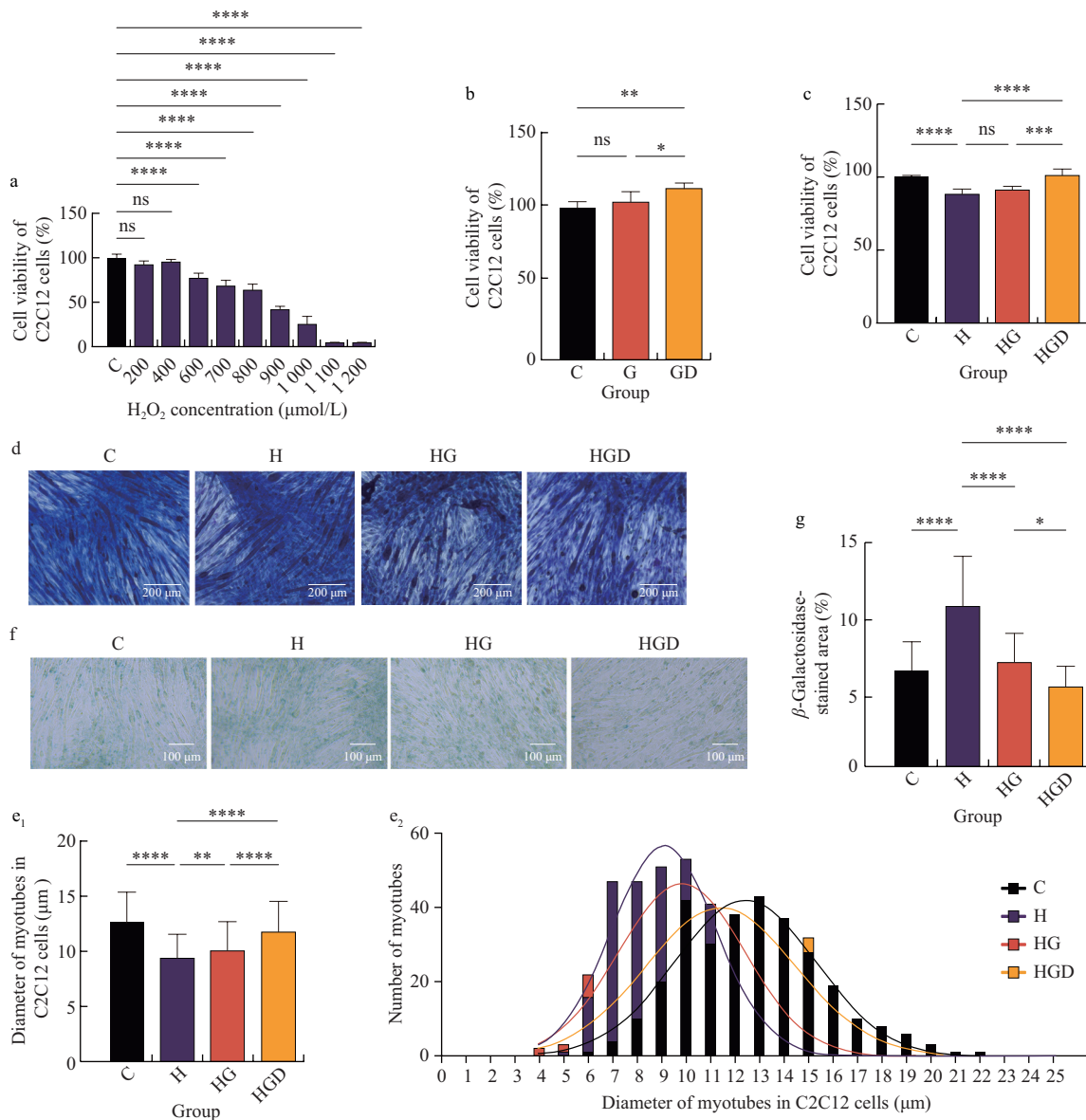


Fig. 3 The effect of goji NFC juice and QiNeng on C2C12 cells. (a) The toxicity of H_2O_2 on C2C12 cells. (b) The effect of goji NFC juice and QiNeng on cell viability of C2C12 cells. (c) The effect of goji NFC juice and QiNeng on cell viability of 600 $\mu\text{mol/L}$ H_2O_2 infected C2C12 cells. (d–e) Quantitative analyses of the mean diameter of myotubes and diameter distribution of myotubes of C2C12 cells. (f–g) Quantitative analyses of the β -galactosidase-stained area of C2C12 cells. C, G, and GD mean C2C12 cells were treated with PBS, goji NFC juice, and QiNeng with 5×10^{10} particles/mL GqDNVs; H, HG, and HGD mean the administration of 600 $\mu\text{mol/L}$ H_2O_2 , 600 $\mu\text{mol/L}$ H_2O_2 + goji NFC juice, and 600 $\mu\text{mol/L}$ H_2O_2 and QiNeng with 5×10^{10} particles/mL GqDNVs. One-way ANOVA analysis and Šidák's multiple comparisons analysis were used in the between-group comparisons. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, and **** $P < 0.0001$; ns indicates $P > 0.05$.

increased the fluorescence intensity of ROS staining, indicating heightened oxidative stress. Both goji NFC juice and QiNeng were able to reduce the ROS-positive fluorescent intensity (Figs. 4e–f). Additionally, H_2O_2 treatment elevated MDA levels and decreased SOD activity in C2C12 cells, indicating an increased oxidative burden. Although neither goji NFC juice nor QiNeng significantly affected MDA levels or SOD, CK, and LDH activity, they did enhance GSH-Px activity (Figs. 4g–k).

These findings suggest that ROS can rapidly attack cellular membranes and lipids, triggering lipid peroxidation (conjugated effects)^[51], even though hydroxyl radicals have an extremely short lifespan of just 10^{-9} s^[52]. The high reactivity and short lifespan of ROS can indirectly deplete the antioxidant defense system and induce

redox imbalance. Both goji NFC juice and QiNeng reduced ROS levels and slightly enhanced the antioxidant defense system.

Oxidative damage is also implicated in age-related mitochondrial dysfunction^[53–54]. After H_2O_2 treatment, ATP levels in C2C12 cells were significantly reduced. While goji NFC juice did not affect ATP levels compared to the H group, QiNeng increased ATP concentration (Fig. 4l). Since both ROS and ATP are primarily generated through oxidative phosphorylation, which involves the transfer of electrons through the mitochondrial electron transport chain^[55], further experiments were conducted to explore the effects of H_2O_2 , goji NFC juice, and QiNeng on mitochondrial quantity and function.

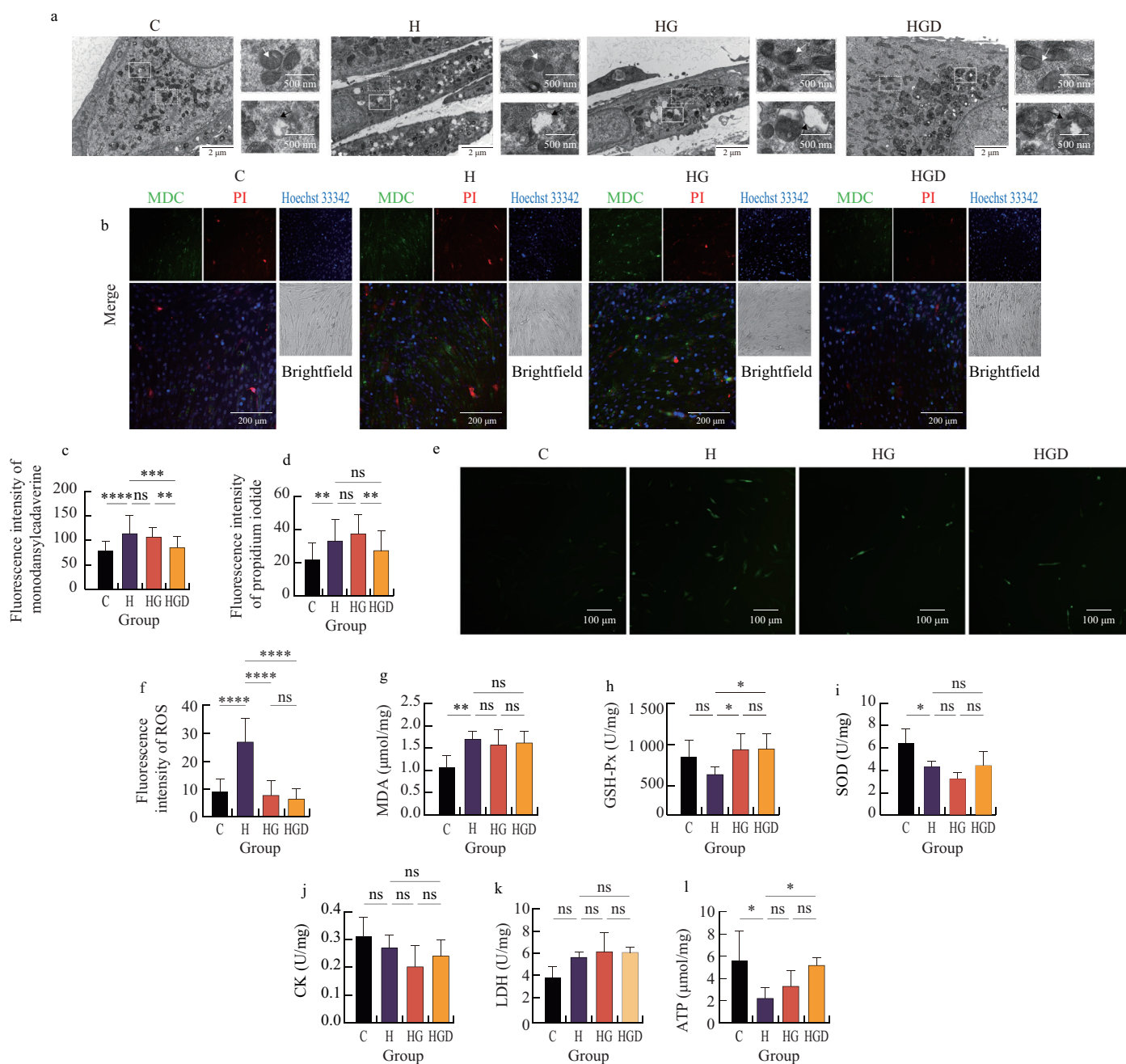


Fig. 4 The illustration of transmission electron microscopy and quantitative analyses of ROS and oxidative stress indicators of differentiated C2C12 myotubes. (a) The illustration of transmission electron microscopy of differentiated C2C12 myotubes. The white arrow points to the mitochondria; black arrow points to the autophagosome. (b–d) The autophagy staining with MDC (green), PI (red), and Hoechst 33342 (blue). (e–f) ROS staining (green) of the C2C12 cells. The fluorescence intensity was measured by ImageJ. (g–l) The biochemical detection of oxidative stress in C2C12 cells after intervention. One-way ANOVA analysis and Šidák's multiple comparison analysis were used in the between-group comparisons. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, and **** $P < 0.0001$; ns indicates $P > 0.05$.

3.7 QiNeng improved the mitochondrial function and quantity in cells

H_2O_2 treatment resulted in a reduction of the mitochondrial fluorescence intensity and an increase in lysosome staining in differentiated C2C12 myotubes (Figs. 5a–c). Goji NFC juice did not significantly affect the mitochondria and lysosomes in H_2O_2 -treated C2C12 cells. However, QiNeng intervention increased the mitochondrial fluorescence intensity and reduced the lysosome fluorescence intensity compared to the H and HG groups. The lysosomal function is closely tied to autophagy, which is implicated in aging and various diseases^[56–57]. The

staining images of mitochondria and lysosomes demonstrated that H_2O_2 activated autophagy and reduced mitochondrial numbers in C2C12 cells, whereas QiNeng helped alleviate these changes.

Mitochondrial membrane potential staining further showed that QiNeng helped stabilize mitochondrial membrane potential in C2C12 cells, while goji NFC juice did not have effects (Figs. 5d–g). Based on these mitochondrial changes, we measured the relative levels of mtDNA in C2C12 cells by assessing the expression of mitochondrially encoded *COI* and β -2-MG^[25]. H_2O_2 treatment led to a decrease in mtDNA content compared to the control group. While

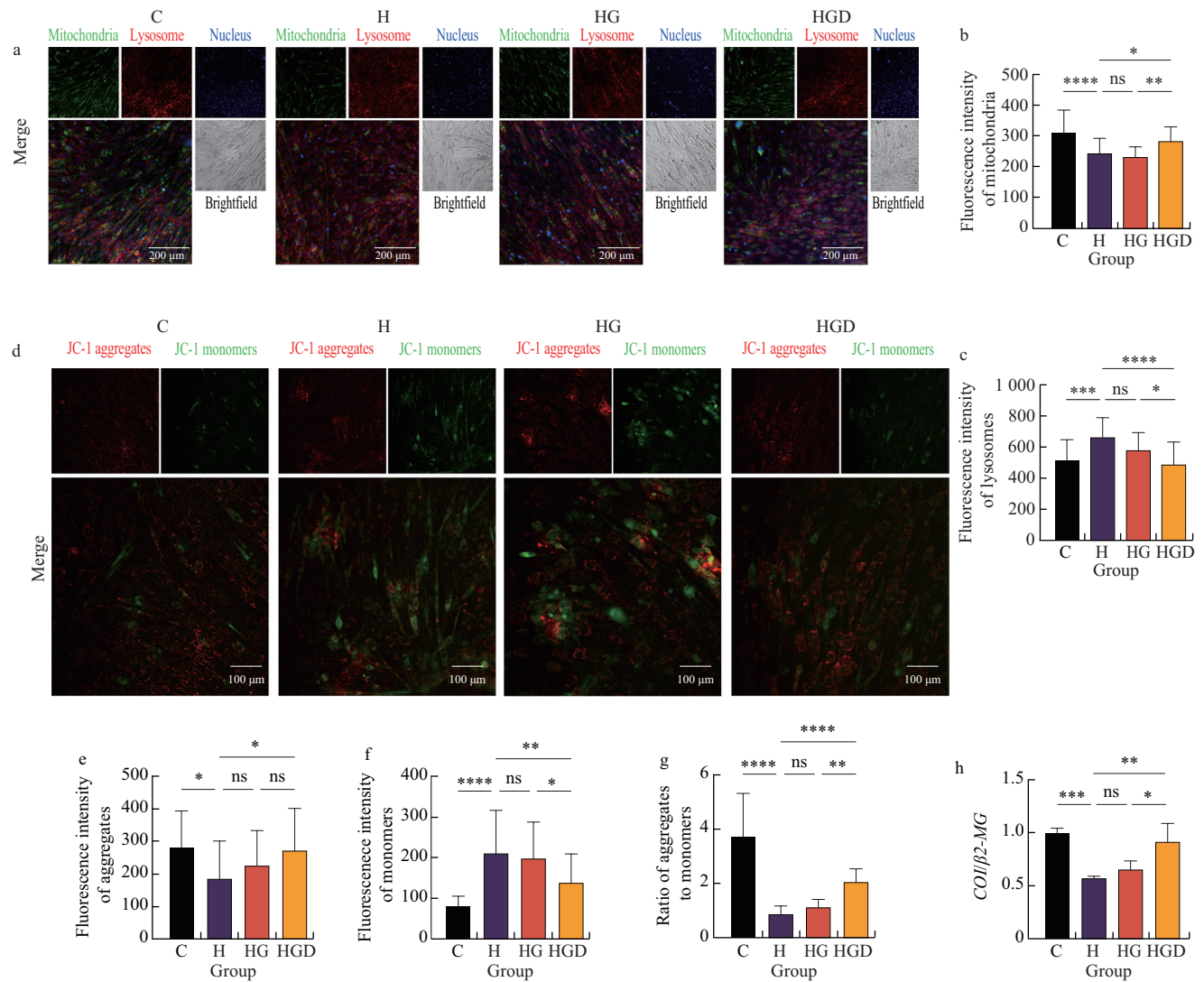


Fig. 5 The quantity and function of mitochondria in the C2C12 cells. (a–c) The staining of mitochondria (green), lysosomes (red), and nuclei (blue). (d–g) The mitochondrial membrane potential of the C2C12 cells. JC-1 aggregates (red) and JC-1 monomers (green). The fluorescence intensity was measured by ImageJ. (h) The level of mtDNA of mitochondria. One-way ANOVA analysis and Šidák's multiple comparisons analysis were used in the between-group comparisons. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, and **** $P < 0.0001$; ns indicates $P > 0.05$.

there was no significant difference in mtDNA content between the H and HG groups, QiNeng significantly increased mtDNA levels compared to both the H and HG groups (Fig. 5h).

Impaired autophagy and mitochondrial dysfunction are key hallmarks of aging^[58]. Our study indicated that H_2O_2 induced excessive autophagy and decreased mitochondrial function and quantity in C2C12 cells. Notably, QiNeng treatment significantly reduced lysosome numbers and autophagosome formation while simultaneously enhancing mitochondrial function and increasing mtDNA content. In contrast, goji NFC juice showed no significant effects on these parameters. Thus, the effects of QiNeng in inhibiting excessive autophagy and mitigating mitochondrial dysfunction were superior to those of goji NFC juice.

3.8 QiNeng upregulated lipid metabolism and significantly improved energy-related metabolism

Metabolomic profiling revealed that goji NFC juice, QiNeng, and GqDNVs contain organic acids and amino acids. Our results indicated

that H_2O_2 could cause mitochondrial dysfunction and oxidative stress in C2C12 cells, while QiNeng alleviated this change. Mitochondria, linked with H_2O_2 and skeletal muscle function, are the most important energy metabolism centers in cells^[59–61]. Consequently, we selected targeted metabolomic profiling on organic acids, amino acids, oxylipins, and energy-related metabolites to further investigate the H_2O_2 -induced damages and the defensive effects of QiNeng and goji NFC juice.

The base peak chromatogram revealed distinct differences among the groups, confirming the reliability of the targeted metabolome results (Figs. S5–7). The PCA demonstrated metabolic distinctions between groups (Fig. 6a–c), with pairwise comparisons shown in Figs. S5–7. OPLS-DA was performed for differential analysis of oxylipins (Fig. S5) and energy-related metabolites (Fig. S7). Differential analysis revealed distinct variations in oxylipins, energy-related metabolites, amino acids, and organic acids among the groups (Figs. 6d–g and S8–9).

3.8.1 Lipid metabolism

In the H and C groups, linoleic acid (LA)-derived oxylipins were the most prevalent, whereas arachidonic acid (ARA)-derived oxylipins were dominant in the HG and HGD groups (Fig. 6d and Table S2). The ω -3 and ω -6 polyunsaturated fatty acids (PUFAs) exert essential biological functions in both animals and humans, encompassing energy metabolism^[62]. These include the n -6 PUFAs LA, ARA, dihomo- γ -linolenic acid (DGLA), and γ -linolenic acid (GLA), as well as the n -3 PUFAs eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and α -linolenic acid (ALA).

H₂O₂ treatment led to increased levels of oxylipins, including prostaglandin (PG) E₂, 20-carboxy-leukotriene B₄ (20-COOH-LTB₄),

20-hydroxyeicosatetraenoic acid (20-HETE), thromboxane B₂ (TxB₂), PGE₁, and PGD₃ in C2C12 cells. However, H₂O₂ reduced levels of PGB₂, 11 β -prostaglandin F_{2 α} (11 β -PGF_{2 α}), 11,12-dihydroxyeicosatrienoic acid (11(12)-DiHET), Resolvin D1 (RvD1), 11-hydroxyeicosapentaenoic acid (11-HEPE), and 17,18-dihydroxyeicosatetraenoic acid (17(18)-DiHETE). Notably, compared with H group, QiNeng increased the metabolites of prostaglandin F_{2 α} (PGF_{2 α}), including 2,3-dinor-8-iso-PGF_{2 α} , 13,14-dihydro-15-keto-PGF_{2 α} , and 11 β -PGF_{2 α} . PGF_{2 α} , along with PGE₂, is involved in the inflammatory response and plays a crucial role in muscle regeneration^[63-64]. Nevertheless, the HG group exhibited minimal alterations compared to the H group. Moreover, goji NFC juice and QiNeng increased RvD1 and 17(18)-DiHETE,

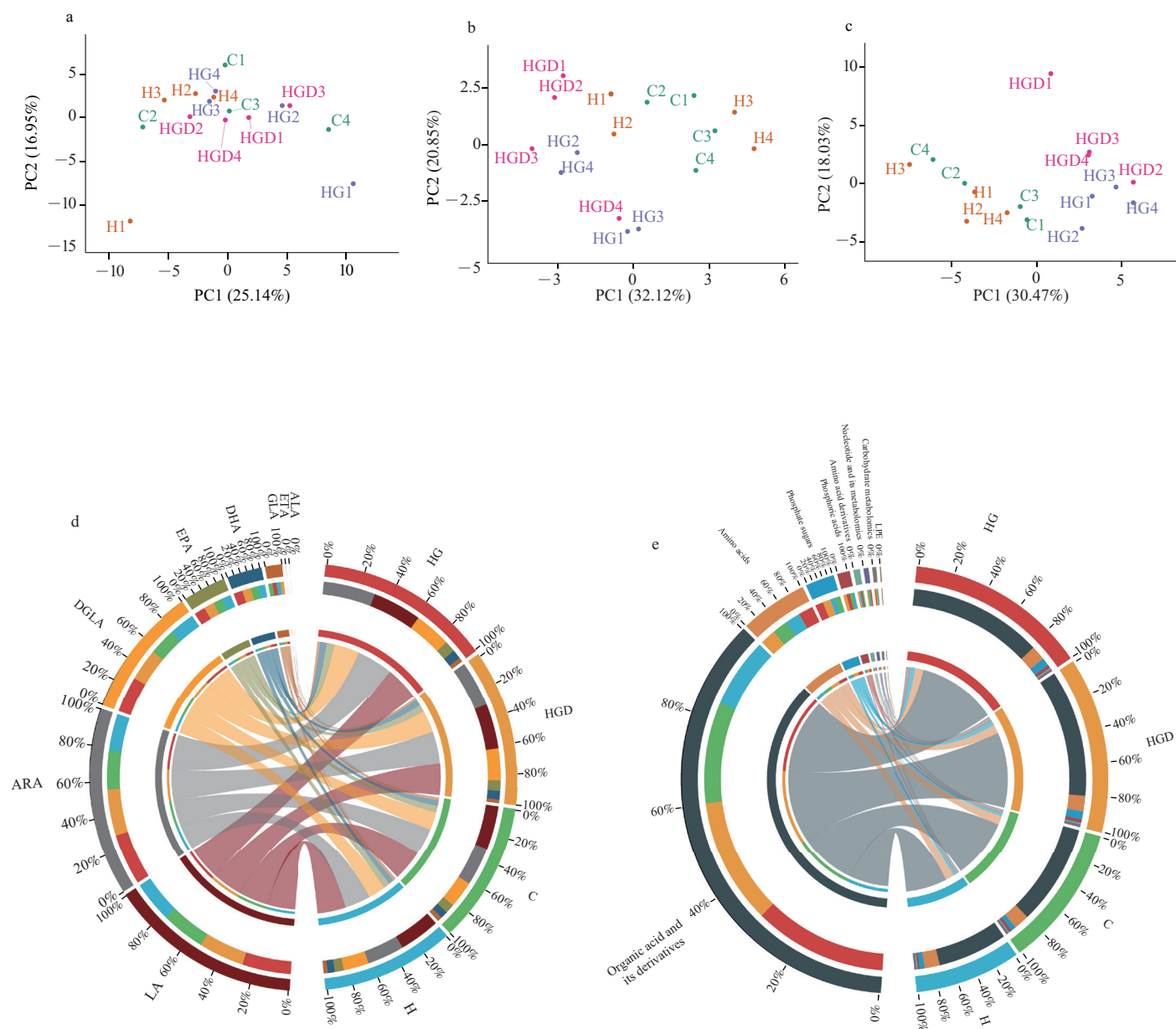


Fig. 6 The illustration of PCA and the percentage chart about oxylipins, energy-related metabolites, and the group of amino acids, organic acids, and their derivatives. The PCA plots of (a) oxylipins, (b) amino acids, organic acids, and (c) energy-related metabolites. The percentage chart and classification of (d) oxylipins, (e) energy-related metabolites, amino acids, organic acids, and their derivatives. The relative concentration of (f) oxylipins, (g) energy-related metabolites, amino acids, organic acids, and their derivatives.

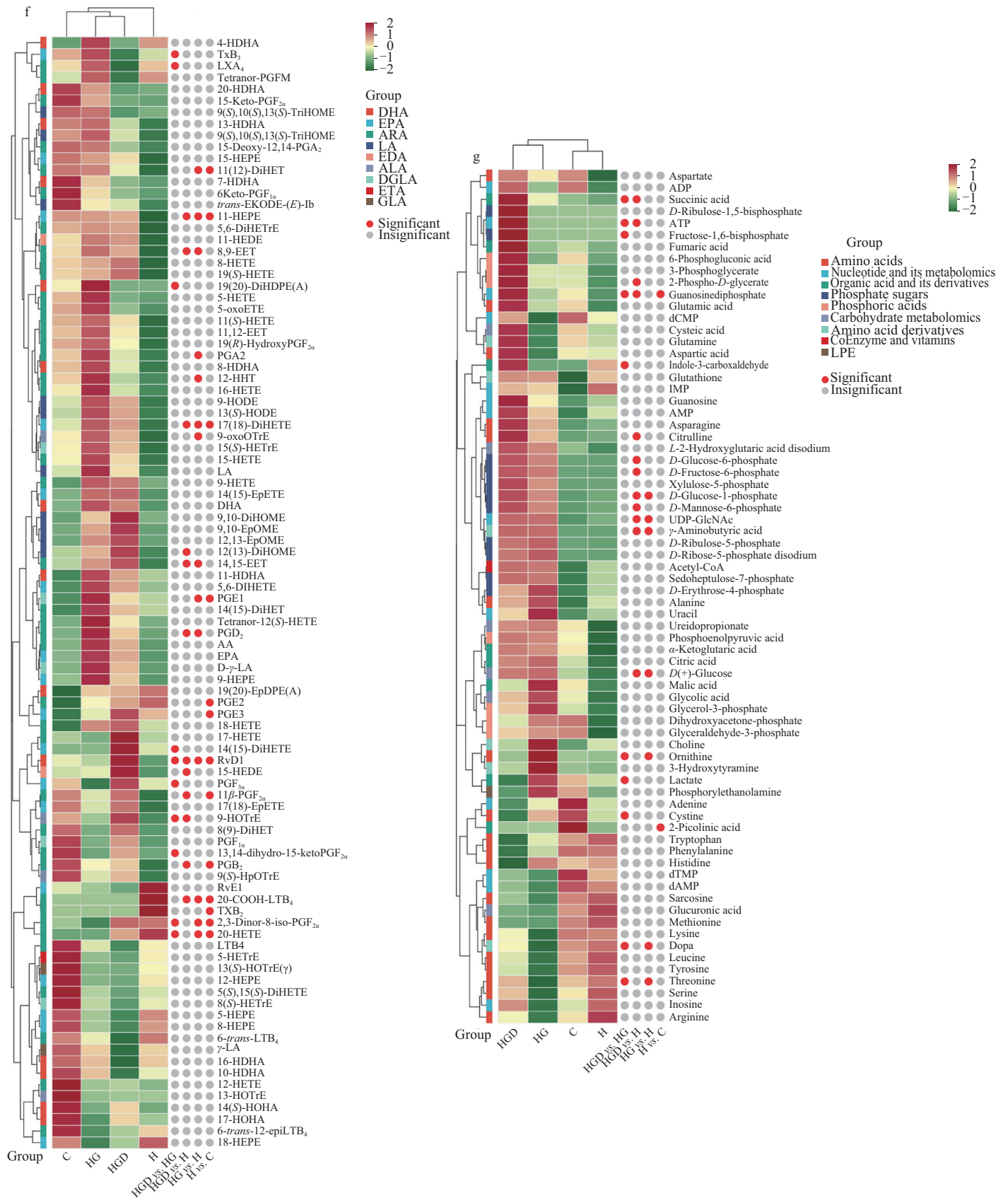


Fig. 6 (Continued)

compared with the H group. RvD1 can attenuate inflammation^[65]. The increase in 17(18)-DiHETE suggested enhanced lipid oxidation and uptake in muscle^[66]. Moreover, RvD1, 17(18)-DiHETE, and 11-HEPE are members of the *n*-3 PUFA-derived oxylipins which can positively modulate inflammatory responses to promote muscle regeneration^[67].

The increase in oxylipins observed in C2C12 cells treated with QiNeng and goji NFC juice indicated both inflammatory and anti-inflammatory responses essential for muscle regeneration^[68]. Lipid metabolism, which primarily occurs in mitochondria, converts fatty acids into acetyl-CoA, producing more energy than other metabolites^[69]. While type II (glycolytic) muscle fibers rely on glucose, type I (oxidative) muscle fibers primarily use lipids as their energy source^[70-71]. These findings indicate a shift in C2C12 cells from type II to type I muscle fibers. Consequently, the oxidized lipids observed in this study likely originated from both H₂O₂-induced oxidative stress and cellular lipid metabolism. The elevated oxylipin levels in the HG and HGD groups may reflect a combination of exogenous oxidative stress and enhanced lipid metabolism, contributing to muscle cell regeneration.

3.8.2 Amino acid and energy-related metabolism

Targeted-metabolomic profiling of energy-related metabolites, amino acids, and organic acids revealed that low concentrations in H group (Fig. 6e and Table S3). The HG group exhibited the highest levels of organic acid and its derivatives and phosphate sugars. In contrast, the HGD group had the highest concentrations of amino acids and phosphoric acids (Fig. 6e).

A decrease was observed in guanosine-diphosphate and 2-picolinic acid levels in the H group compared to the C group (Figs. 6g and S9). The administration of goji NFC juice and QiNeng significantly increased levels of γ -aminobutyric acid, *D*(+)-glucose, uridine diphosphate *N*-acetylglucosamine (UDP-GlcNAc), and *D*-glucose-1-phosphate in C2C12 cells following H₂O₂ treatment. γ -Aminobutyric acid exhibits potential anti-aging benefits^[72]. UDP-GlcNAc serves as a precursor to *O*-linked *N*-acetylglucosamine (*O*-GlcNAc) protein modifications, which are crucial to muscle metabolism^[73]. Notably, levels of 2-phospho-*D*-glycerate, *D*-glucose-6-phosphate, and *D*-fructose-6-phosphate increased in the HGD group compared to the H group, suggesting that QiNeng improved glycolysis^[74], whereas goji NFC juice showed insignificant changes. Guanosine-diphosphate and succinic acid levels increased in the HGD group relative to the H and HG groups, indicating an activated tricarboxylic acid cycle in mitochondria^[74]. Lactate levels in the HGD group were lower than in the HG group. Lactate produced by glycolytic (type II) muscle fibers is transported to oxidative (type I) fibers, where it is converted into pyruvate via mitochondrial metabolism to enter the tricarboxylic acid cycle^[75]. Moreover, the observed increases in ATP levels in the HGD group compared to the HG group suggest that QiNeng may be more effective for energy production.

Interestingly, despite its high presence in both supplements, we noted that betaine was not detected in C2C12 cells treated with goji NFC juice or QiNeng. We hypothesize that betaine, as a methyl donor^[76], was rapidly utilized to enhance lipid metabolism, glucose utilization^[77-78], maintain autophagy^[79-80], DNA methylation, and protein synthesis^[81]. Previous studies have shown that betaine can improve lipid accumulation in skeletal muscle cells^[82], mirroring the lipid metabolism changes observed with goji NFC juice and QiNeng in this study. Additionally, arginine and ornithine were abundant in

the goji NFC juice and QiNeng. Citrulline levels in the HGD group were higher than in the H group, whereas ornithine levels in the HG group surpassed those in the H and HGD groups. Arginine, ornithine, and citrulline can be interconvertible^[83]. Citrulline can enhance muscle strength^[84], while arginine can upregulate mitochondrial mRNA expression and muscle fiber type I^[85]. The variations in these amino acids suggest that C2C12 cells may absorb them from goji NFC juice and QiNeng, potentially influencing their metabolism.

In conclusion, our results indicate that QiNeng enhances glycolysis, the tricarboxylic acid cycle, and lipid metabolism, while goji NFC juice primarily promotes lipid metabolism in cells. These findings correlate with changes in mitochondrial staining, demonstrating increased mitochondrial function and quantity.

3.9 KEGG analysis of the differential metabolites between groups

The differential metabolites between the H₂O₂-treated group and the control group were primarily mapped to the metabolic pathway (ko01100) and ARA metabolism (ko00590) (Figs. S10 and S14a). Compared to the control group, H₂O₂ accelerated the ARA metabolism (ko00590) and slightly inhibited the serotonergic synapse (ko04726) in C2C12 cells (Fig. 7a). Compared with the H₂O₂-treated group, goji NFC juice promoted ARA metabolism (ko00590), vascular smooth muscle contraction (ko04270), serotonergic synapse (ko04726), and amino sugar and nucleotide sugar metabolism (ko00520) (Figs. 7b, S11 and S14b). Goji NFC juice also slightly enhanced sugar-related metabolic pathways, including galactose metabolism (ko00052), biosynthesis of nucleotide sugars (ko01250), and glycolysis/gluconeogenesis (ko00010). Consequently, H₂O₂ primarily affected lipid-related metabolic pathways in C2C12 cells. Goji NFC juice promoted lipid-related metabolisms and slightly accelerated sugar-related metabolisms.

Notably, QiNeng (HGD) significantly upregulated several metabolic pathways, such as galactose metabolism (ko00052), glycolysis/gluconeogenesis (ko00010), amino sugar and nucleotide sugar metabolism (ko01250), starch and sucrose metabolism (ko00500), and biosynthesis of nucleotide sugars (ko01250) in C2C12 cells compared with the H₂O₂-treated group (Figs. 7c, S12 and S14c). QiNeng primarily promoted sugar-related metabolic processes, whereas changes in lipid-related pathways were less prominent based on KEGG pathway annotations of differential metabolites. Compared to goji NFC juice, QiNeng more effectively enhanced metabolic pathways related to oxidative phosphorylation (ko00190), prolactin signaling (ko04917), and tyrosine metabolism (ko00350) pathways (Figs. 7d, S13 and S14d). These findings suggest that QiNeng facilitates energy production through enhanced sugar-related metabolisms and improved mitochondrial function, particularly oxidative phosphorylation. These changes align with the alterations of mitochondrial number and membrane as previously described. The metabolic changes induced by QiNeng were consistent with our previous publication^[12], which may contribute to the regeneration of C2C12 cells.

3.10 GqDNVs suspension also had miRNAs, which indirectly regulated the function of C2C12 cells

Plant-derived small RNAs have been found in the mammalian circulating system, where they regulate gene expression and

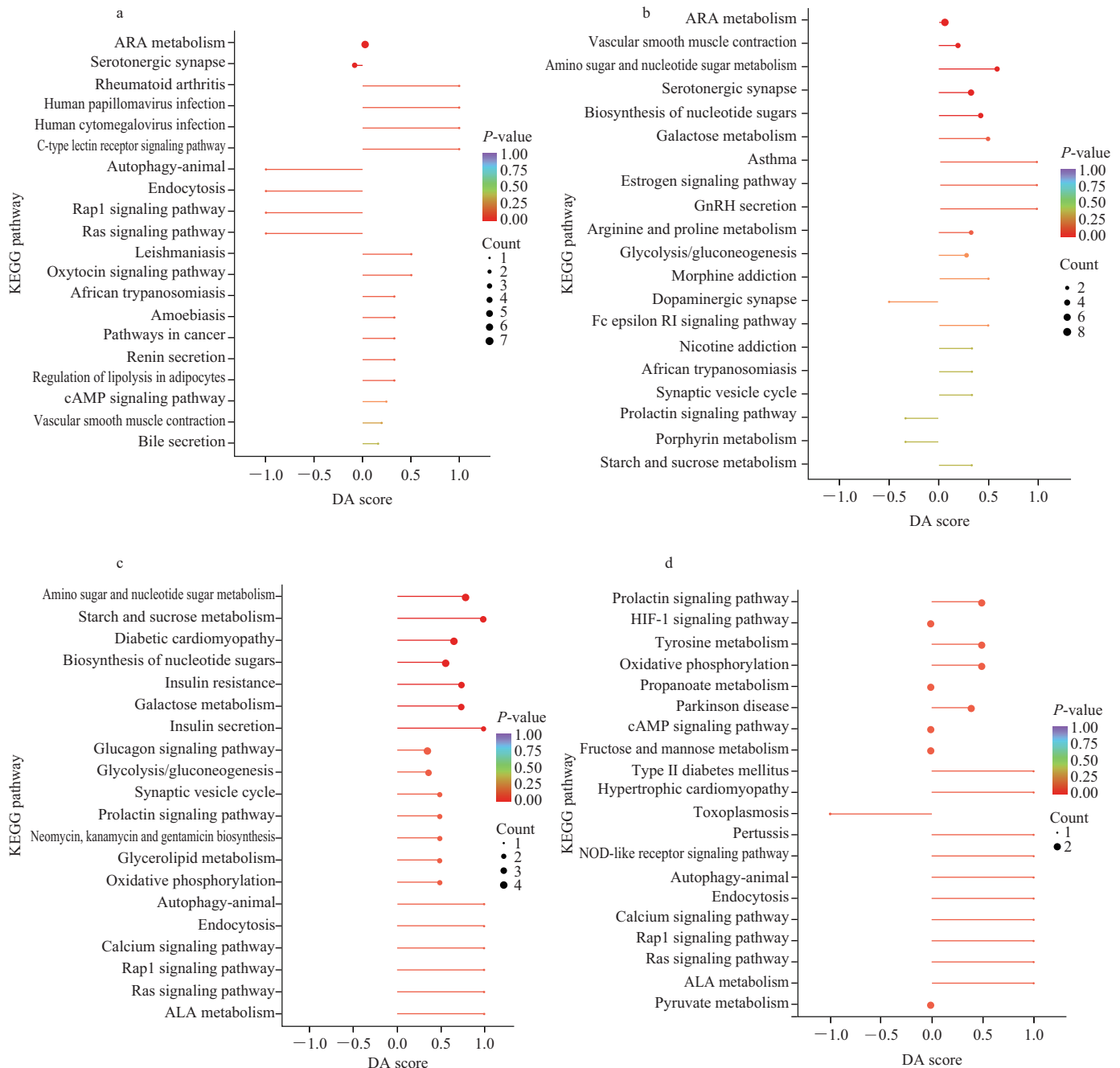


Fig. 7 The KEGG annotation of metabolites. The differential abundance (DA) score of KEGG annotation of differential metabolites in (a) H vs. C, (b) HG vs. H, (c) HGD vs. H, and (d) HGD vs. HG. DA score = (the number of up-regulated differential metabolites in this pathway – the number of down-regulated differential metabolites in this pathway)/the number of all metabolites annotated to this pathway.

participate in physiological processes^[86-87]. Consequently, small RNA sequencing was conducted on the GqDNVs suspension, goji NFC juice, and QiNeng to provide a comprehensive understanding of their functional roles in this study. The distribution of clean reads is presented in Fig. S15. After aligning the reads with Rfam13 and miRBase22^[26-27], we observed distinct miRNA distributions among the GqDNVs suspension, goji NFC juice, and QiNeng (Tables S4–5). The miRNAs were still detected in the GqDNVs suspension, though the concentration in the GqDNVs suspension was significantly lower than in goji NFC juice and QiNeng (Figs. 8a–c). Furthermore,

miRNAs from the GqDNVs suspension were annotated to the *Homo sapiens* cDNA library using psRNATarget (<https://www.zhaolab.org/psRNATarget/>)^[88]. This revealed associations between GqDNVs miRNAs and human genes, including pyruvate dehydrogenase kinase 3 (*PDK3*), NADPH-dependent diflavin oxidoreductase 1 (*NDOR1*), and RNA binding motif protein 4 (*RBM4*) (Fig. S16).

The expression levels of the top four sequences (aof-miR166d, aof-miR167a, vca-miR156a-3p, and aof-miR168a), linked to human cDNAs, were quantified using qPCR (primer details in Table S6). Both qPCR and small RNA sequencing revealed that the

expression levels of these miRNAs in goji NFC juice were higher than in GqDNVs suspension and QiNeng (Figs. 8d–g). Notably, after co-culturing C2C12 cells with goji NFC juice and QiNeng, the expression levels of the goji-derived miRNAs were higher in the HGD group compared to those in the HG group (Figs. 8h–k). This indicates that C2C12 cells absorb more goji-derived miRNAs when administered QiNeng than when treated with goji NFC juice alone.

Due to the observed changes in mitochondrial function and the annotation of goji-derived miRNAs via psRNATarget, we measured the expression levels of related mRNAs in C2C12 cells. Both goji NFC juice and QiNeng increased the expression levels of key genes involved in mitochondrial biogenesis^[54], including sirtuin 1 (*SIRT1*), peroxisome proliferator-activated receptor gamma coactivator 1α (*PGC1α*), nuclear respiratory factor 1 (*Nrf1*), and mitochondrial transcription factor A (*TFAM*). Notably, the expression of *Nrf1* was higher in the HGD group compared to the HG group (Figs. 8l–o). These findings are consistent with previous mtDNA and mitochondrial staining results, indicating that QiNeng enhances both mitochondrial function and quantity in C2C12 cells.

In addition, both goji NFC juice and QiNeng affected the expression levels of RNAs related to goji-derived miRNAs and muscle differentiation (Fig. S17). *RBM4* and polypyrimidine tract-binding protein (*PTB*) are involved in muscle differentiation, regulating transcripts such as myocyte enhancer factor 2C (*Mef2c*). There is an antagonism between *RBM4* and *PTB*, with *PTB* overexpression known to inhibit muscle differentiation-related mRNAs^[89]. *RBM4* and *Mef2c* levels in the H group were lower than in the C group. The expression levels of *RBM4*, *PDK3*, *nPTB*, and *PTB* were higher in the HG and HGD groups compared to the H group, while *Mef2c* expression decreased in both the HG and HGD groups. The HGD group showed a higher expression of *Mef2c* than the HG group. *Mef2c* is a key upstream regulator of muscle differentiation genes myogenic differentiation 1 (*MYOD1*), myogenic factor 5 (*MYF5*), and myogenin (*MYOG*), and they are essential for promoting muscle differentiation^[90–91]. Goji NFC juice and QiNeng increased the expression of *MYF5* in H₂O₂-treated C2C12 cells but reduced the

expression of *MYOD1* and *MYOG*. However, *MYF5* and *MYOD1* expressions were higher in the HGD group than in the HG group. Expression levels of myosin heavy chain 1 (*MYH1*, fast 2X), *MYH2* (fast 2A), *MYH4* (fast 2B), and *MYH7* (slow) were also changed accordingly. It is suggested that goji NFC juice likely promotes the conversion of C2C12 cells from fast-twitch (type II) to slow-twitch (type I) muscle fibers. QiNeng exhibited a similar trend, although the effect was less pronounced than goji NFC juice. The interaction between *PGC1α* and *Mef2c* drives the conversion of muscle fibers from type II to type I^[92–93], which mirrors the changes observed in the HG and HGD groups. This muscle fiber-type conversion induced by goji NFC juice and QiNeng is also consistent with previously discussed metabolic changes.

3.11 The heatmaps based on the Mantel test illustrated associations between differential metabolites and RNAs

The Mantel test, which assesses the correlation between two dissimilar matrices^[94], was employed in this study to verify associations between metabolites, RNAs, and oxidative stress indicators across the four experimental groups. Overall, metabolites such as phosphate sugars and amino acids exhibited the strongest correlations with RNA expression levels and oxidative stress markers in C2C12 cells (Fig. S18). Goji-derived miRNAs were positively correlated with mitochondria-related mRNAs (*Nrf1*, *TFAM*, and *PDK3*), *MYF5*, *SIRT1*, and *PGC1α*. In contrast, they were negatively correlated with muscle differentiation-related mRNAs, including *Mef2c*, *MYOG*, *MYOD*, *MYH1*, and *MYH2*. Notably, *Mef2c* may impact *n*-3 PUFAs in C2C12 cells. These findings suggest that goji-derived miRNAs can influence muscle differentiation and are positively linked with the shift from type II to type I muscle fibers. The differential metabolites among the groups, particularly phosphate sugars, amino acids, and their derivatives, were also correlated (Fig. S19). It is suggested that the alterations in metabolites, particularly energy-related metabolites, were associated with the changes in oxidative stress markers, goji-derived miRNAs, mitochondria-related mRNAs, and muscle differentiation-related mRNAs. Dysfunctional mitochondria

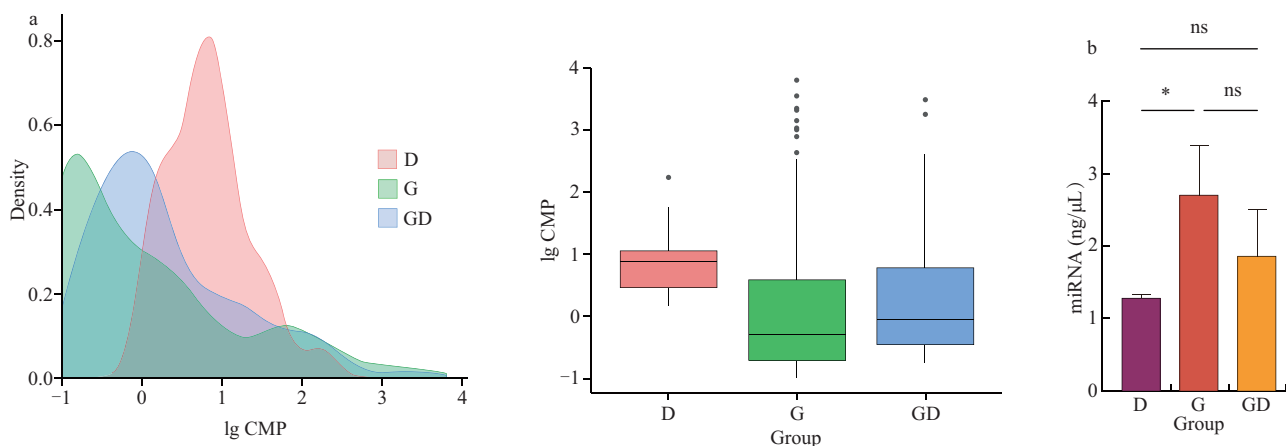


Fig. 8 (a–b) The CPM values and expression levels of miRNAs in GqDNVs, goji NFC juice, and QiNeng. (c) The expression levels of miRNAs in GqDNVs, goji NFC juice, and QiNeng that were detected by small RNA sequencing ($n = 1$). (d–g) The quantitative analyses of aof-miR166d, aof-miR167a, vca-miR156a-3p, and aof-miR168a expression levels in GqDNVs, goji NFC juice, and QiNeng; $n = 3$ in each group. (h–k) The quantitative analyses of aof-miR166d, aof-miR167a, vca-miR156a-3p, and aof-miR168a expression levels in C2C12 cells; $n = 5$ in each group. (l–o) The quantitative analyses of *SIRT1*, *PGC1α*, *Nrf1*, and *TFAM* expression levels in C2C12 cells; $n = 5$ in each group. D, G, and GD represent GqDNVs suspension, goji NFC juice, and QiNeng, respectively. One-way ANOVA analysis and Šidák's multiple comparisons analysis were used in the between-group comparisons.

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, and **** $P < 0.0001$; ns indicates $P > 0.05$.

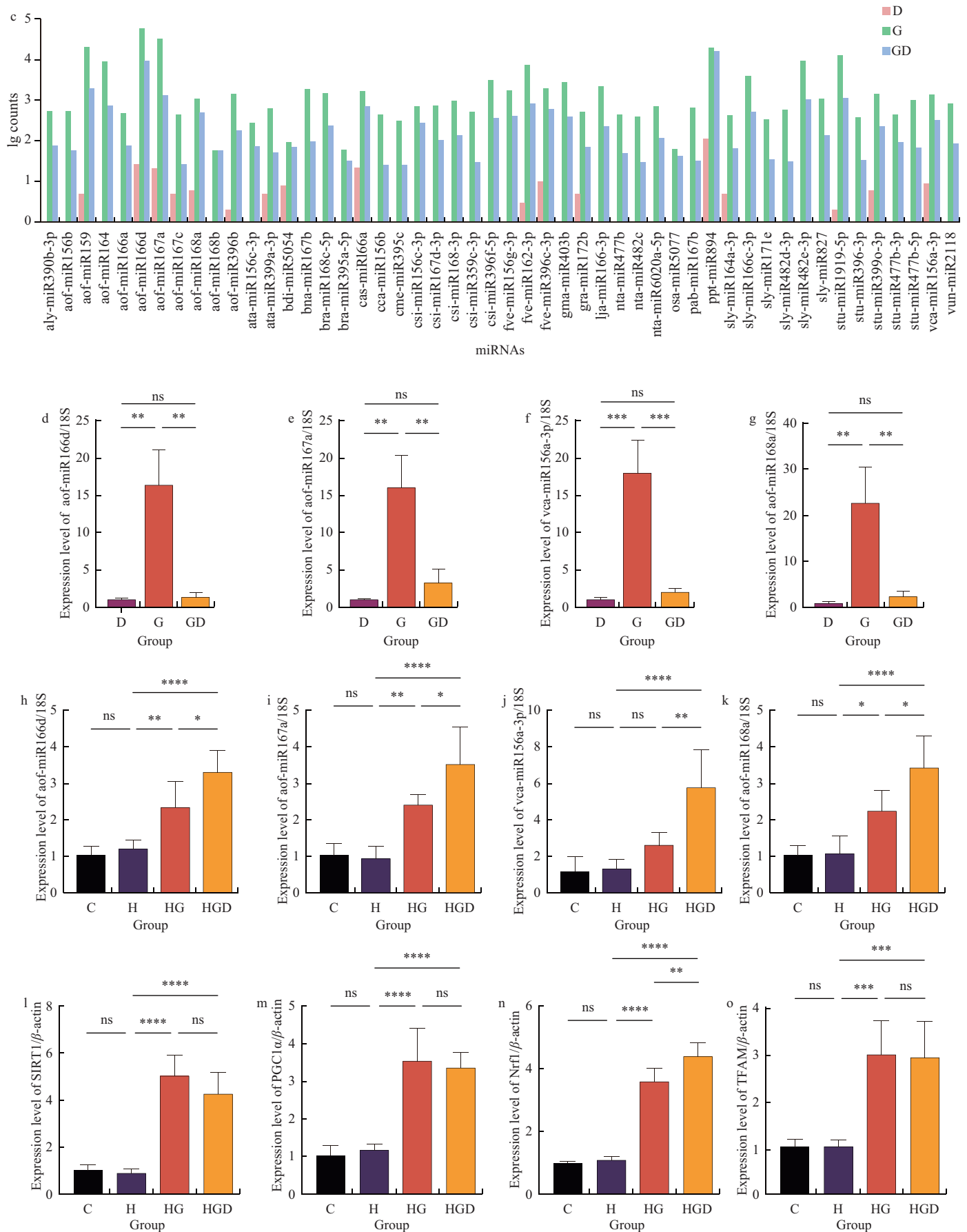


Fig. 8 (Continued)

and an imbalance in energy metabolism are involved in aging and age-related diseases^[95]. Therefore, pronounced increases in energy metabolism and mitochondrial function induced by QiNeng were key in inhibiting senescence caused by H₂O₂ in C2C12 cells.

4. Conclusions

In this study, we developed a prototype beverage called QiNeng, which combines goji NFC juice with 5×10^{10} particles/mL GqDNVs to enhance its health benefits. The GqDNVs in QiNeng are more stable than those in suspension form. Both QiNeng and goji NFC juice reduced oxidative stress induced by H₂O₂. However, after treatment with QiNeng, a higher concentration of goji-derived miRNAs was detected in C2C12 cells compared to goji NFC juice alone. Furthermore, QiNeng demonstrated greater effectiveness than goji NFC juice in promoting cell growth and inhibiting signs of cellular aging, such as excessive autophagy, myotube atrophy, and elevated β -galactosidase activity in H₂O₂-treated cells. Notably, QiNeng significantly enhanced mitochondrial function and energy metabolism in C2C12 cells, as observed through targeted metabolome analysis and mitochondrial staining, whereas these changes were insignificant with goji NFC juice. While QiNeng has demonstrated preliminary evidence of suppressing cellular senescence in an *in vitro* muscle aging model, its translational potential requires cautious evaluation given the multifactorial etiology underlying muscle wasting and/or sarcopenia.

Collectively, QiNeng exerts a pleiotropic effect by improving myogenic proliferation and differentiation, enhancing mitochondrial function, and promoting energy metabolism, thus ameliorating age-related senescence phenotypes in C2C12 cells. Future studies should develop multifactorial *in vitro* models and naturally aged animal models, to comprehensively evaluate the therapeutic potential and mechanism of QiNeng for sarcopenia intervention.

Conflicts of interest

The authors declare that they have no conflict of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://doi.org/10.26599/FSHW.2025.9250642>.

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